components of growth media into a heat stable compound that inhibits the growth of the mutant under aerobic conditions. The compound does not inhibit the growth of the wild-type parent.

- Sonicates of the wild-type parent produce only small quantities of the inhibitory material.
- Mixed cultures of anaerobes have been produced from 30 different soil samples from a variety of sites.
- The mixed cultures of anaerobes have been characterized morphologically and adapted to grow in 2, 2^1 3, 3^1 5, 5^1 and 2, 2^1 4, 4^1 5, 5^1 PCBs.

We intend to continue the development of methods for growing unusual anaerobes. These methods will make use of the enzymatic approach to oxygen removal. New media formulations, mimicking natural environments, will be devised and tested for their ability to support the growth of unusual anaerobes. In particular, attention will be focused on the development of methods that will aid in the isolation of anaerobic bacterial species that are present at very low concentrations in natural habitants. These organisms, although present only in low numbers, may play important roles in the chain of events involved in bioremediation of toxic materials.

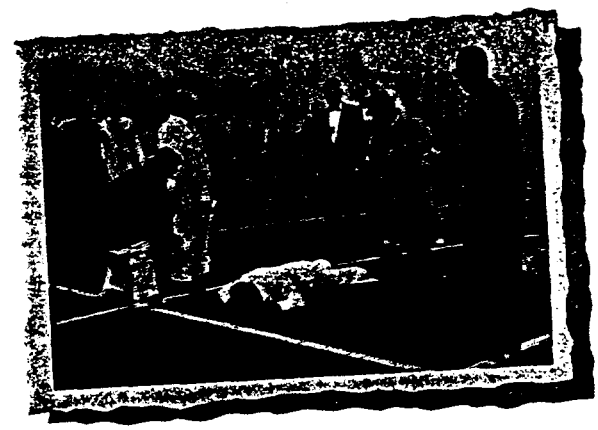
radiation emergency assistance center/training site

Progam Director: ***Robert C. Ricks***

The Radiation Emergency Assistance Center/Training Site (REAC/TS) continued to assist the DOE, Federal Emergency Management Agency (FEMA), World Health Organization (WHO), and the International Atomic Energy Agency (IAEA) in the areas of medical management of radiation accidents as well as assistance in the training of medical and medical support personnel for radiation accident response. The REAC/TS program also provides medical assistance to DOE's Accident Response Group (ARG) and the Federal Radiological Monitoring and Assessment Center (FRMAC) and is prepared to assist DOE emergency response programs following major radiation accidents at DOE-Defense Programs contractor facilities or commercial nuclear power plants.

The REAC/TS emergency response activities continued to include on-site emergency medical assistance and consultation services, among which are:

- An experienced emergency response team consisting of physicians, nurses, health physicists, coordinators, and necessary additional support personnel continued on 24-hour call to provide consultation or direct medical and radiological assistance in the event of a major radiation accident. Approximately 60 domestic requests for assistance were received in the past year. Since June 1976, REAC/TS has documented 1,150 radiation-related events in its registry system, approximately 650 of which were calls for assistance. REAC/TS emergency response teams also continued to provide direct or consultative assistance to WHO, IAEA, and the U.S. Department of State following accidents in foreign countries.



A simulated radiation accident staged to evaluate emergency medical response duringthe early years of MSD (1961). In 1976, the Radiation Emergency Assistance Center Training Site (REAC/TS) was established to study the medical management of radiation accidents and provide training to support personnel for radiation accident response.

- In 1990, REAC/TS continued to provide follow-up assistance to the IAEA and Government of Mexico as a result of the 1989 ^{60}Co accident in San Salvador. Assistance to the IAEA resulted in a post accident review, *The Radiological Accident in San Salvador*, published in early 1990. This document reviews the accident in relation to cause, medical response, and lessons learned. Patient follow-up has also been accomplished through assistance from the Hospital Angeles del Pedregal in Mexico City. In September 1990, samples of bone marrow and peripheral blood were obtained for follow-up cytogenetic analysis. In addition, bone samples obtained after surgical amputation of a victim's heavily irradiated legs have been analyzed using electron spin resonance dosimetry performed at the National Institute of Standards and Technology, Gaithersburg, MD. The results of this analysis will be published soon.

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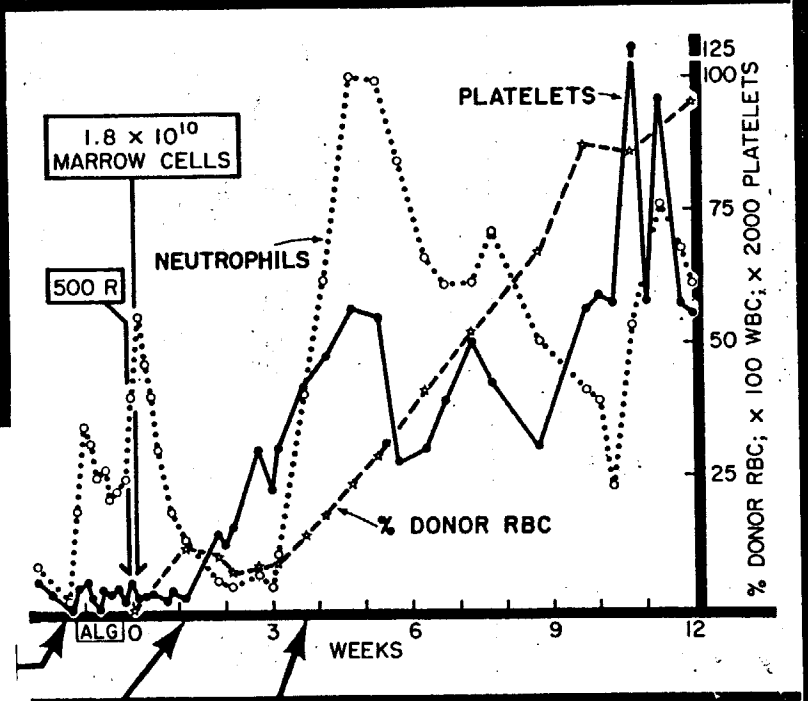
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research report

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ON THE COVER: A diagram showing hematologic evidence of a successful bone-marrow graft. A leukemic patient, immunosuppressed with 500 R of total-body irradiation at 40 R/min and a course of antilymphocyte globulin (ALG), was infused with his brother's bone marrow. At an interval when radiation-induced marrow depression would be most pronounced, an unusual regeneration of blood elements was already well under way, signaling a graft take. It was also confirmed later by a red blood cell marker. *For details of this project please see page 63.*

BIOLOGY AND MEDICINE

ORAU-113

1970

research report

FOR THE YEAR ENDING DECEMBER 31, 1970

Medical Division

Oak Ridge Associated Universities

OPERATING UNDER CONTRACT WITH THE UNITED STATES ATOMIC ENERGY COMMISSION

NOTICE

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ORAU-113

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Printed in the United States of America

Price $3.00

Available from: National Technical Information Service
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ORGANIZATION OF RESEARCH . . . . .

INTRODUCTION . . . . . .

RADIATION EFFECTS AND RELATED TI
Clinical Total-Body Irradiation . . . . . .
Summary of Accomplishments . . . . . .
Infections in Irradiated Patients . . . . .
Dosimetry Studies and Monitoring . . . . .
Applied Radiobiology 47
Radiation Immunology and Marrow Transplantation 56
Experimental Hematology 73

RESEARCH IN NUCLEAR MEDICINE 78
Summary of Accomplishments 78
Radiopharmaceutical Development 79
Clinical Investigations in Nuclear Medicine 100
Whole-Body Counting 117
Instrumentation and Computer Technology 121

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM . 134
Summary of Accomplishments 134
Biological Chemistry 135
Marmoset Research Project 152
Ultrastructural Studies of Tumors 162
Induced Cytogenetic Abnormalities 168
Environmental Pollutants and Altered Metabolism 183
Gold Thioglucose and the Satiety Center 190

PUBLICATIONS 195

MEDICAL DIVISION CONFERENCES–1970 203

LIPID SEMINARS–1970 205

FELLOWSHIPS AND TERM APPOINTMENTS 206

RESEARCH PARTICIPANTS–1970 207

STUDENT TRAINEES–1970 207

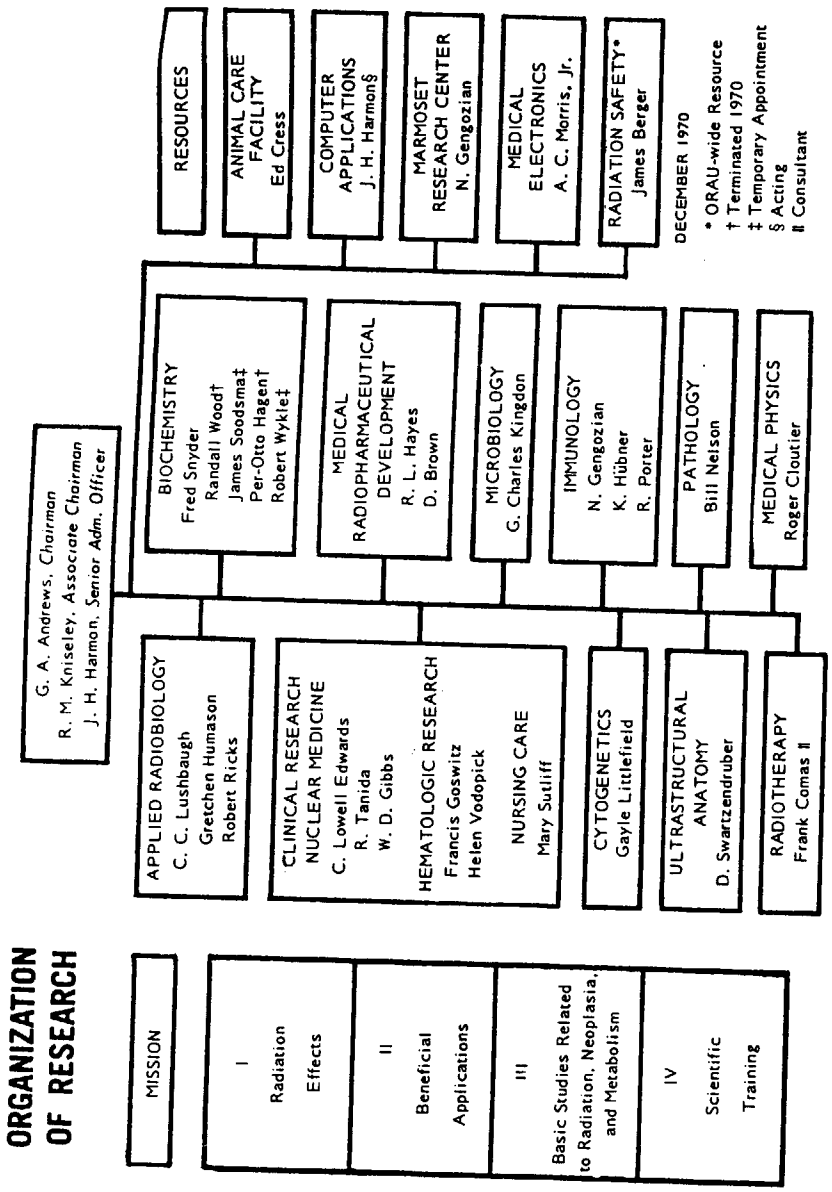
ORAU MEDICAL DIVISION ADVISORY COMMITTEE 207

MEDICAL COMMITTEE, ORAU COUNCIL 208

MEDICAL DIVISION EMPLOYEES 208

CONSULTANTS AND COLLABORATORS–1970 210

NOTE: Numbered references in the text may be located in the list of publications beginning on page 195.



1027893

INTRODUCTION

Since 1949 the Medical Division has conducted a biomedical program, including both clinical and preclinical research. More than 2200 patients have entered our hospital for diagnostic trials as well as therapy of their disorders. Treatment may be by experimental protocols, principally using radiation, but sometimes includes drug or surgical approaches in controlling the patient's disease. Currently in our mission we emphasize (1) mechanisms of radiation injury, especially in man; (2) beneficial applications of radiation for diagnosis and therapy; and (3) pursuit of selected basic studies, particularly ones related to cancer, that arise out of the ongoing program. Further, we see a responsibility to provide training for young investigators and graduate students, since future needs for professionals with research skills will be mounting.

In our 1969 Report we departed from the earlier format hoping to provide a more unified picture of our current activities by emphasizing less the separate units of research. Encouraged by the favorable responses, we have elected to retain this format in 1970. However, in this report we do plan to expand the general introductory parts of the three main sections to identify major advances during the year. Thus a quick overview of the report may be obtained by reading the brief summaries beginning on pages 4, 78, and 134.

In 1970 there were relatively few changes in the scientific staff. Our technical editor, Elizabeth Anderson, reached her date of retirement on July 31 and we held her farewell ceremonies with genuine regret. We were pleased that Marion Garber, a long-time employee of the ORAU library, was able to transfer to our Division and is assuming Mrs. Anderson's duties. Randall Wood, who joined the lipid group in 1965 as a postdoctoral fellow and later was promoted to research biochemist, resigned and accepted a position in the research laboratories of the Neuropsychiatric Research Laboratory, Hines VA Hospital. During his 5 years here he established a reputation among lipid biochemists as a solid research worker.

David Brown, an expert in tissue fractionation, joined our senior staff on a temporary basis to work on the gallium project.

Owing to the strictures in the current funding, we reluctantly reduced training activities somewhat and this partly reflects the general curtailment of funding for training. We could not award the usual two new postdoctoral Assistantships in Clinical Investigation this year, handicapping our capability to care for our customary number of patients. Steven Ominsky served as temporary resident and contributed to the study of intralymphatic ^{67}Ga. Gifford Ammermann, formerly at the Chicago Wesley Memorial Hospital, completed his one-year appointment as Postresident Assistant in Clinical Investigation; we are pleased to note that he is working in nuclear medicine as a member of the Pathology Department at the University of Tennessee Memorial Research Hospital. We had one Medical Student Fellow in Nuclear Medicine, Parham R. Fox, assigned here from the Medical School of Virginia Commonwealth University. Yukio Tateno's fellowship from the Damon Runyon Foundation was renewed; he is working in nuclear instrumentation and came here from the University of Chiba, Japan. From the Comision Nacional de Energia Nuclear of Mexico came Jose I. Gomez-Gallardo, on a short-term appointment for work in whole-body counting. Assigned to the Medical Division from the University Participation Office were four undergraduate students in the college trainee program and two Research Participants.

In immunology with N. Gengozian were five University of Tennessee graduate students, Nancy Basford (until August), Charles Gottlieb (until June), John Kateley, Andrew So, and Mike Yurconic.

Leaving the biochemistry program in addition to Dr. Wood, was Per-Otto Hagen who transferred to the Department of Experimental Surgery at Duke University. Dr. Hagen took with him an NIH research grant which had been awarded while he was here. Ten-ching Lu Lee at the end of the year was completing her Public Health Service Postdoctoral Fellowship under Fred Snyder and we are planning to arrange for a continuation of her work as a staff member. James Soodsma completed his AEC Postdoctoral Fellowship and stayed on to work on grant-supported research. Robert Wykle received his Ph.D. degree from the University of Tennessee Medical Units at Memphis and stayed with us to participate in grant-supported research. Also at the biochemistry project were a new postgraduate fellow, Roger Lumb, from Western Carolina University; and two visiting scientists, Kazuo Kasama, Mie Prefectural University, Japan, sponsored by the Japanese government; and Murray Grigor from Dunedin, New Zealand, sponsored by the Medical Research Council of New Zealand.

The year 1970 saw the prompt publication of the proceedings of our 12th major symposium in nuclear medicine. This meeting, held December 8-11, 1969, was supported jointly by the AEC and the Public Health Service; the topic was Medical Radionuclides: Radiation Dose and Effects, and as in earlier years, publication was possible through the help of the Division of Technical Information Extension of AEC in Oak Ridge. Our next major symposium is scheduled for November 15-19, 1971 entitled Clinical Uses of Radionuclides: Critical Comparison with Other Techniques.

In spite of limited funds for travel, our staff was well represented at important national meetings during the year presenting approximately 20 papers. We were also represented at important foreign meetings by Nazareth Gengozian who attended the Third International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions, Uppsala, Sweden, September 1-4, and the Third International Congress of the Transplantation Society, The Hague, September 7-11. Karl Hübner attended the IV International Congress on Radiation Research at Evian, France, June 28 to July 4. Donald Swartzendruber attended the XIII International Congress of Hematology, Munich, Germany, August 2 to 8, and presented a paper entitled, "Electron Microscopic Studies of Lymphoid Germinal Centers." Fred Snyder and Ten-ching Lee attended the VIII International Congress of Biochemistry, Lucerne and Montreaux, Switzerland, September 3-9, where Dr. Snyder presented a paper, "A New Pathway for the Biosynthesis of O-alkyl Bonds in Glycerolipids." Roger Cloutier presented lectures in the IAEA Radiation Dosimetry Course for Latin Americans at the Puerto Rican Nuclear Center, San Juan, Puerto Rico, October 12 to 23, 1970.

Our base of support is mainly from the United States Atomic Energy Commission, Division of Biology and Medicine, John Totter, Director. We have continued to receive outside funds for some important research areas; the NASA project, "Radiosensitivity in Man," under C. C. Lushbaugh's supervision enters its 7th year. The Department of the Army Medical Research and Development Command has continued our contract to study infections in irradiated patients under Dr. G. C. Kingdon. In marmoset research, Dr. Gengozian continues his immunologic studies under an NIH grant, "Immunology of Hematopoietic Chimerism in Tamarins," and receives supplementary support from ORAU corporate funds to maintain the colony. Dr. Snyder's grant from the American Cancer Society on "The Occurrence, Metabolism, and Function

1027894

of Glyceryl Ethers in Neoplasms" has been renewed, as has been an NIH grant to the University of North Carolina in which he participates with Claude Piantadosi on "Basic Studies on Alkoxy Alkenyloxy Glycerol Ethers and Derivatives." The contract with the National Center for Population Research at NIH supports a project, "Chromosomal Breaks in Women Taking Oral Contraceptives" under Gayle Littlefield's direction. New grant support was obtained during the year by Dr. Snyder from the Multiple Sclerosis Society on a project entitled "Occurrence, Biosynthesis, and Biocleavage of Ether-Linked Lipids in Biomembranes." Raymond Hayes was awarded a 3-year grant from the National Institutes of Health for a proposal entitled "Concentration of Gallium by Malignant Neoplasms." Some other proposals are pending. While the stringencies of current research support have been felt, we take some comfort in knowing that we have fared better than many institutions, and in fact, are encouraged by the new grants that were awarded to us during the year.

We will continue to encourage cooperative activities among our staff members and the Oak Ridge National Laboratory, as well as with our member institutions. A promising, significant event during 1970 was the formation of the Cooperative Group to Study Localization of Radiopharmaceuticals. Formation of the Group arose out of a proposal by our Review Committee and a work meeting of representatives mostly from ORAU-member institutions. Initial meetings were supported by the American Cancer Society and out of it have come some specific protocols for cooperative research in evaluating gallium-67 as a tumor-scanning agent. Proposals for support of these activities are pending.

Gould A. Andrews
Chairman

RADIATION EFFECTS AND RELATED TOPICS

Clinical Total-Body Irradiation

The acute effects of total-body irradiation in man continue to be a major part of the Medical Division's program. Reasons for this continued interest are two-fold: We need better ways for controlling or palliating leukemias, lymphomas, and related disorders with radiation, and we need to devise the most effective treatments for victims of accidental exposures to radiation. Our experience goes back almost 15 years, having started with our participation in the early attempts to achieve marrow transplants in leukemic patients after high-dose irradiation. The Division also has had firsthand experience with radiation accident victims and has collected worldwide information on the responses of man to acute accidental exposures of radiation. In more recent years we have carried out a retrospective study of the responses of patients treated with total-body irradiation at many radiotherapy centers throughout the United States.

Some time ago we completed a program in which single treatment doses of 50- and 100-R of medium-dose-rate total-body irradiation were given to a series of patients. The results now form a base for comparison of responses obtained after protocols of protracted and fractionated irradiation.

With the newly initiated bone-marrow transplant protocol, we have a unique opportunity to analyze the response of patients receiving high radiation doses at a high-dose rate. The hematologic responses of the first patient will be recorded in this section of the report; cytogenetic studies will be described in a later section under the topic "Induced Cytogenetic Abnormalities."

Summary of Accomplishments

Thirteen additional patients with hematologic disorders were given low-dose-rate irradiation with distinct benefit, but no evidence of remarkable advantages over other forms of therapy. These patients and others given moderate doses of total-body irradiation at medium and low-dose rates were found to have greater variability in microflora after treatment, but clinical infections were not a problem. Although not constant, decreases in serum immunoglobulins and complement were often seen after irradiation. A dose of 100 R and one of 150 R at the low-dose rate caused increased fatigability and respiratory stress, as shown by exercise tests.

An adult patient with acute leukemia was given a successful allogeneic graft of marrow from his brother after preparation with 500 R of total-body irradiation and antilymphocyte globulin. Early cytogenetic changes were a good index of the absorbed radiation dose. The laminar flow ultraclean environment was used for this patient and extensive microbiologic observations were recorded. The leukemia went into remission and all red cells became of the donor type. There was no clear evidence of any immunologic complication.

Studies of mice given daily total-body irradiation at different dose rates over long periods of time with the same accumulated total dose, were compared by techniques that attempted to measure the relative rates of damage and repair. Certain strains of mice stressed by chronic irradiation were shown to produce hemoglobin of an altered biochemical form.

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Previously, in the immunology program, it has been shown that for gamma irradiation, a high-dose rate yielded increased immunosuppression per rad; this year we demonstrated that with neutron irradiation the dose rate does not influence the degree of immunosuppression. It was shown that less than a lethal dose of high-dose-rate total-body gamma irradiation would allow successful foreign grafts in mice if the immunosuppression was supplemented by antilymphocyte serum. This gave rise to the marrow graft protocol that was used clinically. Further applications of the Jerne plaque test permitted semiquantitative evaluation of immune responses in xenogeneic and allogeneic marrow transplants in mice. It was shown that the greatest graft-versus-host activity is in the first week after transplant even though the clinical syndrome is most prominent during the third and fourth weeks; pronounced impairment of immunocompetence persists after clinical recovery from this reaction.

Electronic sizing of red blood cells, previously shown to be of value in following the course of patients with polycythemia, was used in a study of stored blood; little change was seen during the first five days but progressive swelling of the red cells occurred after that and appeared to be a valuable indication of their status.

The Effects of Low-Dose-Rate TBI upon Human Peripheral Blood Values

Thirteen treatments were given in the low-exposure-rate irradiator during 1970 bringing our total to 37 since the facility was opened in 1967 (C. L. Edwards, G. A. Andrews, Helen Vodopick, Francis A. Goswitz, C. C. Lushbaugh, and R. M. Kniseley). Table 1 tabulates the 1970 exposures by diagnosis, age and sex of patient, exposure, symptoms and response. Only one patient has experienced nausea and vomiting while being treated with LETBI, a 48-year-old woman with chronic granulocytic leukemia (CGL). She consistently has shown good responses to the total-body irradiation [medium-exposure-rate irradiator (METBI), LETBI, and fractionated METBI] and splenic irradiation, but has experienced gastrointestinal symptoms with each. Other patients occasionally have complained of lethargy or a feeling of weakness and mild anorexia, but no nausea or vomiting.

Experience from previous years indicated that exposures of 250 R in LETBI were probably too much for most patients; possible exceptions are polycythemia vera patients with very high platelet counts or those with very high leukocyte counts due to chronic granulocytic leukemia, provided they had not received previous suppressive treatment. Consequently, only two patients were treated with 250 R in 1970. Both patients had untreated chronic granulocytic leukemia with total leukocyte counts greater than 100,000/cu mm. Both experienced symptomatic improvement along with reduction of the WBC to approximately 15% of the pretreatment value while the platelet counts dropped to about 40% of the pretreatment value.

There were five 150-R treatments of chronic granulocytic leukemia. Each treatment was followed by subjective improvement commensurate with the observed hematologic changes including decrease in the white blood cell count to 1-30% of the pretreatment value. The patient whose WBC count dropped to 1% of the pretreatment value also had an unusually severe drop in her other blood values. This probably occurred because the radiation was given only 12 weeks after the previous exposure of 150 R over 5 days in METBI. The low leukocyte count persisted for 9 months before returning to normal values.

Figure 1 shows the geometric mean of the WBC, normalized to the percent of the last pretreatment value (day zero) for three patients having chronic granulocytic leukemia treated with 250 R in LETBI and six patients treated with 150 R. Both groups show a rapid decrease in total WBC through the first 12 days; after this a transient increase occurs, peaking at about 30 days, followed by a second drop that continues through day 52. When these data are compared with the data obtained from patients with CGL treated with 100 R or 50 R in METBI (Fig. 2) we see a similar pattern, particularly in the 100-R group. We know of no satisfactory explanation of this complex curve of white cell values; the timing is quite different from that seen in normal radiation accident victims who show an "abortive rise" in granulocytes at about 15 days and a nadir at about 30 days. The total WBC count of patients with polycythemia vera or lymphocytic leukemia does not show this change after treatment (Fig. 3).

Table I

LOW EXPOSURE RATE (1.5 R/Hr) TOTAL-BODY IRRADIATION OF PATIENTS DURING 1970

No.*	Diagnosis	Sex	Age	Total Residence Hours	Total Residence Days	Total R	Exposure Hours	Gastrointestinal Symptoms	Blood Count Nadir WBC %	WBC Day	Platelets %	Platelets Day
25	PRV	M	59	115.0	5.0	150	102.3	0	30	45	13	31
26	CGL	F	42	221.0	9.0	250	164.5	0	12	52	44	52
27	CGL	F	69	120.0	5.0	150	98.7	0	1	37	3	110
28	PRV	M	76	116.0	4.8	150	100.0	0	24	45	13	31
29	CGL	F	48	118.0	4.9	150	101.4	Vomited	11	52	78	31
30	CLL	M	69	79.5	3.3	100	67.6	0	22	31	80	31
31	LS	M	45	85.0	3.5	100	68.0	Anorexia	33	14	68	24
32	CGL	M	52	118.0	4.8	150	98.0	0	19	38	32	31
33	CLL	M	61	84.0	5.5	100	68.7	Vomited	40	52	73	31
34	CGL	F	48	120.0	5.0	150	102.0	Vomited**	3	52	50	52
35	CGL	M	53	120.0	5.0	150	102.0	Anorexia	28	14	74	45
36	CGL	M	52	207.0	8.6	250	164.5	0	18	14	44	14
37	PNT	M	64	117.0	4.8	150	100.0		45	31	18	45

* Consecutively numbered (see 1969 Research Report).

** Vomiting associated with splenic infarction.

In the Research Report last year we commented on the failure of a single exposure of 100 R in LETBI to change the platelet count very much. This observation was made from data taken from patients with a variety of different diseases grouped together. However, severe platelet depression is rarely a problem in polycythemia vera or chronic granulocytic leukemia so we chose to check this observation in patients with chronic lymphocytic leukemia and disseminated lymphosarcoma, diseases frequently complicated by thrombocytopenia. Four patients, two with chronic lymphocytic leukemia and two with lymphosarcoma with bone-marrow infiltration, were treated with 100 R during 1970. When data from these patients were combined with that from the three patients with similar diseases treated previously with 100 R in LETBI, we found a significant drop in the mean platelet count. Figure 4 shows the geometric mean of the platelet counts normalized to the percent of the pretreatment value. There is a progressive decrease to approximately 60% by the 38th day followed by recovery to approximately 80% at 52 days. If there is a radiation dose rate at which the lymphocyte count is selectively lowered without causing a drop in the platelet count, it must be even lower than 1.5 R/hr.

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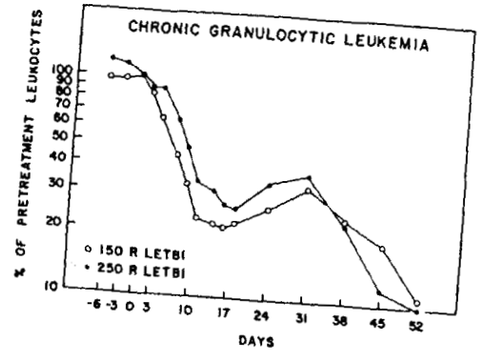


Fig. 1. The effect of 250 R (3 patients) and 150 R (6 patients) total-body irradiation at 1.5 R/hr on the total leukocyte count in chronic granulocytic leukemia shown as the geometric mean for each group. The values for the total leukocyte count are expressed as percent of the value for day zero, the morning before radiation was started. The radiation was given from day zero to 5 for 150 R and zero to 8 for 250 R.

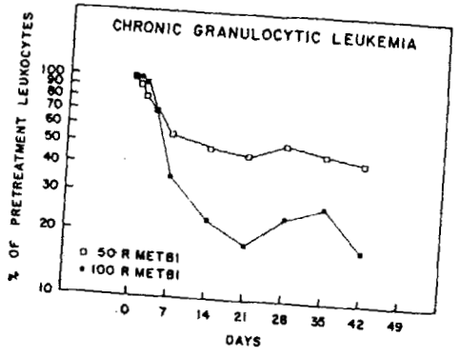


Fig. 2. The effect of 50 R and 100 R total-body irradiation at 1.5 R/min (^{137}Cs) on the total leukocyte count in chronic granulocytic leukemia shown as the geometric mean for each group. The leukocyte counts are expressed as the percent of the average preradiation values.

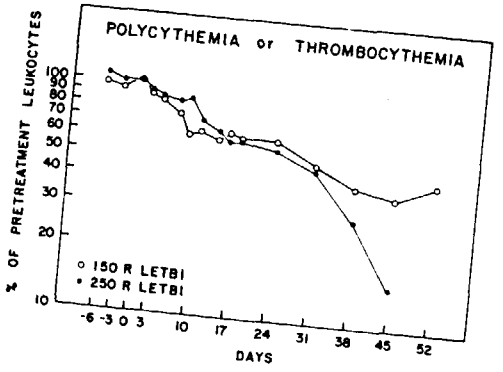


Fig. 3. The effect of 150 R and 250 R total-body irradiation at 1.5 R/hr on the platelet count in patients with thrombocythemia, idiopathic or associated with polycythemia vera. The platelet values are the geometric means for each group expressed as percent of the value on the morning before the radiation, day zero.

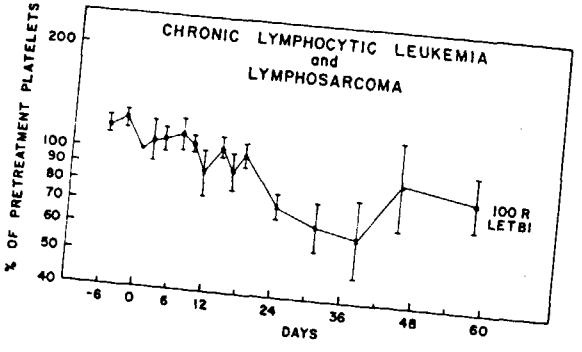


Fig. 4. The effect of 100 R total-body irradiation at 1.5 R/hr on the platelet count in seven patients with chronic lymphocytic leukemia or disseminated lymphosarcoma with bone-marrow infiltration. The platelet values shown are the geometric means and standard errors for each point and are expressed as the percent of the value of day zero, the morning before the radiation.

1027897

Leukokinetic Studies After LETBI

To investigate the mechanism of white blood cell (WBC) changes after irradiation, five studies have been completed before and after low-exposure total-body irradiation (LETBI) in four patients with chronic granulocytic leukemia (Helen Vodopick, Elizabeth Rupp, Francis A. Goswitz, and C. L. Edwards). One patient participated during two treatment sequences. White blood cells were labeled by an in vitro technic with ^{32}P-diisopropylfluorophosphate. A baseline leukokinetic study was performed before treatment and was repeated just at the commencement of therapy or just after completion of irradiation. Total treatment dose given as continuous exposure at 1.5 R/hr was 150 R in three instances and 250 R in two instances.

The two patients who received the 250-R exposure had slight prolongation of the labeled granulocyte survival time as compared to their control values. The control granulocyte disappearance $T_{\frac{1}{2}}$ values were 80 hr and 114 hr; the posttreatment values were 90 hr and 124 hr respectively. A concomitant fall in the circulating WBC values was observed.

When the exposure dose was 150 R, the labeled granulocyte disappearance time did not change at all in two patients although their circulating granulocytes were reduced by therapy. In the third patient receiving this radiation dose, a triphasic granulocyte disappearance curve in the pretreatment study became a biphasic curve after treatment and thus did not allow a satisfactory comparison.

These initial data are hardly conclusive but a tentative interpretation can be made. The lack of discernible change in the disappearance time of the radioactively labeled granulocytes after the 150-R LETBI would suggest that the relative input of unlabeled cells had not changed sufficiently to alter the leukocyte specific activity. But when a larger dose and longer exposure, 250 R, is used, the leukocyte specific activity is prolonged suggesting a reduction of the input of unlabeled granulocytes. In parallel with these changes of radioactively labeled granulocytes, a greater fall in the circulating WBC count occurs with the larger radiation dose. These results suggest that the longer exposure time and the larger total dose produce a greater effect on the dividing precursor cells and thus reduce production to a greater degree.

A Reappraisal of Radiation-Induced Effects on Human Gonads

As a segment of our studies on human radiation sensitivity we have examined the literature and our own experience with gonadal changes after radiation exposure (Robert C. Ricks, Gretchen Humason, and C. C. Lushbaugh). After seven decades of radiotherapeutic exposures of man and other animals there are still few well-established guidelines which mark the radiation-exposure level where severe pathologic consequences are to be expected in normal tissues necessarily exposed in tumorous areas. Most conclusions concerning the doses required to cause normal cell necrosis are extrapolated from animal experiments and introduce a bias due to interspecies variation in response. Moreover, each patient exposed to ionizing radiations presents an individual variation that itself has not yet been studied extensively.

The great volume of literature on radiation response of animals has been searched for such end points but most experimental studies concern single doses at high dose rates (< 10 R/min). Most radiotherapeutic exposures, on the other hand, are not done in one day but are divided over longer periods so that dosage appraisals are complicated

by fractionation, radiation-free interval versus exposure time, partial-body exposures, and varying ways of expressing total dose.

Rubin and Casarett (University of Rochester) have recently pointed out again* the need for guidelines to define for the radiotherapist the range of tolerance doses to the normal tissues. They have defined the maximum tolerance dose (TD 50/5) as that radiation dose resulting in a 50% rate of severe complications during the 5 years postexposure, with all patients exposed under a standard set of fractionated therapeutic conditions. The standard exposure time was fixed at five treatments/wk for 30 equal exposures of about 200 R to small portals. The minimum tolerance dose (TD 5/5) was defined as that resulting in 5% of the patients having severe complications during the 5 years after exposure. They presented a table that provided a rough estimate of such tolerance doses for all normal organs. For the gonads these were:

Organ	Injury at 5 Years	TD (5/5)	TD (50/5)
Testes	Permanent sterilization	500 - 1,500	2,000
Ovary	Permanent sterilization	200 - 300	625 - 1,200

Since we were asked in the course of our studies on human radiation sensitivity to evaluate gonadal-dose-response relations, we used their tentative guidelines as a starting point and reevaluated the literature and our clinical pathologic material.

The tolerance doses of Rubin and Casarett clearly imply that the human testis is more radioresistant than the ovary. Our literature search has failed to support this concept for man and other primates, although it is well documented in studies of mice and most other rodents.

After a single dose of 600 R to the testes, azoospermia occurs in man but persists for less than 5 years. As little as 15 R, however, can produce a short period of azoospermia 46 days after exposure. Following testicular doses of < 100 R in radiation accidents, lower than normal sperm counts have been found for several years. A total-body exposure of ∿400 R in one man caused a 3-year period of sterility as assessed by sperm counts and testicular biopsy.

Disagreement as to whether the human testis is radiation-sensitive or resistant appears to stem from (1) the ease with which a single small dose causes significant testicular damage and from (2) the fact that no single large dose is known to have ever produced permanent sterility in man. Our review revealed that only the 1968 Argentina radiation-accident victim who received > 1,000 R but < 2,000 R to the testes in two 10-hr exposures one day apart has documented complete testicular atrophy. Recently we restudied the changes in the germinal epithelium of five victims who died at various times after accidental total-body exposures. After lethal single exposures, primitive spermatogonial cells capable of later regeneration have survived (Figs. 5 and 6). The testicular tubules shown in Fig. 5 were exposed to about the human LD_{50} level 20 years previously. Regeneration and restoration of the germinative epithelium was complete and did not fail in time. In Fig. 6 A a section is shown of the testis of an accident victim exposed 2 days before his

* Data presented at the Sixth Annual San Francisco Cancer Symposium, October 16 and 17, 1970 at the Sheraton Palace Hotel in San Francisco.

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Fig. 5. Photomicrographs (x370) of testicular tubules of a man exposed accidentally to about 390 R of neutron and gamma rays 20 years previously. The upper photo shows an area of sparse but normal sperm production in thin-walled tubules; least residual damage. The lower photo shows intertubular fibrosis and a lower level of recovery.

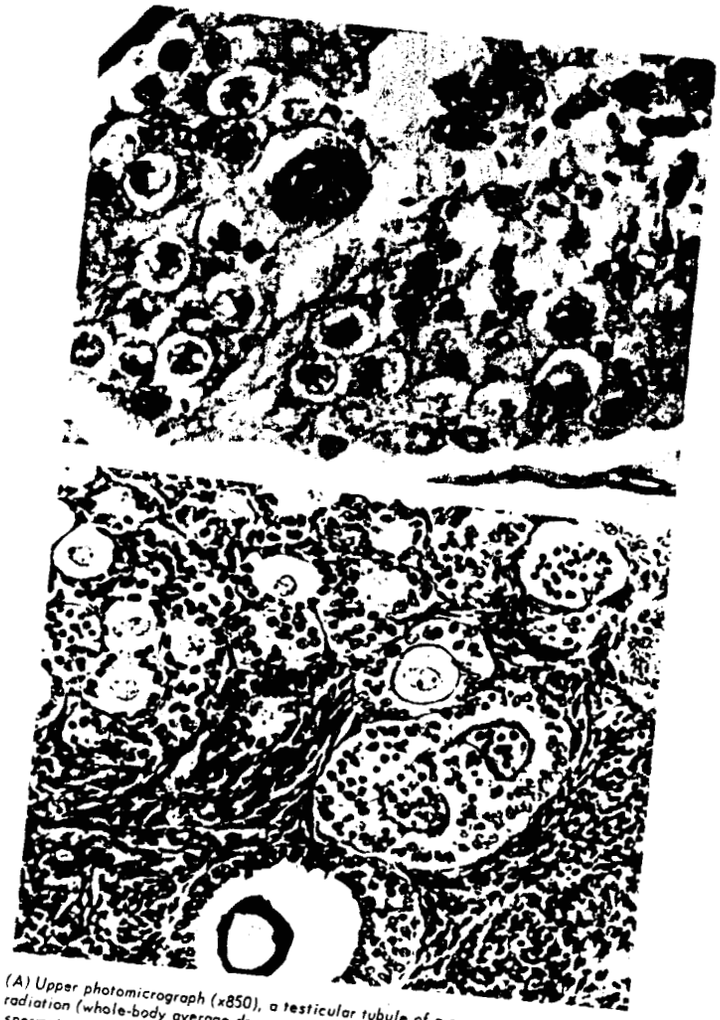


Fig. 6. *(A) Upper photomicrograph (×850), a testicular tubule of a man accidentally exposed to radiation (whole-body average dose estimated to be 8000+ rads) contains degenerating spermatocytes, abnormal multinuclear forms, normal appearing sperm, and rare normal appearing spermatogonia 2 days after exposure.*

(B) Lower photo (×370), the ovarian cortex of a marmoset (S. fuscicollis) that died a hematopoietic death 16 days after a total-body exposure to 200 R. Three degenerating multilayered graafian follicles and their ova are seen next to a field of single-layered follicles contain surviving normal appearing oocytes. This animal had no surviving bone marrow and numerous intestinal mucosal ulcerations.

death to more than 8,000 R. Living, normal appearing spermatogonia and sperm can be seen among the debris of the more radiosensitive cells. Therefore, the radiosensitive human testis contains a pool of radioresistant stem cells that are not killed immediately by these single large doses (lethal to total body).

The evidence from experimental animals shows, however, that if instead of a single large exposure, a series of small doses are given for extended times (as in radiotherapy) these resistant spermatogonia are irradiated as they cycle through a radiosensitive stage of replication and are destroyed. Permanent sterility in the male, as is now well known, is easily produced by the type of fractionated exposure regime suggested by Rubin and Casarett. This phenomenon where radiation damage is enhanced instead of reduced by fractioning the exposures is not seen in most other cellular systems since fractionation permits greater time for intracellular repair of radiation damage.

Apparently, in the human and primate ovary, recovery from radiation damage depends upon intranuclear repair only. There are no stem cells to replicate and thus return of fertility in the irradiated woman depends entirely upon the number of her surviving primary oocytes. Radiation sensitivity in the human ovary depends upon the developmental age of the ovum and the stage(s) of follicular development at the time of irradiation. In addition, the primate ovum itself has an unusually high radiation resistance.

Studies on monkey ovaries show that doses of 4,000 to 5,000 R are required to destroy single layered ovarian follicles and their ova. The minimum sterilizing ovarian dose for the monkey (M. mulatta) is about 2,000 R.

This unusually high cellular radioresistance appears to be the result of the spatial dispersion of the chromosomes of the primate ovum. These lampbrush-like elongated chromosomes offer thin targets to the radiations and therefore suffer few double hits. Fractionation of total dose under these circumstances would increase time for repair of fewer hits and increase the tolerance dose as defined.

In our pathologic studies of the marmoset we found another example of high ovarian resistance relative to the sensitivity of other tissues. N. Gengozian and J. S. Batson have previously reported the unusually high sensitivity of this animal to total-body irradiation (LD_{50} = ∿200 R). Fig. 6 B is a photomicrograph of the ovary of a marmoset at time of death after total-body irradiation of 200 R. Three mature graafian follicles showing death of their ova are seen next to a group of surviving ova in normal appearing one- and two-layered follicles.

Radiation resistance of human ovaries has been inferred for the most part from studies of women treated with portal irradiation for menstrual disorders. In one study about 97% of 2,068 women treated failed to menstruate following two to four fractionated exposures, totaling 360 to 720 R to the ovaries (∿200 R/port). A few women (< 3%) required more than one such course of exposures before permanent sterility was obtained. From this study and others, it appears that the nominal single dose for ovarian sterility may be about 600 R. However, if the exposures are fractionated into 30 equal doses (5/wk) the ovarian tolerance dose would have to total about 2,000 R for a 50% probability of inducing permanent sterility. We feel, however, that the tolerance dose of fractionated radiation would have to be higher if the woman was young and her ovary contained a greater proportion of primary oocytes than of maturing graafian follicles as in these patients with abnormal ovarian function.

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The following radiation tolerance levels of human germinal epithelia are suggested by these studies.

Organ	Injury at 5 Years	TD (5/5)	TD (50/5)
Testes	Permanent sterility	< 450	< 600
Ovary	Permanent sterility	< 650	> 2,000

Radiation Sickness: The Onset of Anorexia, Nausea, and Vomiting

This study deals with anorexia, nausea, and vomiting, the systemic responses to irradiation manifested by patients who receive portal radiation therapy. These symptoms constitute a syndrome that has been called radiation sickness or in those exposed in radiation accidents, the prodromal syndrome. Our purpose was to establish quantitative relations between those responses and several variables: time, radiation dose, type of disease, and anatomical region irradiated (Frank V. Comas and C. C. Lushbaugh). In this report only one particular point will be covered: the onset of each type of response.

This retrospective analysis is based on 194 patients who were irradiated on 346 different occasions, each treatment being regarded as a separate entity.

These patients were selected according to the following criteria: (1) They were irradiated between 1956 and 1965. (2) They were hospitalized at ORAU, Medical Division. (3) Irradiation was given to one part of the body only, excluding total-body irradiation. (4) Observations were available for the duration of the treatment course plus 3 days before, and 3 days after the irradiation.

The basic information was coded from nurses' notes. For instance, a recorded observation like "patient ate poorly", or "refused supper" was encoded as anorexia: YES. Nausea, another subjective symptom, was recorded from the nursing notes when the patient had told the nurse that he was nauseated, either by volunteering the information or by being asked. Vomiting is accompanied by liquid objective evidence, and it was probably recorded with greatest faithfulness to actual occurrence. All information for each patient on any one day was reduced to either YES or NO for anorexia, nausea, and vomiting, no matter how many times a particular response occurred on that day.

The question we wanted to answer was: When, in the course of a treatment series, will the patient first complain of anorexia, of nausea, and of vomiting? Any contributing factor other than time and his preirradiation status was ignored, specifically the kind of disease, where he was treated, and how much radiation he was given. The data for anorexia are summarized in Table 2. This table was prepared in a manner to permit an actuarial analysis, as is done to calculate survival rates. The first column lists the days during which irradiation was given, including any pauses for weekends or any other reason. Day 1 is the first irradiation day. The second column, headed N, records the number of patients that on the designated days had not yet responded. Each entry is the number of nonresponders on the preceding day minus those who the preceding day either first responded or were withdrawn from observation. The third column, R, is the number of patients who first responded on the designated day. Patients withdrawn from observation, W, are those who

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TABLE 2

ONSET OF ANOREXIA

All Cases

Treatment Day	N	R	W	At risk	P(R)	1-P(R)	Cum 1-P(R)	Cum P(R)
1	346	155	-	346	.4480	.5520	.5520	.4480
2	191	36	-	191	.1885	.8115	.4480	.5520
3	155	27	-	155	.1742	.8258	.3699	.6301
4	128	13	-	128	.1016	.8984	.3324	.6676
5	115	8	1	114.5	.0699	.9301	.3091	.6909
6	106	11	-	106	.1038	.8962	.2771	.7229
7	95	7	1	94.5	.0741	.9259	.2565	.7435
8	87	5	5	84.5	.0592	.9408	.2414	.7586
9	77	2	6	74	.0270	.9730	.2348	.7652
10	69	9	2	68	.1324	.8676	.2038	.7962
11	58	3	-	58	.0517	.9483	.1932	.8068
12	55	2	1	54.5	.0367	.9633	.1861	.8139
13	52	2	6	49	.0408	.9592	.1785	.8215
14	44	1	-	44	.0227	.9773	.1745	.8255
15	43	2	-	43	.0465	.9535	.1664	.8336
16	41	1	6	38	.0263	.9737	.1620	.8380
18	34	2	5	31.5	.0635	.9365	.1517	.8483
21	27	1	2	26	.0385	.9615	.1459	.8541
22	24	2	1	23.5	.0851	.9149	.1334	.8666
26	21	1	8	17	.0588	.9412	.1256	.8744
29	12	2	2	11	.1818	.8182	.1028	.8972
30	8	1	1	7.5	.1333	.8667	.0891	.9109
40	6	1	4	4	.2500	.7500	.0668	.9332
49	1	-	1	-	-	-	-	-

were followed up to the designated day without responding. The number of patients at risk is obtained by subtracting one half of W from N. The 6th column is the probability or response for each day (R/at risk), and the 7th column is the probability of not responding for each day. Next to it is the cumulative probability of not responding, which is obtained by transmultiplying all the entries in the preceding column. Finally, the 9th column is the cumulative probability of responding, calculated by subtracting each entry on the preceding column from 1. The information contained in the last column is better visualized if plotted graphically (Fig. 7) where the treatment day is plotted on a logarithmic scale, and the cumulative probability of responding is plotted on a probability scale. In this fashion all data points arrange themselves along a straight line.

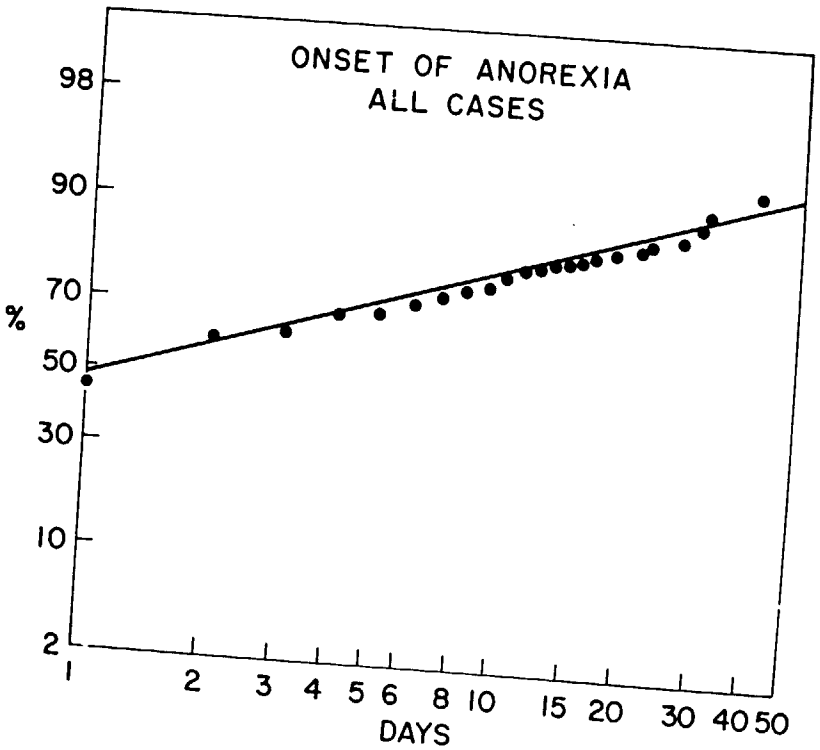


Fig. 7. The cumulative onset of anorexia for all patients, plotted on probability-log scales. The time scale represents treatment days, not number of fractions. In the probability scale is represented the percentage of patients who have had anorexia up to the designated day.

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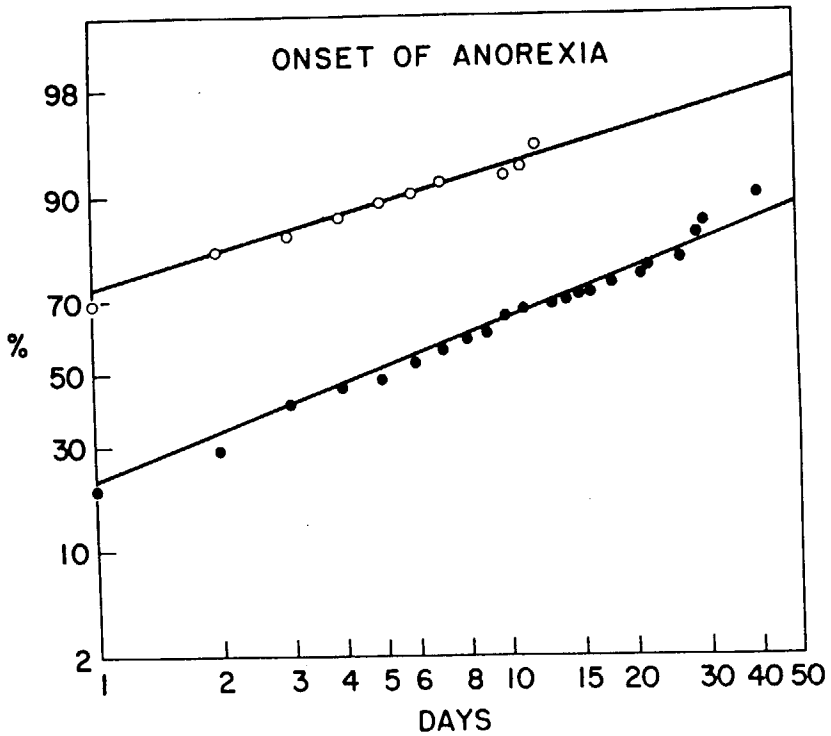


Fig. 8. The cumulative onset of anorexia for patients who were anoretic before irradiation (open circles), and for those who were not previously anoretic (closed circles). See legend on Fig. 1 for further details.

The high incidence of anorexia on the first day of treatment might not represent a radiation effect, but rather continuation of a preexisting condition. Many of the treated patients actually had anorexia because of their illnesses. Conceivably these patients responded differently to irradiation; they might respond to a greater degree because they were already prone to become anoretic, or they might respond less because irradiation partially relieved the pathological condition that made them anoretic.

We decided, therefore, to subdivide the patients into two groups: those who had anorexia before irradiation, and those who did not. The first group includes all patients who on any of the three days preceding irradiation had been anoretic one or more times. There were 176 patients in the first group and 170 patients in the second group. The cumulative incidences are graphed in Fig. 8

The response of nausea was analyzed in the same fashion as anorexia. First,

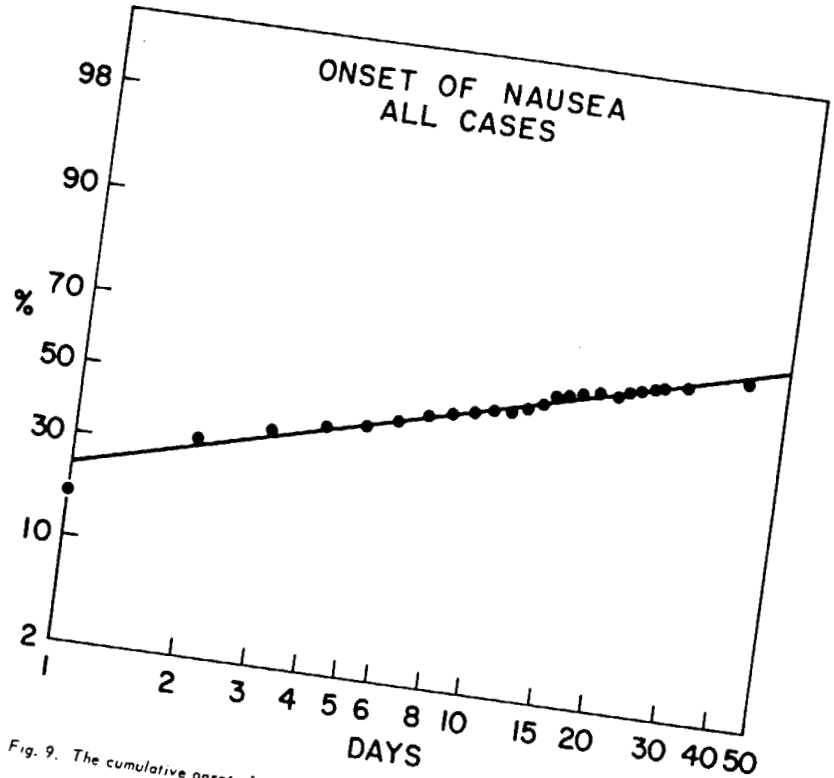


Fig. 9. The cumulative onset of nausea for all patients. See legend on Fig. 7 for details.

all patients were taken together, and then two groups were treated separately: patients who had nausea before irradiation, and those who had not. Again, a patient was included in the first group if he had had nausea at any time during the three days that preceded the beginning of the radiation course. The data are summarized in Fig. 9.

The vomiting response was evaluated as in the two preceding sections. The data are summarized in Fig. 10.

Of the three symptoms of radiation sickness, anorexia appears earliest, next nausea, and vomiting last. The time of onset for each response depends considerably on whether the patient had any of those symptoms before irradiation. If he did, his chances of having more of the same during the treatment course are very high, and if the treatment time lasts as long as 2 weeks, the majority of patients will respond. Patients free of these symptoms before irradiation fare better. Yet their risk of having anorexia, nausea or vomiting, at least once, is high. It is highest for anorexia; at 40 days, 88% of the patients will have had this symptom at least once.

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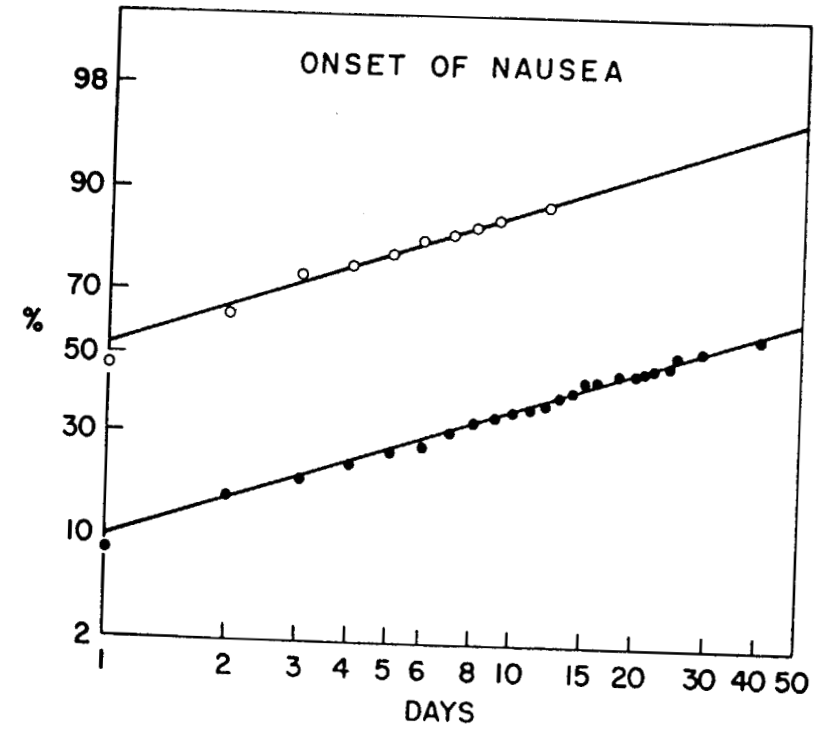


Fig. 10. The cumulative onset of nausea for patients who were nauseated before irradiation (open circles), and for those without previous nausea (closed circles). See legend of Fig. 7 for further details.

Nausea generally appears later, and the risk of developing it does not increase as fast as for anorexia. Still, at the 40th day of treatment 60% of the patients will have experienced it.

Initially the risk of vomiting is only slightly below that of nausea, but as time passes the increment rate for vomiting is less than for nausea. At the 40th day 43% will have vomited sometime since irradiation was started. (Figs. 11 and 12).

It should be underscored that the tables and graphs present cumulative probabilities. On a day to day basis, the probability of response decreases with time. For instance, for previously nonresponders the risk of developing anorexia on the first day is 0.15, on the second day it is 0.12 (0.15 + 0.12 = 0.30). On the third day it is 0.08, etc.

The time scale, in effect, is proportional to radiation dose. Longer treatment times tend to correspond to high total radiation doses, although the clinical data include high single doses and low-dose fractionated courses. We plan to explore

the possible relations between incidence of response and radiation dose. The remarkably good fit of most of the curves, however, indicates that there is, indeed, a good correlation between onset of symptoms of radiation sickness and length of radiation course. It should be stressed that we have commented only on the onset of symptoms. Their frequency and time of recurrence will be reported later.

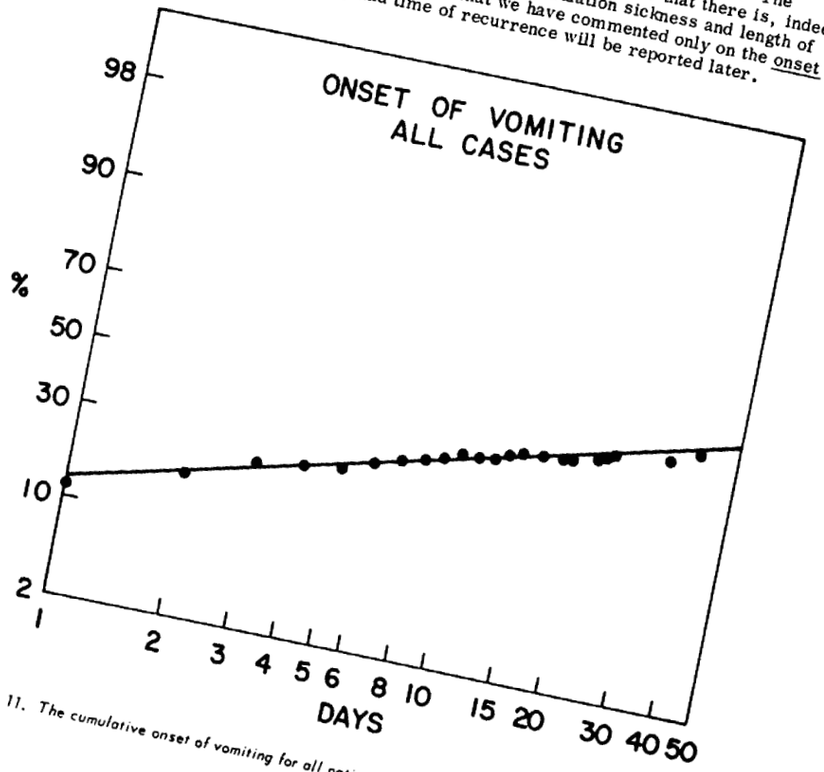


Fig. 11. The cumulative onset of vomiting for all patients. See legend on Fig. 7 for details.

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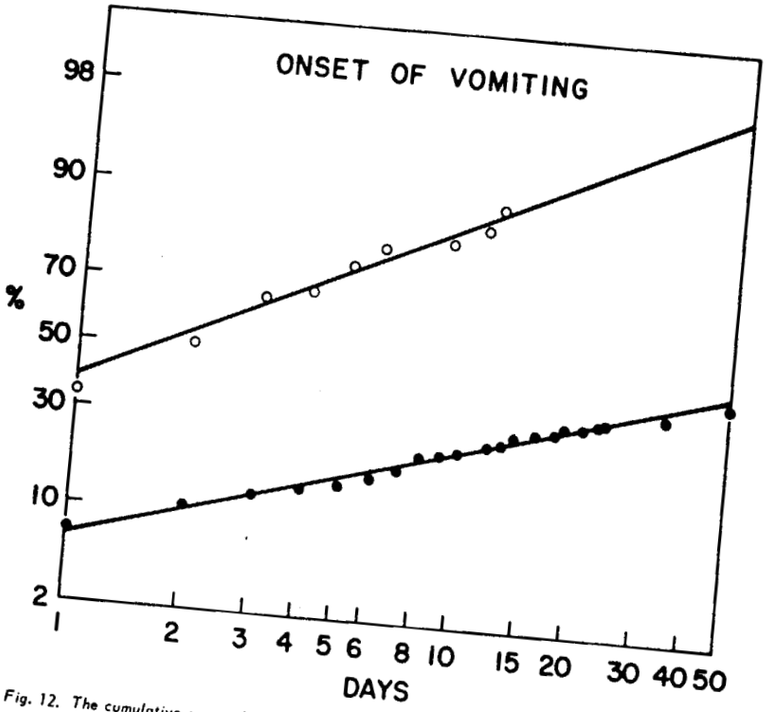


Fig. 12. The cumulative onset of vomiting for patients who were vomiting before irradiation (open circles), and for those who previously did not vomit (closed circles). See legend on Fig. 7 for further details.

Retrospective Study of Radiation-Induced Hematologic Effects in Patients

For several years we have been trying to make quantitative analyses of the radiation-induced hematologic responses of patients who received total-body irradiation at some time during the course of therapy for neoplastic diseases. Our results have not yet been any more quantitative or less narrative than those of numerous other investigators working on this problem. The need for quantitating these effects does not stem from any immediate clinical problems since available descriptions of the time-course of radiation-induced hematologic events afford abundant information to aid in the medical management of any patient irradiated either accidentally or therapeutically. Quantitative formulae are needed, however, to help predict, with some known level of precision, how hematologic damage may be caused by chronic low-dose-rate exposures of normal men.

This work has been a team effort (C. C. Lushbaugh, J. M. Yuhas,* Paulette Aaron, Earl McDow, Jr., Ann Sipe, and Tom R. Stokes) of long duration in which we collected the clinical case histories of 2310 patients from hospitals in the United States and Canada, encoded these records for manipulation and programmed retrieval by computer, graphed the time-course of the changes in peripheral blood cell counts after each TBI, and determined the percent survival of leukocytes and platelets after specific single and multiple radiation exposures. Through analysis of this material we have reported previously the quantitation and dose/response relations of several important physiologic responses such as nausea and vomiting. This year we concentrated our efforts on studying only the most complete hematologic records, culling out those having uncertainties about exposure (total roentgens, fractional doses, total treatment time, intervals between exposures, etc.) and with blood studies insufficient to determine the nadir of peripheral blood cell values and its time of occurrence. We found that at least seven blood cell counts done at weekly intervals for 6 weeks are needed to estimate the percent survival of most blood cells after an exposure, to be certain that a true nadir was observed. Many patients were too ill to afford hematologic data that were not biased heavily by their diseases and terminal infections; these were identified by these data searches and eliminated. In addition, we evaluated the clinical and laboratory evidence that defined the diagnosis in order to group together only patients with the same disease. Only four general disease categories were found that contained sufficiently numerous patients who had received single or multiple exposures to form groups for study. Second and third series of exposure were not included since the responses might be influenced by the previous irradiations. In these ways the single-exposure cases that total 1085 in our data bank were reduced to only 151; the 1225 multiple-exposure cases to 395. Table 3 shows the composition of this study material divided into the four hematologic-disease groups and according to single and multiple types of exposures. The average doses and the exposure duration of the multiple exposures are shown for each diagnostic group. The isolation of a so-called "normal" group was a fundamental need of this problem. The patients in this normal group, however, were not actually normal but either had disseminated solid (but not hematologic) tumors or were in late stages of nonmalignant diseases of the bones, joints, and the genitourinary system. However, in the absence of recorded hematopoietic abnormalities the patients in this group were considered to have "normal" (nonleukemic) marrow and lymphatic systems. The presence in this "normal" group of serious systemic diseases, even though not primarily hematologic, makes our apparent assumption of the existence of completely normal peripheral blood-cell kinetics unquestionably naive. The implication should not be accepted unequivocally that the mathematical description of the response pattern of this "normal" group can be used to describe exactly that of normal young men in the prime of life. It is difficult, however, to believe that normal young men would respond worse than this group did rather than better.

* Biology Division, Oak Ridge National Laboratory.

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TABLE 3

Diagnostic Group	Number of Patient Studies (#)		Average Total Exposures (R)		Average Length of Multiple Exposures (Days)
	Single	Multiple	Single	Multiple	
"Normal"	92	29	195	232	27
Chronic myelogenous leukemia	15	116	117	152	28
Chronic lymphatic leukemia	28	200	*	116	36
Lymphosarcoma	16	50	108	217	31

*Incomplete

The first step in the statistical analyses of this data has just been completed. In it, we chose to ignore the influence of the number of fractions of exposure, for the time being, to simplify our confirming the applicability of multivariant analysis to this problem. Accordingly, we related the individual nadir (%S) of the total leukocyte counts (expressed as percent of the initial total that survived) with the total exposure in R and the total time (days) required for the total exposure to be made. Slope constants (b_1) and (b_2) for a multivarient regression formula (a) were computed first for the single exposures and then for the multiple exposures for each disease group. No radiation dosage groupings within these four diagnostic groups were needed with this method of computation and fitting the regression. In the equation:

$$\%S = \cdot 100\,(D)^{-b_1} \cdot (T)^{b_2} \cdot (F)^{b_3} \quad \ldots\ldots\ldots\ldots\ldots \text{(a)}$$

K is the constant required for the regression line for percent survival to intercept the ordinate at zero dose. This interception point is greater than 100% of initial WBC survival because no effect was observed on cell survival after exposures of less than 50 R; D is exposure in roentgens; T is total length of exposure time in whole days; F is the number of fractional exposures made. For the purpose of this initial analysis the variable F was not used. In single exposures, T by definition is ignored and the equation becomes:

$$\%S = \frac{K \cdot 100}{(D)} \qquad \text{(b)}$$

For exposures longer than one day (as multiple exposures are defined here) the equation is then:

$$\%S = \frac{K \cdot 100 \cdot T^{b_2}}{(D)} \qquad \text{(c)}$$

The computed slope constants for the four diagnostic types of hematologic statuses analyzed here are listed in Table 4 along with the test values for the significance of the fits of the data to the equations (b) and (c).

TABLE 4

SLOPE CONSTANTS AND TESTS OF THE STATISTICAL SIGNIFICANCE OF THE MODELS

Diagnostic Group	Single Exposures		Multiple Exposures		
	b_1	Correlation Coefficients	b_2	Correlation Coefficients	P Value
"Normal"	1.04	0.57	0.63	0.535	<0.025
CML	0.999	0.82	0.392	0.569	<0.0001
CLL	0.91?	-	0.221	0.583	<0.0001
LS	1.119	0.42	0.231	0.567	<0.0005

These results seem encouraging. They show that this method of analysis is applicable to our problem and that the data are statistically well described by the equations. These multiple regression correlation coefficients are exceptionally good for analyses of clinical data. It would seem likely that they will improve even further when the regression equation is expanded to include the influence of other variables such as number of fractions and radiation-free time intervals, etc. The values for the slope constants appear to have interesting biological implications that are medically logical and acceptable. For example, in Table 4 the negativity of b_1 indicates that as dose increases the percent survival of peripheral WBC decreases. The correspondence of this value for each group to 1.0 may indicate that in the leukocytic systems difference in radiosensitivity is not a significant factor. This result is surprising in view of the widely-held clinical belief that the leukocytes in chronic lymphocytic leukemia, for example, are much more radiosensitive than the cells comprising the WBC in chronic granulocytic leukemia or in "normal" persons. This apparent deviation from clinical "fact", however, can be explained by the significant differences found in the values for b_2. The larger this positive slope constant is, the more effective is the length of protraction of exposure in increasing the level of cellular survival; according to these values, in the "normal" the white blood cell level is protected by dose protraction three-fold over that afforded to it in CLL and lymphosarcoma. According to this analysis dose protraction in the latter groups should not decrease the effectiveness of the total dose as much as in the "normal" group or in the CGL group. These interpretations are in agreement with most clinical observations and suggest in keeping with experimental observations that dose protraction aids normal tissue to recover and regenerate more rapidly than pathologic tissues. This concept is, in fact, the rationale for fractionation and protraction of radiation therapy of malignant tissues. Clinically observed differences in WBC survival after the same radiation exposure result more from difference efficiencies of recovery mechanisms than they do from inately different cellular radiosensitivities in these diseases.

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Infections in Irradiated Patients

Infection is a serious complication of sublethal total-body irradiation. A great deal of information is available on the increased susceptibility of laboratory animals to experimental or spontaneous infection following irradiation. However, the underlying causes of infections in irradiated human beings have not been adequately studied, and more information is needed about the effects of irradiation on humoral and cellular defense mechanisms. It is also important to develop more adequate procedures for preventing and treating infections in patients exposed to therapeutic irradiation or after radiation accidents. The objectives of the microbiology project are aimed at these questions.

The Bacterial Flora of Irradiated Patients

During 1970 we have continued to characterize the bacterial flora of irradiated patients before, during, and after treatment (G. C. Kingdon and Jean McIntyre); detailed data were obtained on nine patients who received a total of 12 whole-body exposures. Two patients received 100 R, six patients 150 R, and one patient 250 R in the LETBI facility (1.5 R/hr). Three patients were exposed to 150 R in the METBI facility (1.5 R/min). Cultures of nares, mouth, throat, feces, urine, and blood were taken twice weekly during hospitalization, which averaged less than four weeks, and then weekly until 52 days after irradiation. The most significant changes in bacterial flora are summarized in Table 5.

No infectious complications were encountered during these 12 episodes of irradiation exposure, although the bacterial microflora changed significantly after irradiation. Some aerobic components tended to increase while other components decreased. After irradiation, several patients acquired, presumably from exogenous sources, additional microorganisms in the upper respiratory tract, intestinal tract, and urine. The morphology and physiology of normal aerobic components changed more subtly in some patients. This is illustrated by the strain variability of Escherichia coli isolated from the large intestine of three of the patients. A single biochemical type was isolated from patient 12 before irradiation. This strain disappeared and was replaced by a different biochemical type immediately after exposure; the isolates were biochemically consistent during the remaining 5 months of observation. The numbers of transient microorganisms also increased in the large intestine and nares after irradiation. In a second patient (Number 11, in Table 5), observed for 104 days, we isolated seven biochemical types of E. coli. Only one of these was able to persist in stool specimens while the other six types were present for varying periods. In this patient after irradiation, the incidence of transients in the large intestine and urine increased also. Patient 9 harbored a single biochemical type of E. coli in the stool before irradiation, but a second type appeared after 10 days. Both strains were present for a short time, then the original strain disappeared but the second strain persisted.

It was impossible in most instances to differentiate between changes in the microflora from exogenous microorganisms and those caused by induced alterations in endogenous components. There was little evidence that biochemical variability of E. coli isolates resulted from radiation-induced mutations. This possibility, however, has not been excluded. Microorganisms, presumably exogenous, appeared to become established in the upper respiratory tract and intestinal tract more easily after irradiation. There was also an increased incidence in the number and the diversity of transient microorganisms from the upper respiratory tract, intestinal tract, and urine, suggesting that the patient's ability to maintain a stable microflora had been impaired.

The relative importance of endogenous and exogenous microorganisms in post-irradiation infections has not been completely defined. The introduction of foreign (to the host's system) microorganisms (either genus, species, or strain) before or during leukopenia may suddenly shift the microflora balance to a microorganism with which the host has had no recent immunological experience.

Serum Antibodies in Irradiated Patients

We have examined sera of 12 patients for agglutinating and bactericidal antibody to determine whether the levels were affected by total-body irradiation (G. C. Kingdon and T. A. Pearson). In all assays we used bacteria isolated from the patient's microflora, or a serum sensitive Escherichia coli strain. Washed bacterial suspensions were added to serum and incubated at 37° for 3 hours; we used as a control bacterial suspensions without serum. By determining the number of viable bacteria at selected intervals or by changes of optical density in a spectrophotometer, we measured serum inhibition of bacterial growth and compared it to growth in control cultures. We measured agglutinating antibody by adding a washed bacterial suspension to serial serum dilutions and determining the end point at which agglutination no longer occurred; bacteria without added serum was used as a control. The titer was taken as the reciprocal of the highest serum dilution showing agglutination.

Two patients had no bactericidal or agglutinating antibody against the bacteria tested; several had low or moderate activity against one or more microorganisms. Data from three patients showing representative changes in serum bactericidal activity are discussed here. Sera of patient 2 before irradiation (100 R) moderately inhibited a Klebsiella sp. strain; this inhibition tended to decrease starting 11 days after irradiation and to remain depressed. Good serum activity was also observed against a second Klebsiella sp strain and a Serratia sp strain; this activity was unaffected by irradiation. We tested sera from patient 4 (150 R) against two strains of E. coli, and also a Citrobacter freundii strain, and observed a slight inhibitory activity against one of the E. coli strains but none against the other strain. The sera suppressed C. freundii effectively and the inhibition was unaffected by irradiation. Serum inhibition of the first E. coli strain increased at 7, 11, and 48 days. Sera from patient 7 (150 R) inhibited well an E. coli strain, but showed no activity against a Klebsiella sp strain. Serum inhibition of E. coli gradually decreased starting by day 11 and continued to decrease throughout the study. Agglutinins against two Klebsiella sp strains were present in this patient's sera but E. coli agglutinins were absent. Agglutinin titers were unaffected by irradiation.

We also studied the effect of total-body irradiation on serum immunoglobulins (IgG, IgM, IgA), complement (C'3), and transferrin levels in 15 patients receiving 100 R to 250 R in LETBI or METBI (G. C. Kingdon and Stuart Chaskes). No increases or decreases were observed in two; five of the remaining 13 showed decreases in two or more of these serum components; only one had a decrease in all components. A lowered IgA was the most consistent change, occurring in 10 patients; two of them also developed lower IgG levels after irradiation. In one the IgA drop was associated with decreased IgM levels and in another reduced C'3 levels accompanied the IgA decrease. A decrease in IgA levels unaccompanied by changes in other immunoglobulins or complement appeared in five patients. Reduced levels of IgG were observed in six patients; two also had reduced levels of IgA and one had decreased levels of all serum components. Only two patients showed decreases in IgM; in both this was associated with reduced levels of other immunoglobulins.

Data on three patients representative of the major changes observed in serum immunoglobulin levels after irradiation will be presented here. The serum IgG level of patient 5 (150 R) was in the high normal range before irradiation (Table 6). Starting

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TABLE 5

SUMMARY OF CHANGES IN PATIENT MICROFLORA AFTER TOTAL-BODY IRRADIATION

Patient No.	Dose	Dose Rate	Preirradiation Cultures	Postirradiation Cultures
1	100 R	1.5 R/hr	Microflora normal and consistent.	*Staphylococcus aureus* from nares and throat after day 11. *Diplococcus pneumoniae* in throat and mouth and *E. coli* in urine.
2	100 R	1.5 R/hr	*Klebsiella-Enterobacter* gp and *P. mirabilis* in nares, throat and mouth; *Klebsiella* sp from feces.	*Proteus mirabilis* from feces after day 7. The *Klebsiella* sp population in stool decreased from 10^8 per gram dry weight on day 3 to 10^4 on day 10; enterococci increased from less than 10^4 to 10^9 on day 7.
3	150 R	1.5 R/hr	*Monazella* sp occasionally cultured from nares.	Stable numbers of *D. pneumoniae* in throat and mouth. Urine contained *S. albus* and *P. mirabilis*.
4	150 R	1.5 R/hr	Microflora normal and consistent.	*Pseudomonas aeruginosa*, *Klebsiella* sp. *D. pneumoniae*, *C. albicans* and beta hemolytic streptococcus in throat cultures. *Catenabacterium* sp and *C. albicans* increased in feces. *Catenabacterium* sp remained at a concentration of 10^8 - 10^9 until day 38 when it disappeared.
5	150 R	1.5 R/hr	Microflora normal and consistent.	*Proctus mirabilis* from nares. Urine cultures consistently positive for *S. agalactiae*.
6	150 R	1.5 R/hr	Microflora normal and consistent.	*Candida albicans* from throat, mouth and gastric washings. The number of *C. albicans* increased in feces reaching a peak of 10^8 after 38 days. Urine contained a variety of bacteria.
7	150 R	1.5 R/hr	Microflora normal and consistent.	*P. mirabilis* in urine; the number tended to increase.

TABLE 5 (CONTINUED)

Patient No.	Dose	Dose Rate	Preirradiation Cultures	Postirradiation Cultures
8	150 R	1.5 R/hr	Mouth washings contained low numbers of *D. pneumoniae*.	Increased numbers of *D. pneumoniae* in mouth washings beginning on day 3 and persisted until day 31; also from throat swabs.
9	250 R	1.5 R/hr	A variety of microorganisms in urine. The fecal microflora normal and consistent.	A variety of microorganisms in urine. Many transient microorganisms from rectal swabs and feces.
10	150 R	1.5 R/min	The fecal microflora abnormal and abbreviated.	*Diplococcus pneumoniae* on throat swabs and in mouth washings in a concentration of 10^4 to 10^5 per ml starting on day 7. Urine contained *S. coli, S. alba, S. agalactae, P. mirabilis* and diphtheroids. Many transient microorganisms in feces.
11	150 R	1.5 R/min	Urine contained *S. alba* and diphtheroids.	*E. coli, P. mirabilis, Klebsiella* sp and non-hemolytic streptococci in urine. *Diplococcus pneumoniae* from nares, throat and mouth. A large number of transient microorganism from rectal swabs.
12	150 R	1.5 R/min	*Diplococcus pneumoniae* on throat swabs and in mouth washings. The stable fecal microflora abnormal and abbreviated.	*Staphylococcus aureus* from nares beginning on day 12. The number of *D. pneumoniae* increased in mouth washings. Numerous transient microorganisms in feces.

on day 31 it decreased reaching a low value of 870 mg/100 ml on day 45 but returned to preirradiation levels 7 days later. The IgA level was abnormally low before irradiation; it gradually declined from 193 mg/100 ml to 120 mg/100 ml 45 days postirradiation, then slight increases occurred. The IgM levels of patient 5 were elevated before irradiation remaining above normal, although it tended to decrease after exposure to 150 R (Table 7). The IgG decreased from normal values to a low of 800 mg/100 ml Serum IgM, C'3, and transferrin were elevated before exposure significant decreased to a minimum of 136 mg/100 ml on days 24 and 30, then returning to near normal levels (Table 8). Serum IgG, IgM, C'3 and transferrin were not significantly altered.

Serum immunoglobulin (IgG, IgA, IgM) and complement (C'3) levels changed after total-body irradiation, generally a decrease in IgA levels beginning between 7 and 21 days after irradiation; IgG, IgM and C'3 levels also decreased during this time.

TABLE 6

EFFECT OF 150 R TOTAL-BODY IRRADIATION (1.5 R/hr) ON THE LEVELS OF IMMUNOGLOBULINS (IgG, IgA, IgM), COMPLEMENT (C'3) AND TRANSFERRIN IN A PATIENT'S SERUM

	Serum Proteins (mg/100 ml)					
Time (Days)	IgG	IgA	IgM	C'3	Trans	T.P.*
-5	1550	193	124	270	370	8.4
-3	1435	161	109	255	259	7.6
0	1550	193	109	260	360	8.5
+4	1300	161	97	250	290	7.8
+11	1690	176	-	260	235	8.5
+13	-	-	117	-	-	-
+14	1690	161	131	255	268	9.1
+24	1400	137	92	155	210	7.3
+31	1050	147	87	220	288	7.8
+38	1270	137	92	200	300	7.8
+45	870	120	87	250	210	7.3
+52	1550	147	103	220	227	8.4
Normal Range	1200 ± 300	288 ± 21	80 ± 29	144 ± 22	280 ± 67	6.5 - 8.5
Patient 5						

*Total Protein (gm/100 ml).

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TABLE 7

EFFECT OF 150 R TOTAL-BODY IRRADIATION (1.5 R/hr) ON LEVELS OF IMMUNOGLOBULINS (IgG, IgA, IgM) AND COMPLEMENT (C'3) IN A PATIENT'S SERUM

Serum Proteins (mg/100 ml)

Time (Days)	IgG	IgA	IgM	C'3	T.P.*
-7	1590	257	190	100	9.0
-6	1275	224	160	100	7.9
-5	1160	231	230	108	10.5
-4	1400	260	215	127	9.0
-3	1160	268	185	108	8.4
0	1275	248	185	97	7.8
+1	1050	224	172	97	8.3
+3	890	290	160	124	8.3
+4	800	238	135	137	7.6
+7	950	257	160	124	8.4
+10	1000	238	160	105	7.9
+14	1000	210	172	111	8.4
+17	1275	224	152	94	7.7
+24	1350	238	128	82	7.8
+31	1350	257	110	82	7.8
+38	1350	196	128	105	8.0
+45	1275	196	135	94	8.0
+52	1275	224	135	130	8.3
Normal Range	1200 ± 300	288 ± 21	80 ± 29	144 ± 22	6.5 - 8.5

Patient 8

*Total Protein (gm/100 ml).

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TABLE 8

EFFECT OF 150 R TOTAL-BODY IRRADIATION (1.5 R/min) ON THE LEVELS OF IMMUNOGLOBULINS (IgG, IgA, IgM), COMPLEMENT (C'3) AND TRANSFERRIN IN A PATIENT'S SERUM

Serum Proteins (mg/100 ml)

Time (Days)	IgG	IgA	IgM	C'3	Trans	T.P.*
-5	1760	364	76	185	308	8.7
-3	1660	385	72	185	238	8.0
0	1850	392	81	160	262	9.6
+4	1430	371	76	150	262	7.6
+7	1620	301	72	160	290	9.0
+9	1340	276	76	134	262	8.6
+11	1500	231	57	160	238	7.8
+14	1600	210	67	167	250	7.4
+16	1500	192	72	167	278	8.3
+24	1200	136	76	141	278	7.7
+30	1500	136	81	134	290	8.7
+37	1660	245	72	167	290	-
+49	2000	231	72	185	262	-
+56	1480	217	62	185	238	-
Normal Range	1200 ± 300	288 ± 21	80 ± 29	144 ± 22	280 ± 67	

Patient 13

*Total Protein (gm/100 ml).

Increased levels, however, also occurred in some instances. The data suggest that IgA may be more sensitive to depression by irradiation than IgG or IgM. The IgA level tended to be decreased more frequently, earlier, and to a greater extent and remained so longer than IgG or IgM.

The significance of decreased immunoglobulin and complement levels to increased susceptibility to infections is not yet clear; however, our information suggests that patients with IgG, IgM or IgA deficiences are infected more frequently after total-body irradiation than patients with normal immunoglobulin levels. Secretory IgA

levels in saliva or mucus secretions were not measured. However, it is possible that secretory IgA may be affected by irradiation in a way similar to serum IgA, an explanation for the trend toward instability of the microflora in the upper respiratory tract and large intestine after irradiation.

Bactericidal activity of serum of several patients was good or moderate against the microorganisms tested. Both increases and decreases in inhibitory activity appeared after irradiation: however, frequently no changes were observed. The effect of irradiation on the bacterial agglutinin titers was minimal.

There was no correlation between agglutinin titers and bactericidal activity of the serum. Where changes in bactericidal activity occurred, we could not correlate them with decreased or increased levels of immunoglobulins or with changes in complement (C'3) levels. This was not unexpected since specific bactericidal antibody would account for only a small part of the total immunoglobulins. Also, C'3 is only one of several complement components required for bactericidal activity; changes in bactericidal activity could be due to alteration of a complement component other than C'3.

Ultraclean Environments to Prevent Infections

Preventing infection in irradiated human beings is an important part of their management, especially for those receiving large doses of irradiation or intensive chemotherapy preparatory to bone-marrow transplantation and for those accident victims exposed to near lethal doses. The Medical Division maintains a laminar air-flow (LAF) facility which provides an ultraclean environment for reducing the exposure to infection by exogenous microorganisms. By monitoring the patient's microflora to determine shifts in antibiotic resistance and potential overgrowth of certain components, we hope to reduce the incidence of infection by endogenous microflora. Prophylactic therapy with antibiotics and other antibacterial and antifungal agents is used to reduce the patient's level of endogenous microflora.

The LAF facility is a restricted-entry, self-contained unit composed of two ultra-clean patient rooms separated by a clean preparation room and an anteroom (Fig. 13). The patient rooms, equipped with high efficiency filters covering the entire wall of one end of the room, are capable of excluding all particles over 0.3 microns. Air is forced through the filters at an initial velocity of approximately 90 ft/min and returns to the filters through a ceiling plenum at the opposite end of the room. The rooms are also equipped with the usual hospital room furnishings including chairs, tables, etc. A lavatory, bathtub-shower and plumbed sanitary commode have also been included. We are evaluating these fixtures to determine whether they are compatible with the maintenance of an ultraclean environment.

Everything unsterile, except medical personnel and the patient, is rigidly excluded from the patient room. All foods, medications, and other materials are sterilized or effectively excluded by sterile coverings. Before the patient enters the room, it is sterilized by fogging with an antiseptic solution.

Medical personnel are allowed into the LAF based on need to enter. Sterile gown, cap, mask, boots, and surgical gloves are required, and the personnel are instructed to remain downwind of the patient at all times.

We have examined this ultraclean environment during patient occupancy (G. C. Kingdon, Jean McIntyre, Joan Marks, and William Tankersley). The contamination level of surfaces, furnishings, and fixtures, both in the patient rooms and preparation room, were determined by contact plate at least once each week. Air contamination

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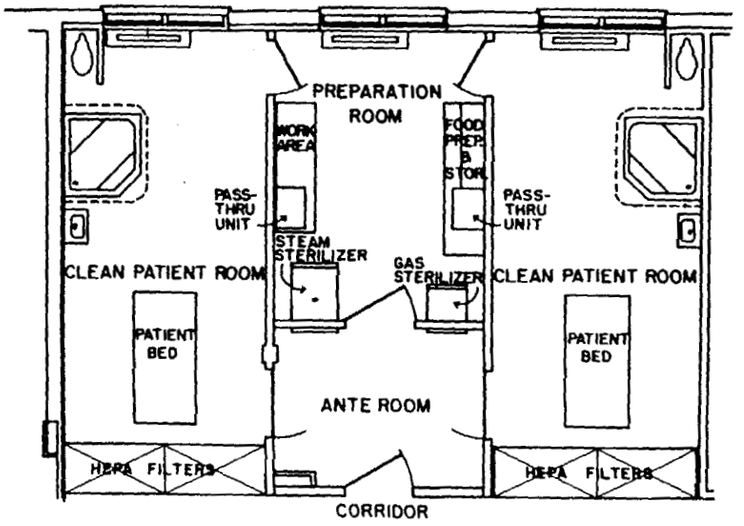


Fig. 13. Floor plan of Laminar Air-Flow Facility.

was determined by liquid impinging samplers five days a week. The microflora of the patient's nose, throat and mouth, skin, and feces were cultured two or three times each week; blood and urine cultures were also done.

The patient microflora and LAF data presented here were obtained from a male patient who was exposed to 500 R total-body irradiation for bone-marrow transplantation: he was confined to the ultraclean environment for 39 days, followed by 4 days of modified clean environment. During this transition time the patient's immediate family was allowed to enter the patient's room in sterile gowns, caps and boots, except for the first day when sterile masks and gloves were also worn. Medical personnel were still required to maintain a sterile barrier between themselves and the patient. The period of modified clean environment was felt to be necessary to reestablish a fairly normal patient microflora before leaving the facility.

Staphylococcus albus, Streptococcus anhemolyticus and Klebsiella-Enterobacter gp predominated on the skin of the patient before he was admitted to the LAF facility (Table 9). Quantitative studies on skin were not done; however, the data indicate that its microflora was not greatly altered by the antiseptic soaps and solutions used to decontaminate, although gamma hemolytic streptococcus was eliminated. The implication that decontamination is not very effective may be somewhat misleading since cultures from external auditory canals and other sites difficult to decontaminate are included as skin samples. Proteus sp was first cultured from skin 10 days after the patient entered the room and persisted for the duration. Pseudomonas aeruginosa was isolated only twice; E. coli was also cultured twice but this was during the time when the patient was in the modified clean environment. The source of contamination by Proteus sp and

TABLE 9

MICROORGANISMS CULTURED FROM SKIN OF PATIENT DURING OCCUPANCY OF LAF ROOM

Days	*S. albus*	Diphtheroids	*Proteus* sp	*Ps. aeruginosa*	*E. coli*	Gamma Streptococcus	*Klebsiella-Enterobacter* gp
-19	X	X				X	X
-12	X					X	X
- 8	X						X
- 5	X						X
- 1	X						
+ 2	X	X	X			X	X
+ 6	X	X	X			X	X
+ 9	X	X	X			X	X
+14	X	X	X				X
+16	X	X	X			X	X
+20	X	X	X				X
+23	X	X	X	X		X	X
+27	X	X	X	X			X
+28	X		X				X
+29	X		X				X
+30	X		X				X
+31			X				X
+34	X		X				X
+35	X	X	X	X			X

Ps. aeruginosa is not certain but it is likely that they were present in low numbers in the large intestine.

Prophylactic, nonabsorbable antibiotics were used to reduce the microbial load in the intestinal tract. Nystatin, paromomycin, vancomycin, and Polymyxin B were administered beginning 8 days before irradiation and were discontinued, except nystatin, on day 27. The fecal microflora was normal before antibiotic therapy (Table 10). Following administration of antibiotics E. coli, S. mitis, Lactobacillus sp, Clostridium sp and fecal streptococcus disappeared from the feces. Bacteroides sp was occasionally cultured from rectal swabs but not from feces after antibiotics were administered. Pseudomonas sp was first cultured from feces on day 6 and increased slowly in number; Proteus sp was also present on rectal swabs. Antibiotics appeared to be unable to suppress the growth of these pathogens. Escherichia coli failed to return to the feces after antibiotics were discontinued. An E. coli culture (10^9 bacteria) derived from the patient's original strain was administered by rectal tube on day 31. The organism became established within 24 hr with a subsequent decrease in Pseudomonas sp concentration and eventually its elimination from the stool. Proteus sp and Klebsiella-Enterobacter gp were isolated after antibiotics were discontinued.

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The bacterial flora of the patient's throat and nose was normal before admission to the LAF room (Table 11). Part of the microflora of the throat, but not of the nose, was eliminated by antibiotics. A beta hemolytic streptococcus was cultured once from the throat. Candida sp was also cultured from the throat several times beginning on day 6 but was never present in numbers greater than 10^2/ml of mouth washings.

The most consistent contaminant in the patient's environment was S. albus (Table 12). This is not surprising since the microorganism was the predominant bacterial component on the skin. The S. albus contamination was recovered almost exclusively from the bathtub-shower, lavatory, and table tops which the patient had touched. There was considerable variability in the number of S. albus cultured from sample to sample, apparently dependent upon the interval between the use of the fixtures by the patient and sampling, and whether they had been cleaned in the interval. Other contamination was at a very low level and was derived mainly from the patient's microflora. Air samples indicated that airborne contamination was reduced 10- to 70-fold in the ultra-clean environment.

The patient experienced neither major nor minor infection during his stay in the unit. We feel that at no time did environmental contamination constitute a risk to the

TABLE 10

MICROORGANISMS CULTURED FROM RECTAL SWABS, FECES AND URINE OF PATIENT DURING OCCUPANCY OF LAF ROOM*

Days	*E. coli*	*Lactobacillus* sp	*Bacteroides* sp	*Pseudomonas* sp	*Proteus* sp	*Klebsiella-Enterobacter* sp	*Clostridium* sp	*S. mitis*	*S. albus*	Fecal Streptococcus	*Pseudobacterium* sp
-19		x	x			y		x	xy	x	x
-12	x	x	x						x	x	
- 8	$x10^8$	$x10^7$	$x10^9$			x		x	x	x	
- 5			x				x				
- 1			x				x				
+ 2						y			x		
+ 6				$x10^4$							
+ 9				$x10^5$							
+14			x	x							
+16				x		y					
+20				$x10^6$	x	y					
+23				$x10^5$	x				x		
+27				x	x	y					
+28			x	$x10^6$	x	y					
+29				$x10^8$	x	y					
+30				$x10^8$	x	y					
+31	(†)			$x10^8$y	$x10^5$y	y					
+32	10^9			$x10^8$	$x10^8$						
+33	10^9			$x10^7$	$x10^8$	10^8					
+34	$xy10^9$			$x10^7$y	$x10^8$	$xy10^9$			y		
+35	$x10^9$			$<10^6$	$x10^8$	$x10^9$			x		

*x = cultured from rectal swabs.

y = cultured from urine.

Numbers indicate microorganisms per gram dry wt feces.

†1 x 10^9 *E. coli* administered.

TABLE 11

MICROORGANISMS CULTURED FROM THROAT AND NOSE OF PATIENT DURING OCCUPANCY OF LAF ROOM*

Days	*S. salivarius*	*S. mitis*	*Micrococci* sp	*Neisseria* sp	Gamma Streptococcus	*Lactobacillus* sp	*Bacteroides* sp	*Veillonellae* sp	*Fusobacterium* sp	Diphtheroids	*S. albus*	*Moraxella* sp	*Candida* sp
-19	x	x	x	x	x	x	x	x	x	y	y	y	
-12	x	x	x	x	x	x	x	x	x	xy	y	y	
- 8	x	x	x	x		x	x	x	x	y	y	y	
- 5		x	x	x		x	x	x		y	y	y	
- 1		x				x	x	x		y	y	y	
+ 2		x				x	x	x		y	y	y	
+ 6		x				x	x	x		y	y	y	
+ 9		x				x	x	x		y	y	y	
+14		x			(†)	x	x	x		xy	xy	y	
+16		x				x	x	x		xy	xy	y	
+20		x				x	x	x		xy	xy	y	
+23		x				x	x	x	x	xy	xy	y	x
+27		x				x	x	x		xy	y	y	x
+30		x				x	x	x	x	x	xy	y	
+34		x	x	x		x	x	x	x	y	y	y	x
										xy	y	y	x
										y		y	

*x = cultured from throat swabs.
y = cultured from nose.
†beta streptococcus (not group A) isolated.

patient. The patient's microflora was well controlled, with the possible exception of Pseudomonas aeruginosa, and was also not an unusual threat to the patient. These preliminary data suggest that ultraclean environment can effectively reduce the risk of infection in immunosuppressed patients by reducing the level of contamination by exogenous microorganisms. Prophylactic antibiotics also appear to be of benefit by reducing the endogenous microflora load.

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TABLE 12

MICROORGANISMS CULTURED FROM AIR AND SURFACES OF LAF ROOM DURING PATIENT OCCUPANCY

	Dates Sampled						
	8-19	8-26	9-2	9-9	9-16	9-23	9-29
Bacillus sp	1		6	4	1		
Diphtheroids	4	1		3			
S. aureus	2						
S. albus	3	191	22	183	1	7	
Pseudomonas sp				1			
Klebsiella-Enterobacter gp					2		
Micrococci sp				3		2	
Mima-Herrellea gp					1		
Fungi (sap.)		1					
Positive samples (+/T)	6/17	6/17	5/17	8/17	3/17	8/17	
Ave. colonies/inch²	0.15	2.80	0.41	2.90	0.07	0.13	
Air samples (colonies/cu ft)							
LAF	0.065	0.070	0.240	0.210	0.220	0.200	0.075
Preparation room	0.700	0.620	0.420	0.625	0.240	0.310	0.225

Dosimetry Studies and Monitoring

The Medical Division has staff members who are skilled in medical physics and radiation safety. They combine their talents to evaluate the risk of external and internal radiation exposures. This requires a careful study of the radiation dose distribution in patients undergoing total-body irradiation and the evaluation of the radiation dose from internally administered radionuclides. Because of the public's growing concern about radiation exposures, the Medical Division studies are becoming a valuable resource, especially in the area of radiopharmaceutical dosimetry.

Radiation Dose from Radioisotopes in the Blood

The radiation dose to the body and to its organs from a radionuclide in the blood depends on the type of radiation emitted, its energy, and the geometric relation of the blood vessels to the organ being irradiated. The absorbed fraction is used to calculate the radiation dose and is the ratio of the energy absorbed in a target to the total energy emitted by the source. It can be easily shown that if a radionuclide located in the blood emits only nonpenetrating radiation, the absorbed fraction value used for calculating the dose to the blood will approach 1 as the blood vessels become larger. This means that the absorbed fraction for the tissue surrounding the blood vessel must be very small and the dose to the other tissues of the organ must approach zero. We have studied the more complex situation involving the smaller vessels, including the capillaries, and have concluded that for many radionuclides in the blood, the spleen (Table 13) receives the highest dose of any organ in the body per millicurie of radionuclide in the blood (Roger J. Cloutier and Evelyn E. Watson). This calculation takes into account both the geometric relation between blood vessels and their sizes (54).

TABLE 13

MEAN DOSE RATE TO VARIOUS ORGANS

(millirads/hr per millicurie*)

Isotope	ORGAN				
	Liver	Spleen	Lungs	Kidneys	Brain
^{32}P	41	150	86	68	3.2
^{51}Cr	0.68	1.6	0.92	0.81	0.16
^{59}Fe	20	42	27	25	6.3
^{99m}Tc	2.9	5.8	3.7	3.3	0.53
^{131}I	16	47	28	23	2.7

*Uniformly distributed in the blood of a standard man.

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We also calculated the radiation dose that a patient receives from two different hematologic tests: ^{59}Fe incorporation into blood cells and ^{51}Cr labeling of red blood cells and found that the dose depends on the radionuclide and the patient's disease. The patient's disease affects the retention of the radionuclide in the body; patients with polycythemia vera and endogenous hemochromatosis receive a bone-marrow dose from ^{59}Fe that is two to three times the whole-body dose. The spleen dose is 15 times normal because the disease causes the early destruction of red blood cells, making the radioactivity in the spleen increase more rapidly than in the normal. The exact dose depends on the stage of the disease.

Unlike iron, which is rapidly removed from the destroyed cells and reincorporated into new cells, chromium does not relabel new cells. The ^{51}Cr tagged cells are destroyed by the spleen and much of the chromium is retained there, resulting in a dose approximately 30 times higher than the dose to the blood for both the normal person and the acute leukemia patient. For the patient with hemolytic anemia the spleen dose, however, is only 15 times higher than normal. Because of its magnitude, the dose to the spleen appears to be the important consideration in evaluating the risk of using red blood cells tagged with radioactive chromium.

Dosimetry for Total-Body Irradiation Studies

In our total-body irradiation program we are using three different irradiators: The LETBI and METBI facilities and more recently the University of Tennessee-AEC Variable Dose-Rate Irradiation Facility (VDRIF). Their radiation characteristics and geometry are given in Table 14. Because of different source-patient geometries and gamma-ray energies, the patient's depth-dose distribution differs. Last year we (W. L. Beck, Tom R. Stokes, Roger J. Cloutier, and C. C. Lushbaugh) reported the results of the organ dosimetry for LETBI and METBI. These studies have been expanded to include the VDRIF.

As a patient analog we used an Alderson Rando phantom. To locate organ volumes, we compared radiographs of the 34 transverse sections of the phantom with bone structure and organ location of anatomy reference works. Individually calibrated LiF dosimeters were placed in all available sites within the volumes defined as organs. Then we reassembled the phantom and exposed it in the same manner as a patient. The rad dose measured by each dosimeter was determined by analysis techniques previously reported (USAEC Report CONF-680920, 1968, 976). With the exception of the bone marrow we assume that the average rad response of all dosimeters in an organ equals the organ dose. Since the active marrow is not uniformly distributed in a single volume, we calculated from our measurements average doses for separate marrow compartments and weighted them by the amount of active marrow in each compartment to obtain the average marrow dose as shown in Table 15. The active marrow distribution was taken from Ellis (Phys Med Biol 5, 255, 1961).

In Table 16, the organ doses and their ranges are given for all three irradiators. Predictably, the bone-marrow dose ranges are the most variable since the bone marrow is both near the midline and near the surface of the body. In LETBI, on comparing the dose distribution with the other two facilities, we find that organs in the head and neck receive about 10% more, in the upper and central chest about 10% less, and in the lower part of the torso about 15 to 30% less. In METBI, as compared with the VDRIF, the organ doses are slightly higher in the head, about the same in the upper chest, and lower in the abdominal and pelvic regions. These same distributions are seen in the marrow compartment doses, Table 15.

Because most studies of total-body irradiation, both in humans and animals,

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TABLE 14

RADIATION CHARACTERISTICS AND GEOMETRY OF THREE TOTAL-BODY IRRADIATORS

FACILITY	SOURCES	RADIATION	EXPOSURE VOLUME	EXPOSURE RATE AND VARIATION IN TREATMENT VOLUME	MODE OF EXPOSURE	SOURCE TO MID-EXPOSURE VOLUME
LETBI (Low Exposure-Rate Total-Body Irradiator)	10 Cobalt-60	1.25 Mev Gamma	16'x16'x16' (room)	1.5 R/hr +10% to -10%	Multi-lateral	19.5'
METBI (Medium Exposure-Rate Total-Body Irradiator)	8 Cesium-137	0.662 Mev Gamma	6x2x1 (above bed)	1.5 R/min +5% to -5%	Multi-lateral	5.8'
VDRIF (Variable Dose-Rate Irradiation Facility)*	5†Cobalt-60	1.25 Mev Gamma	6x2x1 (above bed)	40 R/min +3% to -6%	Bilateral	10' for sources 3 and 4; 22.5' for sources 2,5,6

*This irradiator belongs to the UT-AEC Agricultural Research Lab.
†VDRIF has 6 sources, but only 5 are used during patient therapy.

TABLE 15

AVERAGE BONE MARROW DOSE IN RADS/100 R

Bone Marrow Compartment	Percent Active Bone Marrow	LETBI Average Compartment Dose	LETBI Weighted* Compartment Factor	METBI Average Compartment Dose	METBI Weighted* Compartment Factor	VDRIF Average Compartment Dose	VDRIF Weighted* Compartment Factor
Head	13.1	84	1100	78	1022	68	891
Upper Limb Girdle	6.4	69	442	66	422	78	499
Sternum	2.3	75	173	69	159	77	177
Ribs	7.9	63	498	68	537	75	593
Vertebrae	28.4	58	1647	65	1846	70	1988
Sacrum	13.9	45	626	54	751	75	1043
Lower Limb Girdle	26.1	52	1357	59	1570	72	1879
Totals	98.1		5843		6277		7070
Average Weighted Dose†		60		64		72	

*Weighted factor = average dose x percent bone marrow.
†Average weighted dose = Σ weighted factors ÷ total percent active bone marrow.

TABLE 16

ORGAN DOSES IN THREE TOTAL-BODY IRRADIATION FACILITIES IN RADS PER 100 R AT CENTERLINE OF PATIENT

Organ	Facility					
	LETBI		METBI		VDRIF	
	Dose	Range	Dose	Range	Dose	Range
Bone marrow	60	42-94	64	53-86	72	61-83
Cerebellum	79	75-81	77	74-81	71	66-75
Cerebrum	82	77-87	75	70-79	70	66-74
Heart	66	61-74	66	58-70	73	65-83
Intestines	57	48-64	67	54-76	68	59-75
Kidneys	54	53-57	66	62-70	68	61-78
Lenses of eyes	94	93-96	87	85-90	72	70-74
Liver	62	55-71	67	61-78	72	67-81
Lungs	67	59-81	67	58-77	77	65-90
Skin (above sternum)	86	84-87	73	72-75	78	72-84
Skin (front waist)	77	76-79	74	72-75	79	73-84
Spleen	58	55-60	69	69-73	73	68-79
Stomach	54	49-61	64	59-69	71	66-76
Thyroid	82	80-84	76	75-77	74	73-76

relate response to some "average body-dose," we have estimated the average torso-dose also. Our estimates are: LETBI, 67 rads/100 R; METBI, 68 rads/100 R; and VDRIF, 74 rads/100 R.

The importance of using absorbed dose as the basis of comparison is clear from Table 16; i.e., if two patients were exposed to 100 R, one in LETBI and the other in VDRIF, the VDRIF patient would receive about a 20% greater marrow dose but his dose to the lenses of the eyes would be 20% smaller than the LETBI patient. Depending upon whether the biological end point was marrow suppression or cataract formation, we would either overestimate or underestimate by 20% the effectiveness of equal exposures given in these different irradiators.

Using these dosimetry data we can analyze, on the basis of dose to critical organs the effects of total-body irradiation given in three different irradiators. Estimates of dose-rate effects, repair rates, etc. can obviously be made with greater reliability than if done on the basis of exposure doses.

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Measurement of Performance Decrement and Fatigability Level by Impedance Pneumography During Controlled Exercise

Since the inception of man's space efforts, many exhaustive tests have been performed to determine physiological responses to hostile environments. Recent lengthy space missions, which traversed the Van Allen radiation belts, have increased the necessity for a more thorough understanding of man's performance ability in the event of exposure to ionizing radiations. This need is particularly enhanced by the prospect of orbiting space stations (now in planning stages) powered by on-board nuclear reactors that will expose crew members to low, well-defined levels of total-body irradiation. Less predictable galactic and solar radiations add to the possibility of exposures that might have physiologic effects that decrease performance capabilities.

In order to investigate the performance decrement as a response to total-body irradiation we have measured respiratory function (via impedance pneumography) in patients with chronic leukemia exposed to therapeutic levels of ^{60}Co gamma radiation (Robert C. Ricks, Ann Sipe, and C. C. Lushbaugh). Pulmonary impedance waveform data were analyzed using a computerized data reduction system (power spectral analysis) which we have previously shown can be used to recognize respiratory stress. Since the initial application of this computerized system, the program has undergone slight modifications that provide a more useful data output. These modifications are described later in this volume (page 128).

In evaluating the performance decrement effects of total-body irradiation, we first determined that the stress of exercise modified pulmonary impedance in a way that power spectral analysis could measure. Normal (non-leukemic) volunteers exercised, under controlled conditions, on a bicycle ergometer for specific periods at standard work loads, and were monitored before, during, and after exercise stress. Postexercise monitoring was generally continued for at least 30 min following cessation of exercise. Typical results, shown in Fig. 14 are from a volunteer exercising against a workload of 60 watts, pedaling at a speed of 50 RPM for 8 min. Increased respiratory demand, reflected by deep breathing, during and immediately following exercise is indicated by increases in both continuous and average variances of the power spectrum. The continuous variance plot indicates that periods of deep breathing cycled with periods of normal breathing approximately every 5 min. The average variance plot likewise reflects increased respiratory demand after exercise. Pulmonary impedance was measured in two selected patients exercising on the ergometer before, during, and after total-body irradiation therapy. Only patients in chronic states of their disease without malaise or other symptoms of distress were monitored in this study. They exercised at work levels comparable to non-irradiated volunteers, determined by preexposure tests to be within their capabilities. They were not in the radiation field during the exercise test period. Increased respiratory demand after exercise in the irradiated patient was similar to that of the nonirradiated test subjects. However, the increase was amplified after radiation exposure as illustrated in Figs. 15 and 16. Fatigability increased (performance decrement) on the 3rd day postirradiation and appeared to subside within 10 days to 2 weeks. The exposure rate for both patients was 1.5 R/hr and their accumulated exposures were 100 R and 150 R. These preliminary data illustrate that (1) a decrement in the exercising ability of the irradiated patient occurs postexposure, and (2) this increased fatigability is reflected by changes in respiratory function which can be measured by pulmonary impedance power spectral analysis.

Experiments are in progress to assess the performance decrement effects

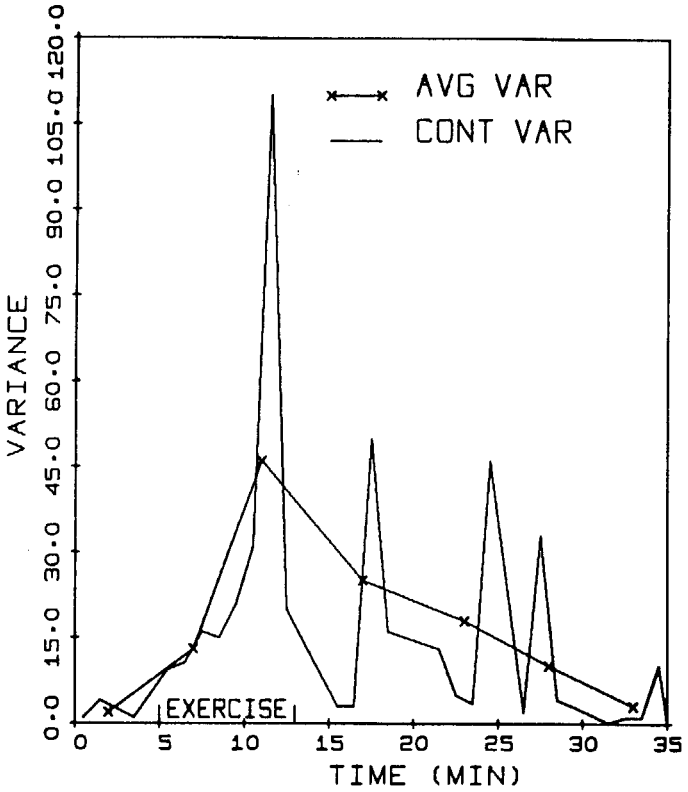


Fig. 14. Changes in pulmonary impedance (power spectral) variance in a normal volunteer monitored electronically before, during, and after exercise on a bicycle ergometer.

of exposure fractionation (e.g., 10 R/day at a rate of 1.5 R/hr) and to correlate these changes (as well as those already found with continuous exposures) in performance ability with measurable biochemical changes such as carbon dioxide (expired air), lactic acid dehydrogenase (LD), and creatinine phosphokinase (CPK).

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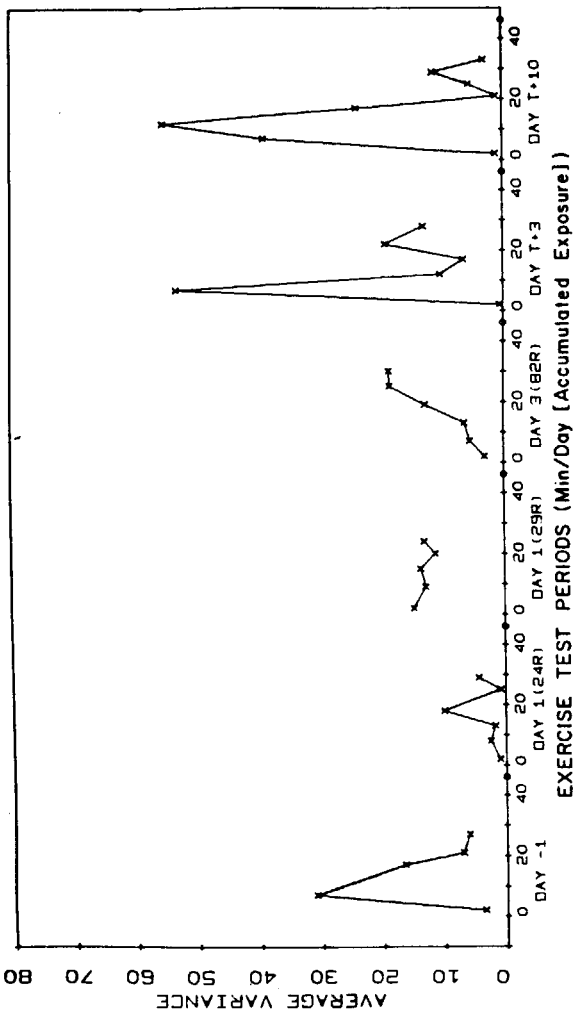


Fig. 15. Average changes in pulmonary impedance (power spectral) variance during standard exercise periods obtained before, during, and after total-body irradiation at 1.5 R/hr in a patient treated with 100 R for chronic leukemia. (Exercise load was 60 watts, 9 min duration.)

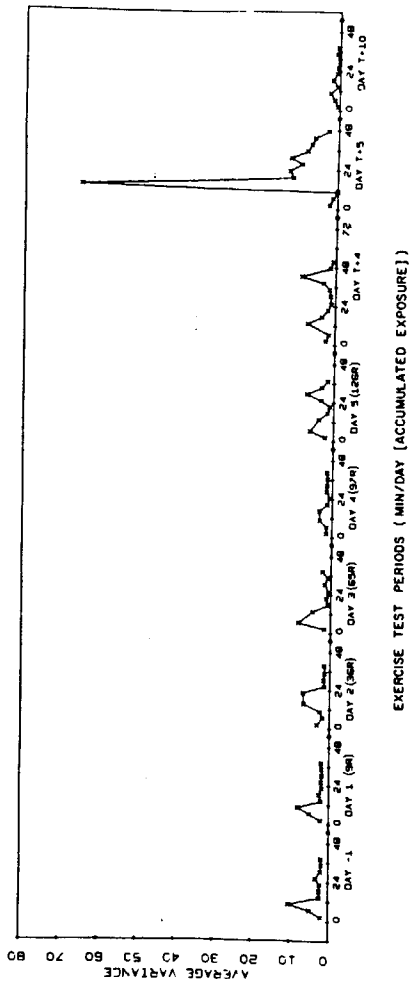


Fig. 16. Average pulmonary impedance (power spectral) variance for exercise periods obtained before, during, and after total-body irradiation at 1.5 R/hr in a patient treated with 150 R for idiopathic thrombocythemia. (Exercise load was 25 watts, 5 min duration.)

1027916

Applied Radiobiology

The efforts in applied radiobiology span human and animal studies. One cardinal topic has been the monitoring of physiologic responses in patients who have received total-body irradiation; the results are important not only to those working in nuclear energy, but also of vital interest to the NASA program which must anticipate the deleterious effects of radiation during prolonged space flight. The animal studies attempt to answer specific questions arising out of the human studies and to provide data that cannot be obtained directly from man.

Serum Trypsin-Inhibitor Levels in Patients with Hematopoietic and Other Malignancies

We continue our search for objective measurements of cell substances in the blood with levels related to hematopoietic functions (C. C. Lushbaugh, James F. Soodsma, Dorothy Lushbaugh, and R. D. Harlow with the clinical collaboration of Francis A. Goswitz, R. Tanida, and C. L. Edwards). We need such measurements to evaluate the effects of various therapeutic regimes and to eliminate the excessive reliance on quantitation of highly subjective symptoms. Commonly, we depend on a battery of chemical determinations and peripheral blood counts. The latter still reveals the most about how the hematologic patient is responding, for example, to total-body irradiation. Their prognostic, as well as diagnostic value, is high but in many of the diseases we study and attempt to treat, the blood cell values are highly pathological and define the disease, but may obscure reaction to the disease or to concurrent infections. The pretreatment levels of specific cells in patients with the same disease, for example, chronic lymphocytic leukemia, also vary so much from patient to patient that therapeutically-induced responses are difficult to compare. It would be desirable to have an additional reference measurement that would be normal in healthy people, but progressively abnormal as a person becomes ill and approaches death; the pathologic results in this measurement should subside toward normal when clinical management is successful or spontaneous recovery occurs. The study reported here to see whether measurements of serum trypsin inhibitor levels could be applied for these purposes.

The importance of serum trypsin-inhibitor (TI) levels in health and disease is highly speculative. Numerous teleologic reports suggest that TI plays a role in promoting normal growth processes by limiting random nonspecific proteolysis, and that its most important role is in protecting the pancreas and the small intestine from undesirable tryptic autodigestion. Measurement of serum trypsin-inhibitor levels has been useful in clinical laboratory only in pancreatic disease where its correlation with clinical status has been established.

We report here the results of a 5-month period of measuring TI levels using the serum and plasma of all persons who had some other measurement made on samples of their blood in the Medical Division laboratories. Experimental design was limited to that required for making the determinations. After all the measurements were completed, the sources of the serum samples were identified by disease and clinical condition and the results graphed in relation to calendar date. Those measurements from the same patient were connected by lines to see how the levels changed as the disease progressed or was arrested.

The amount of trypsin inhibitor was determined by measuring the amount of hydrolysis of α-N-Benzoyl-L-Arginine ethyl ester (BAEE) obtained under standard conditions by 0.653 units of trypsin with and without 10 microliters of serum present. The method was modified and adapted in our laboratory.

As shown in Fig. 17, normal persons and patients without serious diseases, or without symptoms of a previously established malignant disease, had similar TI levels around the normal mean of 32.5 TI units/ml, ranging up to ∿45 units and down to ∿20 units. Patients hospitalized because of uncontrollable progression of such diseases as acute and chronic myelocytic and lymphocytic leukemia, myelomas, lymphomas, myelofibrosis, Hodgkin's disease, and carcinomas had levels commonly above 50 units, and those who died of their disease during this time had progressively rising levels occasionally in excess of 90 units (∿3 x average normal). Several persons with persistent values above 60 units have survived but have been difficult therapeutic problems. Two patients who died of vascular accidents associated with their malignant disease process or its therapy, were the only ones to succumb with approximately normal levels.

Metastatic spread of epithelial neoplasms has been reported to be accompanied by a progressively rising TI preceding death; an example is shown in Fig. 18.

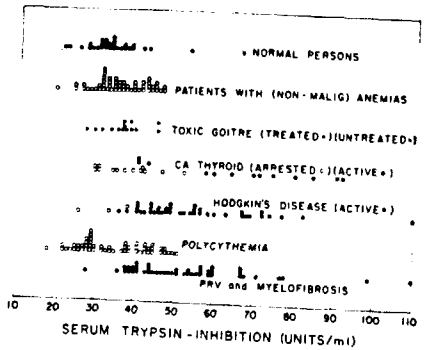


Fig. 17. Histographic distributions of the serum trypsin-inhibitor levels found in 30 determinations in normal persons, 56 in patients with anemias but without hematopoietic malignancies, 15 in patients with active or inactive toxic goiter, 13 in 5 patients with active thyroid carcinoma, 13 in 10 patients whose thyroid carcinoma is arrested, 46 in 4 patients with active Hodgkin's disease, and 3 with Hodgkin's disease suppressed by therapy, and multiple determinations in 4 patients with myelofibrosis and 5 patients with polycythemia vera. All were measured in the same 5 consecutive calendar months. See text for further explanation.

We also saw this premortem rise in patients who died from Hodgkin's disease and myelofibrosis. However, such a rise is not always irreversible; a patient with malignant plasmocytoma, who had had moderately elevated TI levels for about two months, developed rapidly rising values paralleling a similar rapid increase in blood urea nitrogen (BUN) and oliguria. Intensive cytotoxic chemotherapy produced a return to normal TI values over a 2-week period. The TI value remained normal during the rest of the study period of one month, during which the patient was relatively symptom free. Similar reductions of TI values have followed radiation and chemotherapy in other patients. It is not yet clear whether Prednisone has an effect on TI levels; it seems so far that corticosteroid therapy is accompanied by TI levels in the 50 to 60 unit range but this correlation has not been sufficiently studied. In chronic lymphocytic leukemia (CLL) and polycythemia vera, when active

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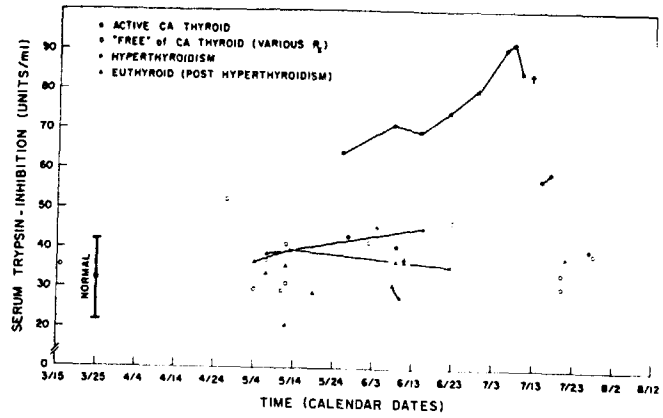


Fig. 18. Graph of the serum trypsin-inhibitor measurements in patients with thyroid disease (shown in Fig. 17) according to the calendar dates of their measurement. The dots connected by lines represent measurements in the same patient. The mean normal value and 2 standard deviations of the mean are shown at 3, 25. The cross designates the death of one patient.

disease was present but life was not immediately threatened, TI levels were often found to be much less than normal (20 down to 10 units). The clinical significance of this observation eludes us since the patients with CLL and acute leukemia who died during this observation period developed abnormally high TI levels shortly before death in spite of initially low base lines.

This retrospective study of the relation of TI level in malignant diseases shows that the level of this substance (probably in alpha globulin) is increased as clinical status worsens and decreases as improvement is obtained. This observation has encouraged us to continue these measurements and to extend our efforts to correlate changing levels with clinical events.

The Dose-Rate Dependence of Endogenous Splenic Colony Formation and Proliferation in Mice During Protracted and Fractionated Irradiation

Endogenous colony-forming cells are able to proliferate in the rodent spleen in response to radiation injury forming macroscopic colonies in the red pulp that resemble in all respects the colonies formed in the spleen of the irradiated mouse after injection of bone-marrow cells. This response is considered part of a recovery mechanism and has been used to study in vivo stem-cell survival after radiation exposure (Radiology 90, 363, 1968). From such studies the D_0 (37% survival dose) has been estimated by extrapolation to be 115 R; however, a minimum acute exposure of 250 R was necessary to elicit spleen colony formation. Above 400-900 R, increasing numbers of stem cells are killed and colony formation is suppressed. After 300-400 R, the proliferative response of the mouse stem cell is so extensive that the colonies become too numerous to count macroscopically. From these

observations this in vivo system did not appear to be particularly suitable as a tool for quantitating stem-cell survival at low doses. However, we thought the system might provide a possible end point with which to determine the time and extent of hematopoietic recovery from large radiation doses at low exposure rates.

Previously we reported that variable changes in spleen weight occurred in mice during daily exposure to gamma irradiation at low dose rates. As dosage or damage accumulated, spleen weight first decreased, then increased, and finally decreased. The correlations of these changes with accumulated exposure were satisfactory. Good correlation was seen, however, between splenic changes and the exposure rates in terms of R/day. This year we extended these observations to studying the onset time of splenic colony formation, the number and size of colonies found, and the corresponding changes in splenic weight (Lydia S. Corrill, Tom R. Stokes, and C. C. Lushbaugh) (77).

C_{57} black 6/cum male mice were placed in irradiation groups under two regimens: (1) mice were irradiated continuously (20 to 24 hr/day) at three rates of exposure (0.8, 1.6, 3.2 R/hr) and received 16-19, 32-38, or 64-77 R/day; (2) other mice were pair-irradiated at 3.6 R/min, daily receiving the three same total exposures per day. Animals were killed when they had accumulated specific doses. Spleens were weighed, fixed in Bouin's fixative, and colonies counted. Although it is usual procedure to ignore the small colonies, all were counted. Small ones are difficult to count and their quantitation is less dependable than that of those greater than 1 mm in diameter but their appearance time is biologically significant.

In Fig. 19, these results are shown as spleen weight, time of spleen colony appearance (S), and time of large colony development (L) in relation to accumulated exposure in R. In mice exposed at 18 R/day, spleen weight decreased initially about 25% and then remained constant until approximately 2,200 R had accumulated. Then spleen weight began increasing in mice exposed in both the continuous and discontinuous modes. Although the rate of increase in spleen weight was greater in continuously irradiated mice, formation of discrete colonies was not observed in them, but was seen at this time only in the discontinuously irradiated mice (2,200 R).

At 35 R/day (the approximate human therapeutic exposure rate in LETBI) mice exposed in the continuous mode respond to an accumulated dose of 700 R with a 30% decrease in spleen size and with initiation of colony formation. Those exposed discontinuously respond dramatically to the same accumulated exposure as exhibited by reduced spleen weight (50% of normal) and by the initiation of spleen colony formation. With both modes of irradiation at this daily dose rate, as well as at 70 R/day, increase in spleen weight was related to colony proliferation (L), not colony formation (S). Colony formation was begun during or immediately following the time of minimum spleen weight; maximum proliferation corresponded to greatest spleen weight.

Spleen weight in 70 R/day mice never achieved the maximum values observed in the two lower exposure rate groups. Only at this highest exposure rate, however, did colonies become sufficiently large and numerous (up to 40/spleen estimated) to become confluent over the surface of the spleen. Mice exposed to 35 R/day in the discontinuous mode were the only other group that approached this degree of proliferation of spleen colonies.

We interpret these results in terms of radiation damage and recovery to mean that: at 18 R/day, the rate of damage induction is depreciated by cellular repair mechanisms so that accumulated R "overestimates" residual damage. The level of accumulated residual damage does not induce formation and proliferation of splenic colonies until 2,200 R or more have accumulated. Since this "turn on" occurs only in mice

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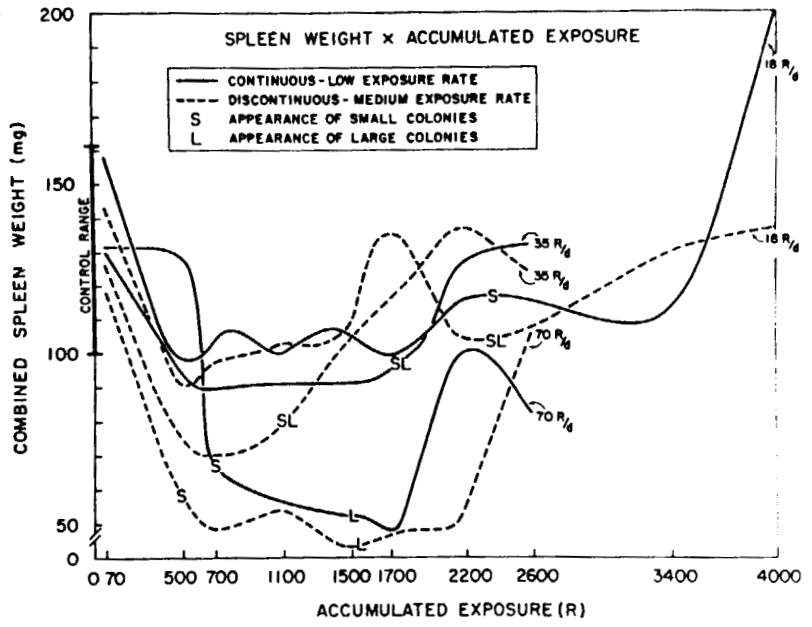


Fig. 19.

irradiated discontinuously, and since the delayed peak in spleen weight is great in the continuously exposed mice, we assume that residual damage has outweighed repair in the discontinuously exposed mice while in the continuous regimen repair has maintained at least an advantage over damage and may even be producing a state of "over compensation."

At 35 R/day the rate of damage induction apparently exceeds reparability early (1,100 R) and formation and proliferation of splenic colonies is a vigorous response to systemic demands for blood cell formation, a response which produces an abortive splenomegaly and macrocytosis of the RBC, and which temporarily delays the onset of a lethal anemia.

At 70 R/day the reparative processes are quickly overwhelmed and damage accumulates sufficiently to elicit formation of spleen colonies almost at once (500 R) but their proliferation, as shown by the appearance of large colonies, is suppressed until 1,500 R have accumulated. At this level the 70 R/day mice began to exhibit the severe radiation-induced anemia which eventually was the main cause of death.

These results would suggest that at these low dose rates, the physiologic level of hematopoietic damage depends upon dose rate and not dose, that recovery from such radiation damage is triggered by the rate at which residual or unrepaired

damage is accumulated, and that endogenous stem-cell proliferation is also highly dose-rate dependent. Our findings concerning spleen colony formation and proliferation in this study have confirmed previous observations in which we compared the response of mice to continuous and discontinuous radiation using survival, splenic weight, and total-body weight as end points. Mice in the discontinuous mode receive the radiation over a short period of time at a medium-exposure rate (3.6 R/min) and have a long radiation-free period during the day, while the mice in the continuous mode were exposed at a low-exposure rate and had a short or no radiation-free interval. Spleen colony formation and proliferation in mice irradiated in these two modes at 18 R, 35 R, and 70 R/day demonstrated that dose-rate effects predominate regardless of the length of radiation-free periods. Discontinuously irradiated mice exhibit damage earlier (spleen colony formation) and repair later (colony proliferation) than the continuously irradiated.

Production of "Stress" Hemoglobin by Endogenous Splenic Colonies During Continuous Irradiation at Low Dose Rate

Previously we reported that continuous low-exposure-rate total-body irradiation induced macrocytosis as well as pancytopenia in C57B1/cum mice. Electronic RBC sizing indicated that the red cells approached the volume of those of the newborn mouse. We interpreted this to mean that the splenic stem cells, proliferating extensively then, were bypassing the usual number of cell divisions and were consequently making fewer but larger RBC as described by Lajtha (Effects of Ionizing Radiations on the Haematopoietic Tissue, Proceedings of a Panel, Vienna, 1966. International Atomic Energy Agency, 1967, p. 3) to stress. In addition, we showed histologically that the bone marrow was almost completely atrophic at this time and that the splenic erythroblasts were arising from hemocytoblasts that must be originating in the spleen during the radiation exposure. We wondered, consequently, if these "new" stem cells could be from a more primitive anlage than those destroyed by irradiation and if they might be synthesizing hemoglobins which resemble the embryonic and fetal hemoglobins found in the macrocytic RBC of prenatal animals. A collaborative study was undertaken (Lorraine Kraus,* Asri Rasad,* Tom R. Stokes, and C. C. Lushbaugh) to provide the sophisticated hemoglobin biochemistry required to answer these questions.

Electrophoretic analysis of hemoglobin of normal adult mice has shown two general types of fractionation patterns: diffuse (two or more components) and single (a discrete homogeneous band). Each inbred strain has an inherited specificity for one of the two. The hemoglobin of the C57B1/cum mouse used in our previous experiments is classified as the single type. To augment the hemoglobin study, a mouse (BALB/c) with the diffuse (three components) type of hemoglobin was included.

Equivalent groups of 120-day-old mice of these two strains were exposed to ^{60}Co gamma radiation at 1.6 R/hr for an average of 22 hr/day (Tables 17 A, 17 B). After an accumulated exposure of 2100 R was reached the first sample was removed for analysis. Starch gel electrophoresis of spleen, bone marrow, and peripheral-blood hemolysate produced normal patterns in all cases. Gross appearance of the spleens was also normal. The second sample was made at 3000 R. The bone marrow and peripheral blood of both strains remained normal, but the splenic hemolysate of the C57B1/cum mice contained a definite new fast band in addition to the normal band. The BALB/c spleen hemolysate also showed a trace of a fast band. The

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1027919

HEMOGLOBIN ELECTROPHORETIC PATTERNS OF CONTINUOUSLY IRRADIATED MICE

TABLE 17 A

C57B1/cum 120 Days Old

	2100 R	3000 R	3550 R
Spleen	Normal	Definite Fast Band	Definite Fast Band (Decreased Amount)
Peripheral Blood	Normal	Normal	Normal
Bone Marrow	Normal	Normal	Trace of Fast Band

TABLE 17 B

BALB/c 120 Days Old

	2100 R	3000 R	3550 R
Spleen	Normal	Trace of Fast Band	Normal
PB	Normal	Normal	Normal
BM	Normal	Normal	Increased Amount in Faster Bands

spleens at the time had a rough appearance which indicates early nodule formation. The last sample was taken at 3550-R accumulated exposure. The peripheral-blood hemolysates showed no change in hemoglobin fractionation pattern. The bone marrow of the C57B1/cum contained a trace of a fast component while the BALB/c bone marrow had a normal pattern but an increased concentration in its fastest band. The new band was still present in the C57B1/cum spleen hemolysate but in a decreased amount. The BALB/c splenic hemolysate had returned to a normal pattern. The spleens showed distinct nodule formation in both strains at this 3550-R level of exposure.

Since the C57B1/cum with the single hemoglobin type showed the clearest and most

TABLE 18

C57Bl/cum 120 Days Old

	2060 R	3045 R	3460 R
Spleen	Trace of Fast Band	Definite Fast Band	Definite Fast Band (Increased Amount)
PB	Normal	Normal	Normal
BM	Trace of Fast Band	Definite Fast Band	Definite Fast Band
Liver	Definite Fast Band	Definite Fast Band	Definite Fast Band (Decreased Amount)

definite picture of the new fast-moving component, the experiment (Table 18) was repeated with these mice in an attempt to obtain additional material for more detailed biochemical analysis. Samples were taken at the approximate accumulated exposures (2060 R; 3045 R; 3460 R) of the first experiment (2100 R; 3000 R; 3550 R). Again, the peripheral-blood hemolysate failed to show any deviation from the normal electrophoretic pattern. The spleens which were showing early nodule formation at the 2060-R level showed a trace of the fast component. The new band was also found in increasing amounts in the spleen samples from the animals that had received the higher doses. The bone-marrow fractionation pattern coincided with that of the spleen, that is, a trace of the new band in 2060 R the first (2060 R) sample and definite fast bands in the last two samples. In the second experiment liver hemolysate was found to contain the fast component at 2060 R and 3045 R, and, to a lesser degree, at 3460 R.

Parallel studies (Tables 19 A, 19 B) with 50-day-old C57Bl/cum and BALB/c mice produced electrophoretic patterns which coincided with those of 120-day-old mice. However, the accumulated exposure necessary to induce this response was ~50% lower (1120 R, 1640 R, 1900 R) than that required in the older mice (2100 R, 3000 R, 3550 R). Again the peripheral-blood hemolysate was normal in all three samples in both strains. In C57Bl/cum, bone marrow showed the new fast band in trace amounts in the 1120-R sample and in increasing concentrations in the 1640-R and 1900-R samples. The BALB/c bone-marrow hemolysates were within normal limits in the first two samples. The third sample (1900 R), however, showed a slight shift toward more concentrated bands in its lead components. The spleens of both strains were nodular at all sample levels. In the lowest dose C57Bl/cum spleen hemolysate, a trace of the fast component was seen and in the intermediate dose group a definite fast band appeared; insufficient material prevented analysis

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TABLE 19 A

C57Bl/cum 50 Days Old

	1120 R	1640 R	1900 R
Spleen	Trace of Fast Band	Definite Fast Band	Insufficient Material for Analysis
PB	Normal	Normal	Normal
BM	Trace of Fast Band	Definite Fast Band	Definite Fast Band (Increased Amount)
Liver	Definite Fast Band	Definite Fast Band (Decreased Amount)	Trace of Fast Band

TABLE 19 B

BALB/c 57 Days Old

	1120 R	1640 R	1900 R
Spleen	Normal	Normal	Trace of Fast Band
PB	Normal	Normal	Normal
BM	Normal	Normal	Increased Amount in Faster Bands
Liver	Definite Fast Band	Definite Fast Band	Insufficient Material for Analysis

at the 1900-R level. The BALB/c spleen patterns remained normal at the lower two levels, but the highest dose sample had a slight trace of a fast band. The liver hemolysates of both strains contained several fast components in the first samples (1120 R). Decreased quantities of these bands were present in the 1640 R group, and in the last (1900 R) group of C57Bl/cum only a trace remained. Not enough material was recovered from the BALB/c to permit analysis at the 1900-R level.

Peripheral-blood counts and hemoglobin levels indicate different degrees of anemia among the various groups, with the BALB/c mice maintaining slightly higher values in all parameters. In general, however, all groups show depressed WBC, RBC, HcT and Hb content with a slight (BALB/c) to moderate (C57Bl/cum) reticulocytosis.

Phenylhydrazine induced macrocytic anemia of Tennessee Swiss mice (diffuse type hemoglobin) is accompanied by changes in the electrophoretically fractionated materials from spleen, bone marrow and in the hemoglobin fractions of the peripheral blood (L. Kraus). These results are similar to those of the irradiated BALB/c mice except that in the latter the peripheral-blood hemolysate has always been normal. The blood of the Tennessee Swiss Mice is characterized by a very pronounced reticulocytosis whereas that of the BALB/c mice shows only a slight increase in reticulocytes. It has also been demonstrated that very old C57Bl/6 cum mice (single type hemoglobin) develop spontaneously an altered hemoglobin electrophoretic pattern which contains a fast band like that of the irradiated C57Bl/cum mice. In the irradiated BALB/c, as well as the hemolysing Tennessee Swiss, there is a tendency toward a change in the distribution of the regular components rather than the synthesis of new ones. Traces of possible new bands are seen, but the decrease in the normal slow components and increase in the fast ones are definite. In the irradiated C57Bl/cum mice a new hemoglobin is clearly evident.

These altered electrophoretic patterns of hemoglobin do not resemble those found in normal fetal tissue; fetal hemoglobins have a slower mobility than the normal adult hemoglobins of the single or diffuse types. Since the abnormal components in the irradiated animals move electrophoretically faster than normal hemoglobins, they must represent a new hemoglobin species that is a manifestation of stressed erythropoiesis. The underlying mechanism is obscure at present but is clearly related to the spleen colonies that this type of irradiation induces.

Radiation Immunology and Marrow Transplantation

In 1970 we undertook bone-marrow transplantation in the human being; this was a renewal of an effort that had been discontinued several years ago because of lack of success. The new attempt used a protocol based on animal work done in our laboratories in which the administration of high-dose-rate total-body irradiation and antilymphocytic globulin were combined to give intense immunosuppression with minimum threat to life from the procedure itself. The success of this first new clinical trial is indicated in the subsequent section and in the illustration on the cover of this report.

Our basic immunology program continues with experiments to understand antigen-antibody reactions as related to transplantation of cellular elements, and the nature of the host-graft relationships in radiation-induced and naturally occurring chimeras.

1027921

The immunology program has evolved from the objective of obtaining therapeutic marrow transplants, particularly as a means of sustaining persons who have been heavily irradiated. During the past year basic studies related to this goal have extended the information on the role of exposure rate in radiation depression of the immune mechanism. High radiation exposure rates in mice have yielded maximum immunosuppression, as measured by the ability to obtain foreign marrow grafts and reduced responses to antigenic stimuli. Initial exposure rate studies were performed with ^{60}Co gamma radiation, but more recently this effect has been evaluated with other qualities of radiation. Exposure-rate-dependency of the immune response to both foreign antigens and grafts has been demonstrated with 250 kvp X rays but could not be shown with fission spectrum neutrons.

A combined form of immunosuppression was explored. Although foreign grafts are obtained with high rate ^{60}Co gamma radiation, the total dose of radiation used was such that control animal mortality was uniformly 100%. Administration of anti-lymphocyte serum (ALS) to mice in conjunction with high-exposure-rate radiation, however, permitted use of a lower total exposure dose to procure foreign marrow transplants. Thus, sublethal radiation could now be used, enhancing the potential application of this procedure to patients. An immunosuppressive protocol based on these experimental findings was applied to a patient with acute granulocytic leukemia with the intent of grafting marrow from his brother. This goal was achieved, and more important, no sign of leukemia was evident during a 4-month period of survival after this treatment. Fortunately, graft-versus-host (GvH) incompatibility so often observed by others in both clinical and experimental grafting situations was not grossly evident in this patient. Its absence, however, may only be apparent because of the lack of any direct objective test for its detection. The use of the Jerne plaque test for detection of an early GvH or HvG reaction in mice bearing foreign marrow grafts was demonstrated previously in our laboratory. Further refinement of the technique has now permitted a semiquantitative evaluation of this disease process in both xenogeneic and allogeneic transplant models. Interestingly, this assay method reveals the greatest GvH activity to occur during the first week after marrow transplantation, not during the 3rd or 4th week when the clinical signs of the disease are most evident. Recovery of animals from the GvH-mediated secondary disease syndrome is not paralleled by a return to normal immunocompetence. The implications of this observation to patients bearing a foreign marrow transplant are readily apparent and emphasize the need for systematic studies on their immunological status.

Role of Exposure Rate in Immunosuppression

The importance of radiation exposure rate in achieving immunosuppression was initially demonstrated in our laboratory by attempting xenogeneic (rat) marrow grafts in mice exposed to ^{60}Co gamma radiation (Science 146, 663, 1964). Briefly, several animals exposed to 900 R at a high-exposure rate of 53 R/min showed acceptance of a permanent foreign marrow graft, while those exposed at the rate of 4 R/min showed only a temporary graft take with subsequent rejection and formation of hemagglutinating antibodies specific for the graft antigens. Extension of these studies to animals receiving a higher level of radiation, 1200 R, still revealed a similar dose-rate effect in transplantation even though this dose of irradiation was 95-100% lethal (Transplantation 7, 259, 1969). Subsequent experiments measuring the immuno-competence of ^{60}Co-irradiated animals by challenge to foreign antigenic stimuli amply verified the role of radiation exposure rate in achieving immunosuppression (Donald Carlson* and N. Gengozian).

* Public Health Service Fellow.

Extension of these studies to other qualities of radiation, namely 250 kvp and fission spectrum neutrons, indicated that there was a similar rate dependency of the immune response only with the former type of radiation (Charles Gottlieb* and N. Gengozian). The similarity of the dose-rate effect with ^{60}Co gamma and 250-X-ray radiation was demonstrated in two well defined experimental protocols. It is well known that the degree of immunosuppression obtained following total-body irradiation is dependent upon the time of antigenic challenge relative to time of radiation exposure. In such a previous temporal study with ^{60}Co gamma radiation, it was found that the greatest exposure-rate effect on the immune response occurred when antigen, sheep RBC, was injected 12 hr before irradiation (Carlson and Gengozian), and a minimal-rate effect was noted with antigen given 24 hr after irradiation. As shown in Fig. 20, a temporal study with X irradiation gave results comparable to that observed with ^{60}Co gamma radiation. Note that the greatest differential effects of exposure rate occurred in the early inductive stages of antibody formation, i.e., when antigen was injected from 1 day before to 2 hours after irradiation. The absence of any dose-rate effect with neutron irradiation is emphasized in a similar protocol which is shown in Fig. 21. Other differences to be noted are the slightly enhanced responses observed when antigen was given either 10 or 5 days before neutron radiation, and also the significant depression when antigen was given 3 days before irradiation, neither of which was observed with X irradiation. These contrasting effects, enhancement and depression, emphasize the differences in the damaging effects of neutrons as compared to those with X irradiation. These contrasting effects, enhancement and depression, studies. Spleen cells from controls and from animals exposed sublethally to X rays and neutrons at varying exposure rates injected into lethally (950 R) irradiated animals which were then injected with sheep RBC antigen. The animals were bled 6 days later and the agglutinating titers were determined as a function of dose of spleen cells. As shown in Fig. 22 (A,B) there was a straight line relationship between antibody titer and number of cells injected, permitting an evaluation of the effects of the varying radiation exposure rates and quality of radiation given the test spleen cells. Note that with X rays, a total dose of 275 rads given at 67 rads per minute produced a greater depression of the antibody-forming capacity of the transferred spleen cells than the same dose delivered at 7.6 rads per minute. These data verify that the varying radiation exposure rates affect the immunocompetent cells and the responses observed previously in the intact animal (Fig. 20) are not merely a reflection of a differentially irradiated environment. Similar results had been obtained previously with ^{60}Co gamma radiation. With neutron radiation, 50-rads exposure to the spleen cells produced significant depression also but without any apparent rate effects, in agreement with data obtained earlier in the intact animals (Fig. 22). It should also be noted that while the slopes of the regression lines obtained with X-irradiated spleen cells paralleled tnose of the nonirradiated control cells, the slopes for the neutron irradiated animals were significantly different. The reasons for this are still obscure.

The dose-rate effect observed with 250 kvp X irradiation on humoral antibody formation prompted marrow transplantation studies similar to those done following ^{60}Co gamma radiation (N. Gengozian and John Kateley*). Only preliminary information can be given, but the results obtained thus far are entirely analogous to those obtained with the higher energy gamma rays. For example, no permanent rat marrow grafts were obtained in mice exposed to 900 R at 4 R/min, while all animals receiving the same dose of radiation at 50 R/min had a transplant when tested 30 days

* Predoctoral Graduate Student, University of Tennessee.

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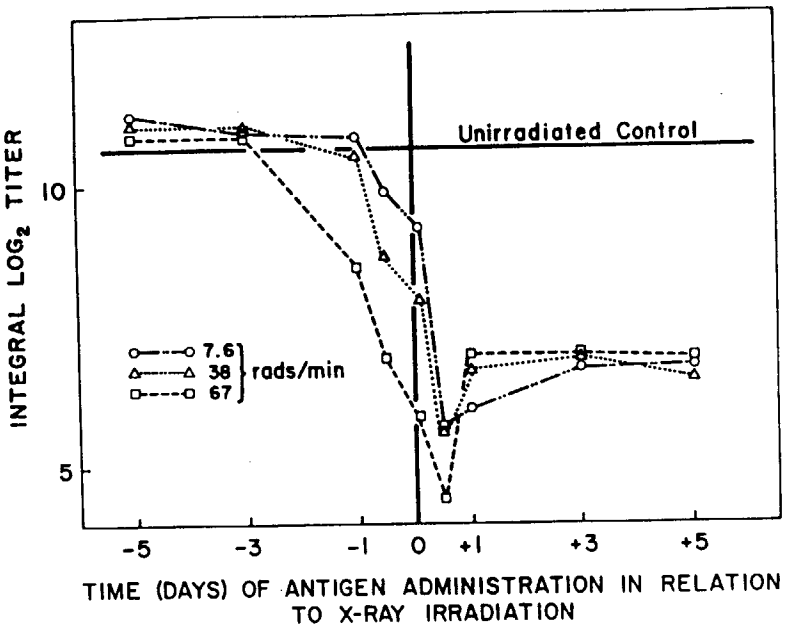


Fig. 20. The effect of X-ray-irradiation exposure rate on antibody formation as a function of time of antigen administration relative to irradiation at 500 rads.

posttreatment. This rate effect appears to be operating at a total exposure of 1000 R also. Since with both ^{60}Co and X-ray irradiation the effect of dose rate on humoral antibody formation was less pronounced 24 hr after than at 2 hr after radiation, it was imperative to determine the importance of delayed marrow injections in producing a permanent graft. Thus, in all our previous transplant studies the foreign marrow was inoculated within 2 to 4 hr postirradiation. While the data are only preliminary, it is important to note that (a) regardless of the rate of irradiation, an injection of bone marrow 24 hr after irradiation is more likely to result in a marrow graft than if given 2 hr after irradiation, and (b) regardless of the time of marrow injection, more permanent and therapeutic grafts are obtained following a high rate of irradiation. These data strongly indicate that to obtain optimal therapeutic value of a foreign marrow graft (a) the recipient should be exposed to radiation at a high rate and (b) the marrow should not be injected immediately but should be delayed 24 hr or more after exposure.

Marrow Grafts with Sublethal Irradiation

In adult animals, it is well known that with radiation as the sole immunosuppressive agent, a foreign marrow graft is best obtained following a lethal dose of radiation. Programmatically, the potential application of our basic findings to a clinical situation is one of our primary missions; therefore, it seemed necessary

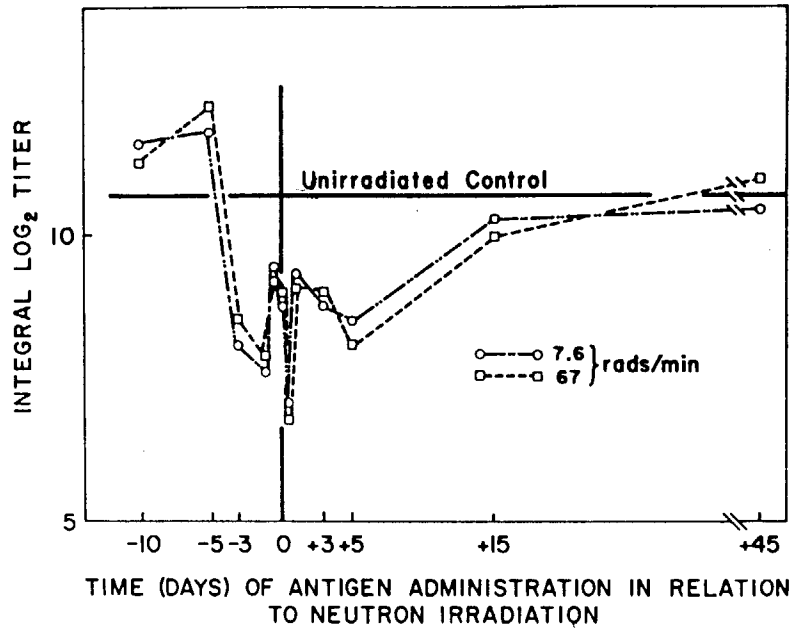


Fig. 21. The effect or neutron X-irradiation exposure rate on antibody formation as a function of time of antigen administration relative to irradiation at 150 rads.

to explore means of obtaining foreign marrow grafts without recourse to use of a lethal level of radiation. To achieve this, studies were made on the combined use of sublethal radiation and injections of antilymphocyte serum (ALS) as means of immunosuppressing an animal for acceptance of a foreign graft (N. Gengozian and Elizabeth Allen*). Groups of mice were injected with ALS for 7 consecutive days and then given a sublethal dose of X irradiation ranging from 500 R to 800 R at a rate of 100 R/min. Twenty-four hours after radiation, they were injected with rat bone marrow and then tested at 10-day intervals for the presence of a graft. In the control studies, i.e., those receiving radiation only, evidence for temporary grafts were obtained only after the highest dose, 800 R, all survivors eventually showing complete absence of any foreign cells. With the ALS pretreatment, permanent marrow grafts were obtained after as little as 600-R total-body irradiation. In addition, all grafted animals experienced a GvH-mediated secondary disease. In other control studies it was found that the ALS-radiation regime alone did not enhance or decrease the percentage 30-day mortality beyond that observed with radiation only, this procedure thus meeting the essential requirement of being no more injurious than either treatment alone. Current studies using this approach

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are on ^{60}Co high-rate irradiation and the transplantation of allogeneic as well as xenogeneic bone marrow in mice.

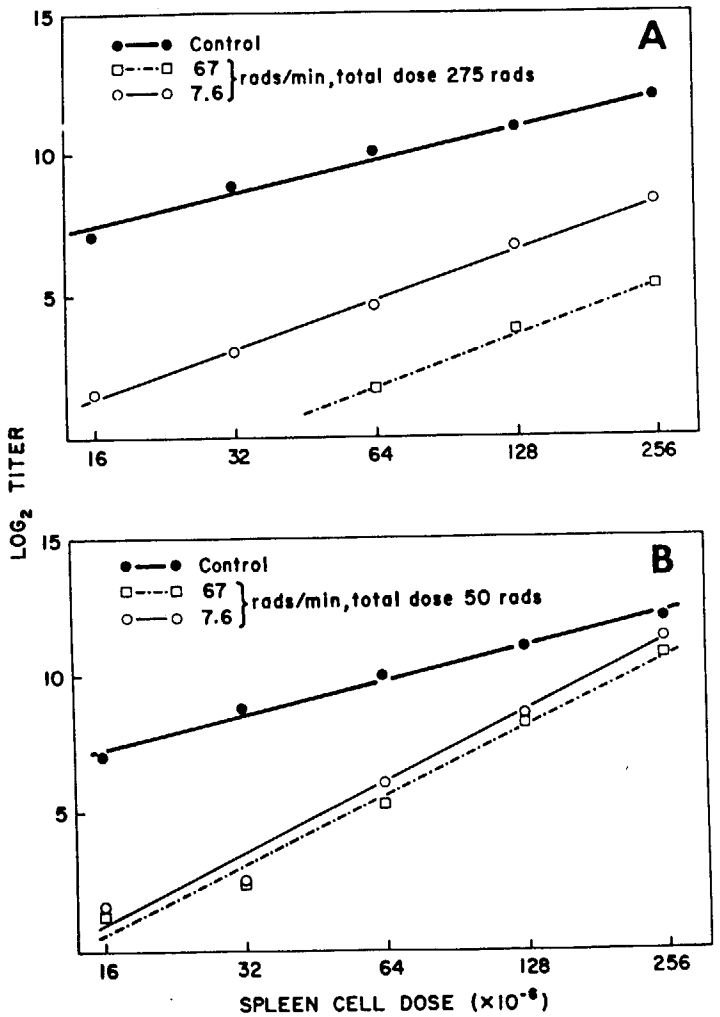


Fig. 22. (A,B). Primary antibody formation of transferred spleen cells that have been exposed to (A) 275 rads of X rays and (B) 50 rads of neutrons.

The more recent advances in human tissue histocompatibility testing procedures, namely the definition of leukocyte antigens and the in vitro mixed lymphocyte culture, and the encouraging clinical evaluations of ALS as a selective biological immunosuppressive agent, have spurred greater interest in transplantation of tissues and organs in patients. These factors, plus the development of an ultraclean facility for strict bacteriological monitoring of a heavily immunosuppressed individual, warranted initiation of marrow transplant studies in selected patients at the ORAU Medical Division. Further, the information obtained from our basic animal studies concerning the role of radiation exposure rates and ALS in enhancing procurement of a foreign marrow graft suggested a feasible immunosuppressive protocol that would hopefully present minimal risk to the patient if the transplant failed. Fig. 23 shows the basic immunosuppressive regime devised for this effort: administration of antilymphocyte globulin (ALG) intravenously for 8 consecutive days, total-body irradiation of 500 R at 40 R/min on day 9, and injection of bone marrow on the 10th day. In addition, methotrexate was to be administered at frequent intervals during the first few weeks posttransplant; this drug has been reported to reduce the severity of the early GvH reaction in experimental animal transplants. With the exception of that time required to transport the patient to and from the irradiation facility, he would be maintained in the ultraclean laminar-flow room while receiving the ALG and also immediately after radiation exposure and marrow injection.

Extrapolation of information gained from the experimental animal, particularly a rodent, to man in the development of such a protocol is obviously not without its reservations. Specifically, in this situation the primary questions or uncertainties would be: (a) the efficacy of the antilymphocyte serum used in the human, relative to that used in the experimental animal model, and (b) the immunosuppressive effectiveness of radiation (dose? rate?) in the human, relative to that in the experimental animal. Other variables, perhaps as important as the first two, but over which the investigator would have little or no direct control, may also be considered: reaction of the patient to the ALG; his physiologic response to a fairly high level of total-body irradiation, both during and after exposure; and most important, the amount of bone marrow that could be obtained from the donor for transplantation. The final variable is the underlying disease of the patient and his health status at the time the procedure is initiated, conditions which can be controlled easily with the experimental animal.

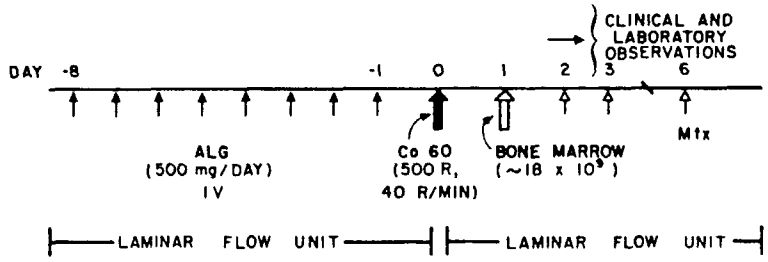


Fig. 23. Protocol for immunosuppression in man for allogeneic bone-marrow transplantation.

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This year a 52-yr-old man with acute granulocytic leukemia who was experiencing an apparently uncontrollable relapse of his disease following an 8-month period of complete hematologic remission was treated with allogeneic marrow under the new protocol (N. Gengozian, C. L. Edwards, Helen Vodopick, and Karl E. Hübner). The antilymphocyte serum used was obtained from the Upjohn Company (Michigan) and specifically was a horse antihuman thymocyte gamma globulin (H-AHTGG). The radiation source was the variable-dose-rate irradiation facility at the UT-AEC Agricultural Research Center, capable of providing the required high exposure rate (Figs. 24-27). The marrow was obtained from the patient's brother who, on the basis of HL-A typing (human leukocyte antigen) and the in vitro mixed lymphocyte test, represented a good histocompatibility match.

Approximately one-half hour after completion of the initial infusion of the H-AHTGG the patient experienced a temperature rise to 39.5° C. He became nauseated and vomited repeatedly throughout the night, but by morning he was feeling well. Aspirin and benadryl were given before each subsequent dose of the H-AHTGG and the patient showed no further side effects from the next seven infusions. Prior to, during, and after irradiation the patient was sedated with chlorpromazine; he experienced only mild nausea the evening after irradiation but had no vomiting. Twenty-four hours after the irradiation he received intravenously 1.8×10^{10} bone-marrow cells from his brother without complications. Prior to the immunosuppression the patient was skin tested against horse globulin, purified protein derivative (PPD), and histoplasmin. He had strong reactions to both the



Fig. 24. Portable sterile isolator for transporting patient from ultraclean room to irradiator. Patient, dressed and draped in sterile attire, receives sterile air through absolute filters by means of a battery-driven fan and flexible air pipe.



Fig. 25. An ambulance transports the patient from the Medical Division to the entry portal of the UT-AEC agricultural project facility which permits exposures of 40 R/min.

PPD and histoplasmin, but no reaction to the horse globulin. Following the H-AHTGG and the irradiation the skin tests to both PPD and histoplasmin failed to elicit a reaction.

The hematologic profile, and the drug management of the patient before and after treatment, are shown in Fig. 28. During the course of the immunosuppression and the first 8 days after the marrow injection, the platelet count ranged from 1200 to 10,000/mm^3. During this period he was given several platelet transfusions to prevent bleeding. Thirteen days after the marrow transfer his platelet count spontaneously increased to 28,000 and, as shown on the chart, rose to more than 100,000 by the 11th week. In the 16th week posttransplant, the platelet level was approximately 175,000. Similarly, the granulocytes, after reaching a nadir of 400/mm^3 on the 16th day showed recovery beginning in the 4th week and in the 16th week were 5,625/mm^3. Lymphocytes were reduced after the first infusion of the H-AHTGG, and showed a minimal but consistent trend toward more normal levels after the 3rd week. The low lymphocyte levels may be attributed to the continued administration of Prednisone and Methotrexate. The erythropoietic response, as measured by the hematocrit and reticulocyte indexes, is shown in Fig. 29. The hematocrit dropped to a low of 27% 4 weeks posttreatment then rose slowly to about 40%. Significant numbers of reticulocytes appeared in the blood during the 3rd week, and the peak level of approximately 6% occurred at the end of the 4th week. A definitive marker for a graft "take" was available only in the erythrocytic cell line, the patient having MN antigens on his red cells, and the donor being MM,

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i.e., lacking the N antigen. As shown in Fig. 29 the percentage of cells lacking this marker increased almost in a straight line fashion from the 3rd week on through the 10th. The 50% level of MM (donor) cells occurred at about the 8th week, and 12 weeks posttransplant all cells lacked the N antigen.

The hematologic responses noted above for the other cell lines are highly suggestive of a transplant also, particularly the platelets, where the marked increase observed at the end of the second week is too early for recovery from irradiation injury. A chromosome marker for the patient's leukemic granulopoietic cells, found in almost 90% (21/24) of the marrow cells karyotyped before the transplant attempt, has not been detected beyond the 1st week posttreatment (see report by G. Littlefield). All granulopoietic cells examined from the 8th through the 76th day after marrow infusion showed the absence of this marker; whether these normal granulocytes were of host or donor origin, however, is not known. Although a direct marker for the lymphocyte population is also unavailable, the patient and donor differed genetically for their immunoglobulin proteins, the patient being Gm (1)+ and Gm (12)-, and the donor Gm (1)- and Gm (12)+. Efforts are now being made to test for the presence of donor-type immunoglobulins in the serum of the patient at varying intervals after the transplant.

In addition to the successful engraftment and the apparent absence of any leukemic cells, this clinical case was remarkable in that the patient experienced no symptoms

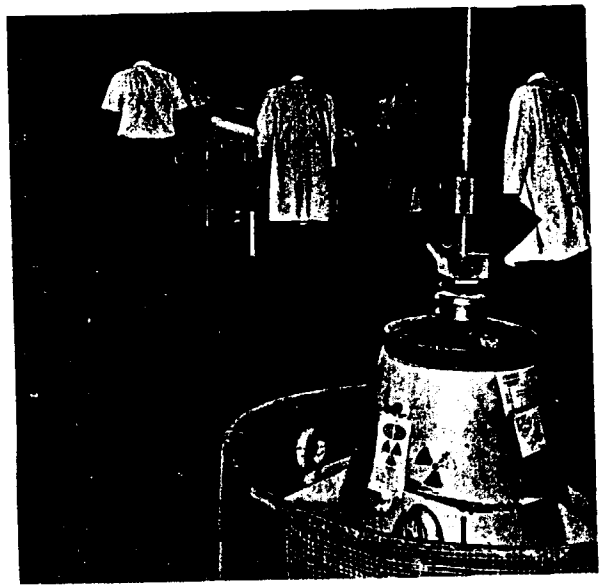


Fig. 26. A trial run. "Patient" in portable isolator wheeled into place at the UT-AEC variable-dose-rate irradiator. A shield for one of the 12,500-Curie sources is seen in the right foreground.

readily attributable to a graft-versus-host reaction, normally quite severe in primate and human marrow transplant attempts. The intermittent doses of Methotrexate administered throughout the posttransplant period may account for the absence of any signs of this syndrome.

Demonstration of Graft-Versus-Host Reaction

Although there are well accepted symptomatic and pathologic lesions associated with the graft-versus-host reaction, the extent of the actual damage detectable with the methods of histopathology is not impressive and is in contrast to the often fatal outcome. Thus, it has been difficult to delineate with certainty the sites and time course of the initial activity of the grafted immunologically competent cells. In the previous report we (Hübner and Gengozian, 1969) described a technique demonstrating graft-versus-host reactivity in spleens of lethally irradiated mice after injection of allogeneic or xenogeneic spleen cells. In those experiments the activity against host-type red blood cells could be seen as complement-dependent hemolytic foci over thin spleen slices. The results obtained were of a qualitative nature, but with the significant finding that a GvH reaction was demonstrable during the first 3 days after inoculation of histo-compatible spleen cells. Subsequently we attempted to quantitate this hemolytic response and to show such graft-versus-host (GvH) activity following the injection of foreign bone-marrow cells. Furthermore, we extended our test system by using host thymus cells, in addition to red blood cells, as target cells.

Mice were exposed to 950-R X rays and injected intravenously with either allogeneic or xenogeneic bone marrow or spleen cells 18 hr later. Groups of



Fig. 27. Control panel (right) of the UT-AEC variable-dose-rate irradiator. On the left is the ORAU physiological monitoring unit for recording EKG, respirations, and pulmonary impedance changes during the exposure to 500 R at 40 R/min.

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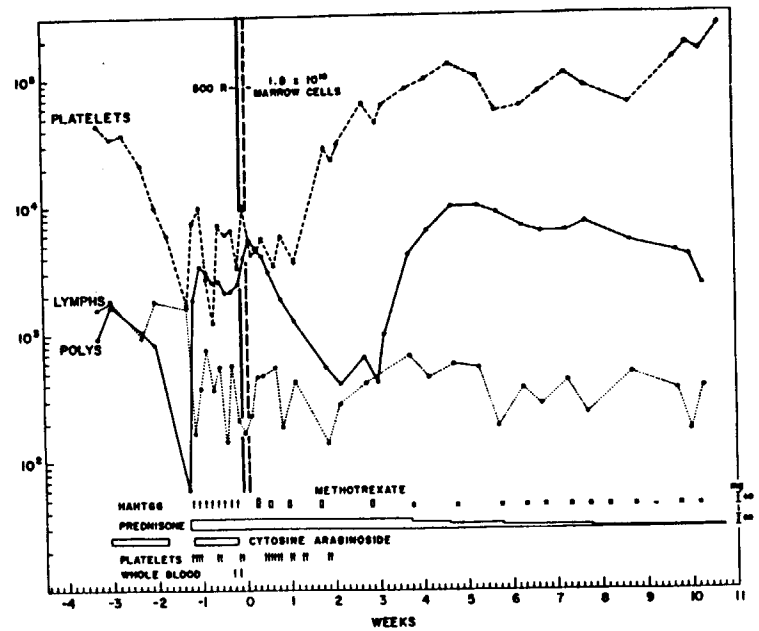


Fig. 28. Hematologic profile and drug management of patient with acute granulocytic leukemia injected with allogeneic bone marrow after the immunosuppressive regime outlined in Fig. 23. H-AHTGG=Horse antihuman thymocyte gamma globulin.

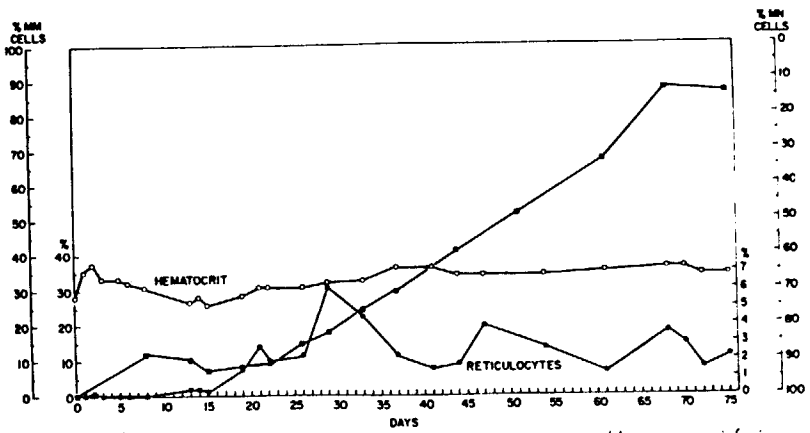


Fig. 29. Erythropoietic parameters in patient after immunosuppression and bone-marrow infusion. Increase in percentage donor-type MM cells (left ordinate, ■———■) offer definitive evidence for graft "take."

the treated animals were sacrificed daily for one week, their spleens removed and frozen in a Cryo-Cut Microtome*. Adjacent pairs of 8-micron-thin longitudinal sections, cut at 250-micron intervals throughout the whole spleen, were placed on microscope slides and covered with agar containing the target cells (host RBC or thymocytes) and complement (human C' for the xenogeneic system and guinea pig C' for the allogeneic system). After incubation at 37° C for 60 to 90 min the preparations were scored for lytic foci. The data obtained with this assay system have been expressed as number of hemolytic foci (red cell) or cytolytic (thymocyte) foci per whole spleen.

After injection of 50 x 10^6 xenogeneic spleen cells, hemolytic foci were observed in some of the test spleens as early as 24 hr after injection (Fig. 30 A). This minimal antihost activity of approximately 3 foci per spleen did not change during the next 3 days. Peak activity was seen on day 6 when 15 of 15 spleens showed an average of 32 foci per spleen. The same spleens also showed antithymocyte foci with a peak number of 19 per spleen on day 5. With both target cell types we observed great individual variation. In additional groups of mice treated with rat spleen cells, we observed the development of an anemia and beginning mortality on days 6 and 7 when focal hemolysis and cytolysis was most active in the animals used for the spleen assay (Fig. 30 A).

Injection of 100 x 10^6 xenogeneic bone-marrow cells also resulted in focal hemolytic and cytolytic activity in spleens of irradiated mice (Fig. 30 B). In this system, the peak hemolysis occurred on day 7 (average of 15 foci) and the number of thymocyte foci was greatest on day 6, with little difference between RBC or thymocyte foci. With marrow transplantation no mortality or anemia was seen during the 8 days of observation.

In the allogeneic ($C3BF1 \rightarrow CAF_1$) situation hemolytic antihost activity developed after injection of spleen cells (50 x 10^6) but not with bone-marrow cells (30 x 10^6). The peak response, after a lag period of low responsiveness as seen in the xenogeneic combination, was 42 foci on day 5, 1 day earlier than after injection of rat spleen cells. No antihost activity could be demonstrated with thymocytes as target cells. This allogeneic donor-host combination resulted in a clinically more severe GvH reaction as documented by an early mortality of all animals within 8 days. However, the anemia in these animals was not very severe, probably because in vivo conditions were not optimal for an immune hemolytic process.

These results indicate that foreign immune competent cells react early against the host. Especially, an injection of xenogeneic or allogeneic spleen cells causes early focal activity in the spleen which is accompanied by a severe and hyperacute graft-versus-host disease. Xenogeneic bone-marrow cells also gave rise to active cell clones directed against host tissue. In this situation, however, the antihost reaction could be demonstrated in the absence of clinical evidence of a GvH reaction. We were unable to detect this activity with allogeneic marrow cells.

The active component in this assay for GvH reaction is clearly related to humoral substances, presumably antibodies, which are capable of the destruction of host target cells. The detection of cytolytic antibody at the cellular level indicates a humoral component in GvH reaction in contrast to the well accepted

* American Optical Company, Buffalo, New York.

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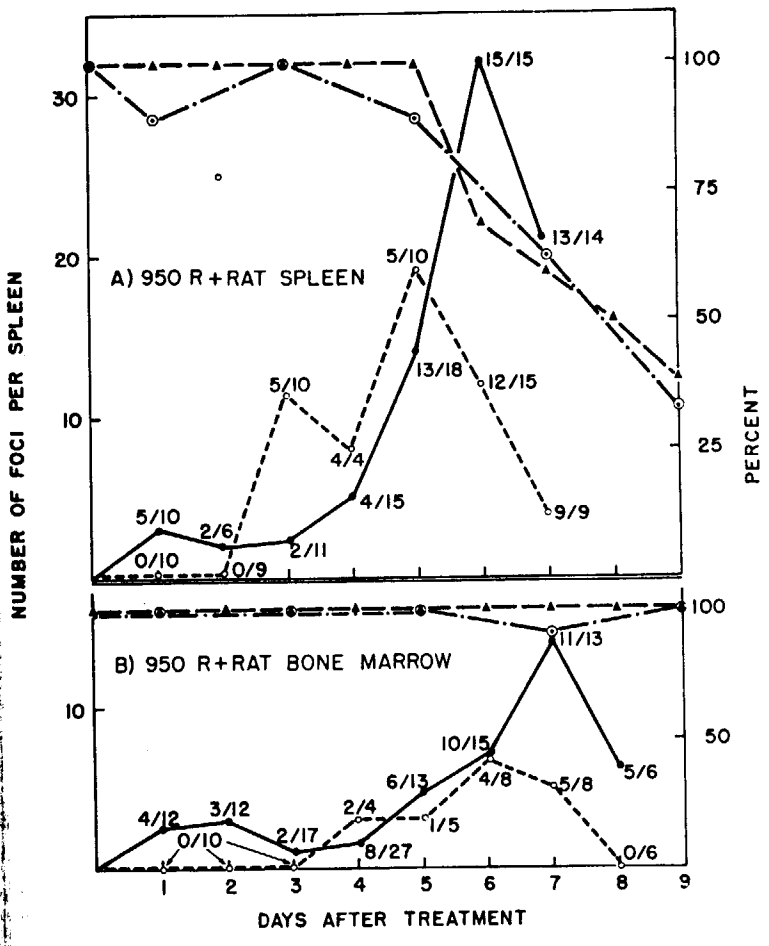


Fig. 30. Development of GvH hemolytic (red cell) and cytolytic (thymocyte) foci in spleens of lethally irradiated mice injected with either (A) rat spleen cells or (B) rat bone-marrow cells. Left ordinate: hemolytic foci, ●———●; cytolytic foci, o · · · · · · o. Right ordinate: survival, ▲——— ———▲; erythrocyte count relative to that of normal animals, ⊙— · — · —⊙. Numbers represent fraction of spleens tested showing hemolytic or cytolytic foci and the points plotted represent the mean number obtained from only the foci-bearing spleens.

concept of cellular immunity as the key factor in GvH disease. As yet, we are unable to assign any pathogenic significance to the humoral factors detected with this assay system, but the ability to localize reaction sites in GvH reactions should contribute to our understanding of the disease process. Furthermore, this technique should permit us to recognize the cells involved and to investigate the kinetics and interrelations of active antihost centers. The potential application to human transplant studies is obvious; thus, early and sensitive detection of a GvH reaction would aid in the clinical management of a patient with a graft.

Immune Status of Allogeneic Chimeras

Mice surviving the critical GvH-mediated secondary disease period, 3 to 4 weeks posttreatment, show a gradual recovery in weight, and finally in gross appearance are comparable to mice that had been grafted with isogeneic marrow. Studies in our laboratory, however, have demonstrated that these long-term allogeneic chimeras, in contrast to isogeneic chimeras, respond poorly to antigens (N. Gengozian and Elizabeth Allen). Ninety to 120 days after lethal irradiation and bone-marrow treatment, i.e., well beyond the secondary disease period, groups of mice received multiple injections of either rat or sheep RBC antigen. They were bled weekly from the retro-orbital sinus and serum samples were titrated for agglutinins before and after in vitro treatment with 2-mercaptoethanol (2-ME). This permitted determination of 2-ME sensitive or 2-ME resistant antibody, hereafter referred to as 19S and 7S antibody, respectively.

Two groups of B6D2F$_1$ → 1C3F1 chimeras were injected with either sheep or rat RBC (Fig. 31). With sheep RBC, the primary response was lower than the normal and isogeneic controls (see footnote Fig. 31), but 7S antibody was detected on the 14th day. The second injection resulted in an increased titer but it was not until the third injection that almost all the antibody was of 7S type. This is in contrast to the responses in normal and isogeneic chimeras where 7S antibody was detected 7 days after the primary injection and by the 35th day appeared to be the major antibody produced. The response of allogeneic chimeras to rat RBC was subnormal even after three injections.

The individual agglutinin responses of some allogeneic (B6D2F$_1$ → 1C3F1) chimeras to sheep and rat RBC are shown in Fig. 32. Also indicated is the maximum body-weight loss experienced by each during the critical period 3 to 7 weeks after treatment. The agglutinin curves show no correlation between severity of "secondary disease" and subsequent ability to respond to antigens. For example, the animal that had lost 7 grams showed early formation of 7S antibody and an overall greater response than the animal which had experienced only 1-gram loss. Also, the animal that had lost 9 grams yielded an almost completely normal primary response, both in terms of titer and conversion to 7S type of antibody; this is in contrast to the completely 19S primary antibody formation by the animal that had shown only a 1-gram weight loss. Among those injected with rat RBC, a very subnormal response was obtained in an animal that had escaped "secondary disease" as evidenced by no weight loss and also in an animal that showed a 9.5-gram weight loss.

This study extends our earlier work that indicated an abnormal immune mechanism operating in allogeneic radiation chimeras. The degree of abnormality revealed depends upon the test antigen used. With sheep RBC and S. typhosa a spectrum of completely normal to severely abnormal immune functions can be discerned; with rat RBC antigen, a normal response is never obtained, even after multiple injections. Our observation that animals that had escaped "secondary disease" may still be deficient immunologically emphasizes the fact that the absence

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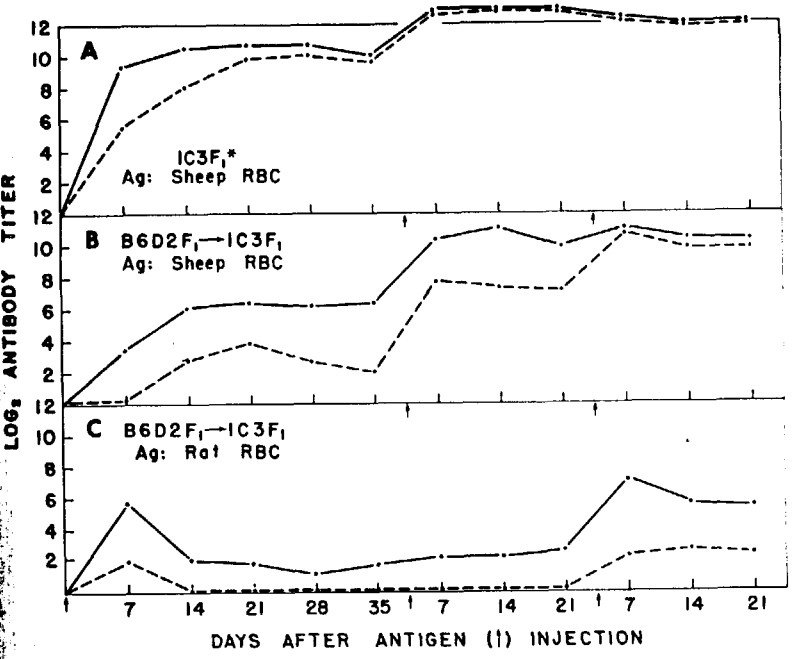


*Fig. 31. Agglutinin response to sheep and rat RBC in normal, unirradiated mice (A) and allogeneic (B,C) radiation chimeras. *Data in (A) are responses to sheep RBC in normal IC3F$_1$ mice; responses to rat and sheep RBC antigens are almost identical in this strain and compare also to the responses in IC3F$_1$ isogeneic chimeras. Solid line: total antibody titer; dashed line: antibody titer after 2-ME treatment of sera.*

of any gross sign of stress (e.g., weight loss) does not connote the absence of a basic physiologic lesion.

The abnormal immune functions among long-term allogeneic chimeras leads one to question the application of the concept of immunologic tolerance to these animals as defined by Medawar (Transplant. Bull. 4, 72, 1957). Evidence that "tolerance" in radiation chimeras (dogs) may be explained in another manner has been given recently by Hellstrom, et al. (Proc Nat Acad Sci 66, 65, 1970). Their results suggest the presence in chimeras of a serum factor (antibody?) that effectively inhibits or blocks the action of chimera (donor) lymphocytes against host-type fibroblasts. If this observation can be substantiated it will support our original concept for the subnormal immune capacity of allogeneic chimeras: "an in vivo antigen-antibody reaction persists in these animals and consequently affects their ability to respond normally to other antigens." (Gengozian, et al., Proc Nat Acad Sci 44, 560, 1958).

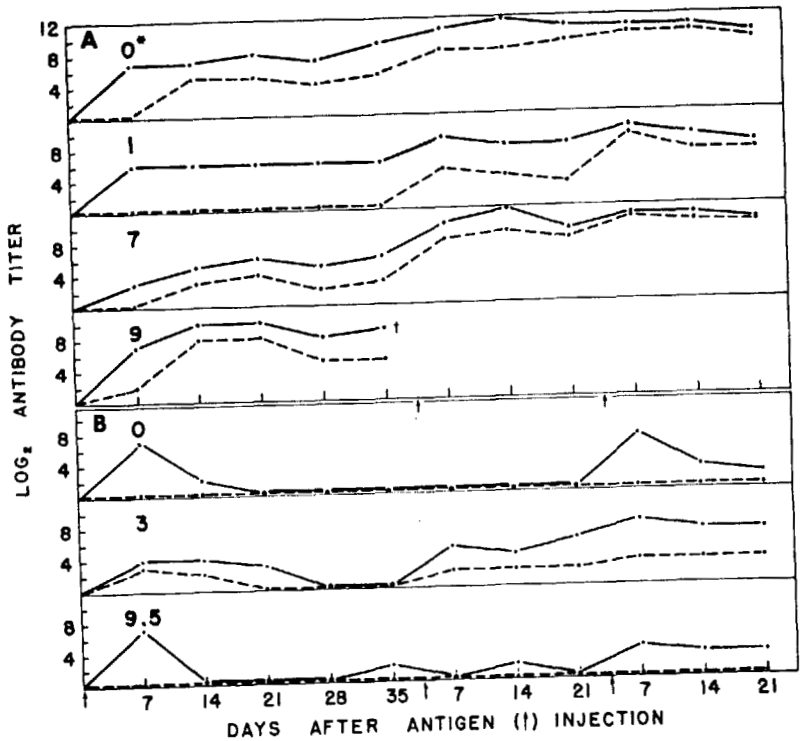


*Fig. 32. Antibody titers obtained in individual allogeneic radiation chimeras (B6D2F1 ⟶ IC3F1) after multiple injections of either sheep (A) or rat (B) RBC. *Numbers represent in grams the maximum weight loss (relative to pre-irradiation level) recorded for each animal from the third through the seventh week after irradiation and marrow injection. (Solid and dashed line, as in Fig. 31).*

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Experimental Hematology

Most of the patients in the Division suffer from leukemia, lymphoma, or related disorders and usually receive irradiation as part of their treatment. The studies in experimental hematology use radioactive cell-labels in characterizing changes in the hematopoietic organ after irradiation and also employ modern electronic cell sizing and counting apparatus for detecting end points for radiation effects. Some of the work that might be categorized as experimental hematology is reported in other sections; the reader is referred to parts of the report on Clinical Total-Body Irradiation, Radiation Immunology, and Basic Studies Related to Radiation, Neoplasia, and Metabolism.

Electronic Red Blood Cell Sizing

We continued to investigate the usefulness of electronic cell-sizing to clinical and experimental hematology (Dorothy Lushbaugh and C. C. Lushbaugh). Our studies can be classified as (1) clinical and experimental applications, and (2) experimental observations on the nature of the electronic two-peak red blood cell (RBC) population phenomenon. In clinical hematology the technique was used: (a) in routine diagnostic work-up of new patients to appraise the status of their erythron, and (b) during therapy of RBC diseases to gauge the rate of return of RBC populations to normal size distribution. Our observations on the change in RBC size distribution in polycythemia rubra vera (PRV) after radiation therapy were extended by studies of new patients and in long-term patients after repeated therapeutic trials over long periods. The results substantiate our tentative conclusions (reported last year, Research Report, 1969, ORAU-110) that remission induced in PRV by irradiation, and other methods of suppressing erythropoiesis, is accompanied by the production of new RBC of normal size. As these cells with a life expectancy of 120 days are delivered into the blood and old microcytic cells are removed, the initial abnormal sized RBC distribution changes progressively toward a normal one. Whether or not a completely normal size profile is obtained, however, depends on the duration of the period during which red cell production rate and iron stores are in balance; if iron deficiency is present (because of intestinal hemorrhage, for example) and goes untreated, iron stores are adequate for only a short time and production of microcytes soon begins and reverses the trend toward normal in the RBC size profile. In other blood dyscrasias with suppressed RBC production and iron stores normal or above, we find no abnormality in RBC size distribution and total-body radiation exposures are not followed by change in RBC size.

In experimental radiation therapy and hematology we used this technic to measure the macrocytosis of mice chronically irradiated at low dose rates where colonies of stem cells are formed in the spleen (see page 49) and abnormal hemoglobin is produced (see page 52). The macrocytic shift in RBC size distribution correlates well, temporally, with splenic stem-cell colony proliferation and abnormal hemoglobin production but not with splenic stem-cell colony formation without proliferation. This finding suggests that when these colonies proliferate under stress they form macrocytes containing abnormal hemoglobins. This correlation allows electronic RBC sizing to be used for selecting irradiated mice for biochemical hemoglobin assay.

Our electronic cell-sizing apparatus, appropriately set upper and lower analyzer gates, was also used to exclude agglutinated RBC masses from being counted in studies (see page 64) of the rate of replacement of an irradiated human recipient's own RBC by new RBC from transplanted bone marrow. Since the size distributions of the two RBC populations (donor versus recipient) differed, we could also follow the growth of the new RBC mass by obtaining sequential RBC size distribution studies. These seemed to correlate well with the findings of the in vitro hemagglutinin system, but were not as easily quantitated in percentages.

We continued our studies on the nature of the well-defined two-peaked distribution of electronic pulses generated from normal blood when the electrical current is high (>1.0 but < 3.0 microamps). We have justified our use of high currents in practice because it allows us to differentiate RBC of two or more different model sizes easier than lower currents do. Sometimes we wish to compare the RBC of two persons with different MCV's by mixing a sample from each and comparing the two-peaked size distribution visually; the presence of two bloods in the sample is more clearly demonstrable with a high current than with a small one.

While there are numerous explanations for the presence or absence of two peaks dependent upon current, none have been shown experimentally to hold true under all the conditions of our measurements. Most explanations are based on physical theory and mathematical solutions rather than direct experimentation. The latest purports to show that the two peaks result from tumbling of some of the saucer-shaped red blood cells in the flow through the sensing volume of the aperture tube. We have shown experimentally that this explanation is inadequate. When glutaraldehyde is buffered and in less than 2.5% concentration, it can fix RBC rapidly (within 15 minutes) without microscopically detectable alteration in their size or shape. With 4% formalin in saline similar RBC fixation without distortion is obtained in 18 hours. We sized unfixed RBC suspensions and those fixed in these two solutions during periods in which all the variable parameters of the electronic sizing system were kept constant. By the time the RBC membrane became "fixed" the two-peaked distribution was not obtained even though the fixed RBC's were still disc shaped and just as capable of tumbling as unfixed cells. Previously we have shown that ball-shaped solid cells like lymphocytes either living or fixed show the same volume or single-peaked size distribution with low and high aperture currents. We believe that the two-peaked RBC size distribution results from alterations in the amount of intracellular fluid in the RBC caused by an effect of the electrical current on the ionic charges of the living, pliable RBC membrane that affects the sodium-ion water transfer pump. The single direct experiment that proves our explanation, however, continues to elude us. We attempted to gain more clues by using the well-known progressive chemical alterations that occur in blood-bank blood stored in acid citrate dextrose solution. Here the selective permeability of the RBC membrane decreases progressively and transport of substances across it depend finally only on Donnan state of equilibrium. The amount of intra-RBC sodium rises progressively over a 30-day period and mean corpuscular volume increases as the RBC swell. We obtained electronic RBC size distributions of blood from nine patients with PRV drawn into ACD at intervals over 30- to 70-day periods of storage at 4° C (Fig. 33). While the changes observed in cell volume were progressive, they were not linear with time, being slow at first and gradually becoming more rapid. These studies answered a practical question for us: How soon after a blood sample is drawn from a patient must the RBC sample be sized if in vitro changes are to be avoided? Sizes or distribution in the specimens did not change significantly during the first 5 days, but after that, all the RBC size distributions shifted progressively toward the right as the cells swelled. In Fig. 33 the squared difference index for the consecutively determined RBC size distributions of these bloods are shown. The index of zero can be assigned to each bottle of blood at the beginning of each experiment because the initial size distribution of each blood served as its own reference point for comparison with subsequent measurements of the same (aging) sample. On comparing the repeated frequency distribution histograms of these samples with the originals one sees that the higher peak comprised of the smaller cells in the initial RBC distribution became progressively lower, broader, and shifted further to the right (large size), finally merging with the original low broad peak of large RBC. These changes logically fit our hypothesis: that passage through electrical current alters the cell membrane charge so that sodium and water leave the cell, making the cell shrink; that small cells with relatively little water and sodium show the greatest proportional shrinkage induced by electrical current while large cells

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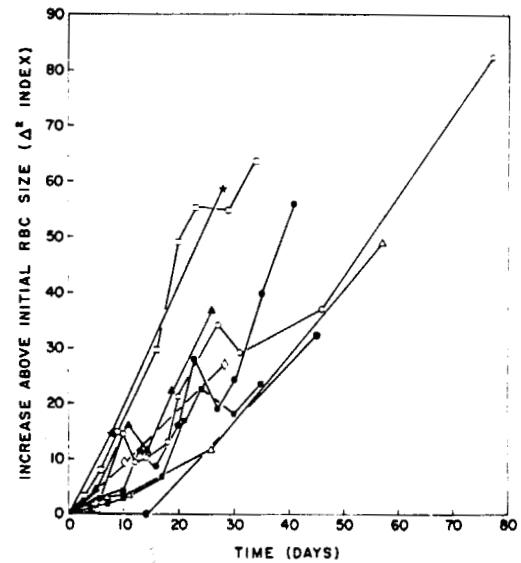


Fig. 33. Graph showing the rates of increase in erythrocyte sizes in blood stored in ACD at 40° F for 30 to 75 days. The blood was obtained by phlebotomy during the course of therapy of nine patients with polycythemia. Initially all were microcytic. The increase in size is expressed by the squared difference index, a statistical measurement of the degree of difference between the initial and each new measurement.

swollen with water and comparatively rich in sodium are decreased least in relative size. Because passage time through the high density current in the aperture tube is constant, only a constant amount of sodium and water can be removed per passage. This is relatively faster and is a greater percentage of the total present initially in small dense cells. After blood bank storage RBC imbibe sodium and water when their ability to glycolyze fails in parallel with a fall in high energy phosphates; the amount imbibed is limited only by the cell membrane and all cells of the same individual swell toward this limit, hemolyzing when it is exceeded. Consequently the RBC remaining in the bottle become more alike in sodium and water content and size; the proportional effect of the current upon the cells decreases and the differentation of the RBC size into two peaks of distribution becomes progressively poorer.

These studies suggest that electronic RBC sizing might be useful in studying the pliability of RBC membranes and their hemolytic fragility as well as variations in size of individuals in total RBC population variations. In addition it would seem feasible to use this system to rapidly detect undesirable changes in normal blood during blood-bank storage. This might have an advantage over other methods because of its simplicity and the rapidity with which results can be interpreted.

Spontaneous Cyclic Leukocytosis and Thrombocytosis in Chronic Granulocytic Leukemia

Since our previous report in the 1968 Research Report, ORAU-107, we have been able to observe another patient with chronic granulocytic leukemia who has had spontaneous oscillations of her leukocyte and platelet count over a period of 18 months. (Helen Vodopick, Elizabeth Rupp, C. L. Edwards, Francis A. Goswitz, and J. Beauchamp*).

This patient, a 68-year-old woman, had received only one course of treatment, a single dose of splenic irradiation (710 rads) in October 1968. Her white blood cell count and platelet count had fallen to normal values with this treatment. A gradual recurrence of the leukocytosis and thrombocytosis developed over the next 4 months. When the white blood cell value had reached 100,000/cu mm, additional treatment was planned. Before treatment was started, a spontaneous drop in the white blood cell count to 20,000/cu mm occurred. Further observation showed regular oscillations of the leukocyte and platelet count with a periodicity of 56 days (Fig. 34). The amplitude of these oscillations increased with each consecutive cycle although the values at nadir remained about the same. Granulocyte and platelet cycles were asynchronous although of the same periodicity. Anemia was not a major problem; however, she did require 3 units of packed red blood cells during these 18 months. When the white blood cell count rose to over 200,000/cu mm, busulfan was begun because of the patient's increasing symptoms of weakness, fatigue, and anorexia. This drug was able to completely shut off the cyclic phenomena. However, with discontinuation of the drug, oscillations of the white blood cell count and platelet count recurred.

We had the opportunity to perform leukokinetic studies using an in vitro technic to label the granulocytes with ^{32}P-diisopropylfluorophosphate. The first study was done before she had received any treatment for her chronic granulocytic leukemia. The second study was done at the peak of one of the leukocyte cycles and the third at the nadir of a cycle (Fig. 35). No evidence for accelerated destruction of granulocytes could be found to explain the oscillations of the white blood cell count.

Computer analysis of these data confirmed the identical periodicity of the granulocyte and platelet changes as well as the asynchrony of the phases. The oscillations did follow a true sine wave with what appeared to be an antidamping effect on the oscillations since each consecutive cycle increased in amplitude.

Plasmaphoresis performed at various times during the course of her illness will provide material with which to determine whether a humoral substance controlling granulocyte and platelet production does exist in her plasma and, hopefully, to identify it.

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* ORNL Statistics Department.

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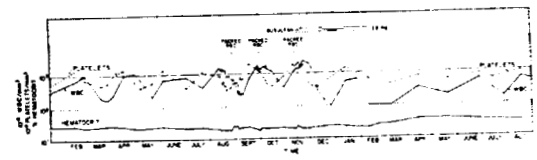


Fig. 34. Spontaneous cyclic changes of the leukocytes and platelets of a patient with chronic granulocytic leukemia. The periodicity of the cycles was 56 days but the leukocyte and platelet oscillations were asynchronous. After the cycles were stopped temporarily with busulfan, spontaneous oscillations again occurred.

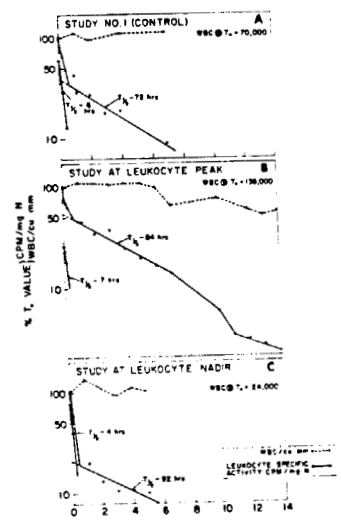


Fig. 35. Leukokinetic studies at various times during the patient's granulocyte oscillations. Granulocytes were labeled with ^{32}P-diisopropylfluorophosphate.

RESEARCH IN NUCLEAR MEDICINE

The mission of this group is to develop radioisotopic agents that can be used for diagnosis, principally in scanning procedures, but also for other in vivo and in vitro clinical tests. No less important, but presently less promising, is the goal of developing effective therapeutic radiopharmaceuticals. In recent years our effort has moved through several phases, including bone-marrow scanning, skeletal scanning, and tumor scanning. The much needed improvement in reticuloendothelial labels has not yet been achieved. We have deferred major attention to this topic because of the high priorities placed on answering the many questions about gallium-67 in tumor scanning. This latter activity grew out of our search for better bone-scanning agents using isotopes of gallium.

Summary of Accomplishments

In animal experiments further progress was made in identifying factors that influence the distribution of gallium-67 in tissue. This has now been investigated in 22 different types of animal tumors. The Morris minimal deviation hepatoma transplanted in the Buffalo rat has thus far shown the greatest absolute and relative affinity for gallium-67. Concentration in hepatoma cells exceeded that in most normal cells, but phagocytes in the stroma of the tumor often had even higher concentrations. Reducing the amount of citrate by a factor of 100 had no effect on tissue distribution, but if it was reduced by a factor of 1000, some significant differences occurred; therefore, the presence of some citrate (~0.1 mg per kg) may be desirable for optimum concentrations of gallium-67 in tumor. In young animals, the distribution in males and females of equal age differs and preliminary evidence suggests differences dependent upon animal strain. To test the influence of oral administration of stable gallium, rats were given daily oral doses of 5 mg before, and for 12 days after, intravenous gallium-67. No differences in whole-body retention or fecal excretion were detected. We have extended our comparisons of gallium with other tumor-scanning agents in experimental animals and in general find the concentration of gallium superior to other agents.

We have pursued the observation that low levels of scandium enhance the rapid clearance of gallium from soft tissues, thereby enhancing the tumor-to-nontumor ratios. The effect seems to be general in both rats and mice at early time periods, but at 24 hrs it is not present in mice. The optimum dose is approximately 0.5 mg of scandium per kilo and its effect is seen when given as much as 2 hours before the gallium-67. The toxicity of scandium has been studied in rabbits at doses up to 135 mg of scandium per kilogram.

We continued to search for the sites of gallium-67 uptake in the cell, using homogenates of transplanted RFT rat tumors. The gallium is identified in a macromolecular complex which is being characterized in detail; it has a molecular weight of approximately 80,000. In cell fractions obtained with the zonal centrifuge, the isotope-binding particle is a small electron-dense single-membrane organelle classified as 'lysosome-like' and having acid phosphatase activity.

During the year, the Cooperative Group to Study Localization of Radiopharmaceuticals was constituted, its members predominately representatives from ORAU member schools. The initial objective of the group is to study gallium-67 as a tumor-localizing agent, and a first protocol apparently will be supported jointly by the NCI and AEC.

In human studies, our experience has been extended to approximately 165 intravenous doses of ^{67}Ga given to 116 patients. Gallium does not localize in malignant tissue in general, but rather in tumors of specific cellular types; it appears to vary in affinity from one type of tumor to another and indeed from site to site in a given patient. Patients with positive scans have reverted to negative after chemotherapy or radiation leading us to consider the possible clinical use of gallium in assessing the effectiveness of therapy. The size of the tumor is an important factor in detectability. Small tumors may escape detection, particularly if deeply located and not precisely at the focal plane of the detector. After preliminary trials in dogs the intralymphatic injection of ^{67}Ga was tested in four patients; the results suggested that there is no advantage to injecting the nuclide by this route even to demonstrate tumors along the draining lymphatic vessels.

We studied the distribution of gallium in human tissues at autopsy and have detailed information for calculating the radiation dose to the tissues, analyzed according to time after dose, age, sex, nutritional state, tumor localization, presence of inflammation, and the amount of stable gallium carrier. Among the various normal tissues, the five with the highest values are the spleen, kidney cortex, adrenal, marrow, and liver. Important from the standpoint of radiation dosimetry is the fact that the concentration varies considerably from patient to patient and in different portions of the same organ.

Work continued this year on the turnover of cobalt metal in guinea pigs, a species susceptable to cobalt ion toxicity. Results of the study using whole-body counting followed by necropsy has led to a working hypothesis that cobalt toxicity is due to an initial binding of inorganic cobalt by the thyroid and brain during blood - interstitial equilibrium.

In medical instrumentation, a number of electronic devices have been interfaced with our computers. Our computer resource has also been expanded during the year through creation of new programs to increase our capabilities for processing, storing, retrieving, and automatically graphing of data.

Radiopharmaceutical Development

Factors Influencing the Tissue Distribution of Gallium

The distribution of ^{67}Ga has now been investigated in a total of 22 different types of rat, hamster, and mouse tumors (R. L. Hayes, Bill Nelson, B. L. Byrd, and J. E. Carlton). Table 20 shows selected data on those not previously reported (18,31). The RFT tumor in the Fischer 344 rat is included for purposes of comparison, since this particular transplanted tumor has been used as a model in many of our studies. Tumors in rats have shown by far the greatest affinity for ^{67}Ga while those in mice have shown the poorest with the hamster tumors intermediate. (Consult footnote to

1027932

Table 20 when comparing tumor concentrations.) The concentration in the mouse tumors relative to that in normal tissues was also lower than that in the rat and hamster.

Microscopic autoradiograms of Fischer 344 rats with transplanted RFT tumors showed fairly uniform ^{67}Ga activity in viable tumor cells, but small concentrations of considerably greater activity were attributable to scattered macrophages in the tumor, especially in degenerating areas. Similar foci of greater concentration were found in

TABLE 20

Tissue Distribution of ^{67}Ga in Animals Bearing Experimental Tumors*

Tumor Type	Tumor Concentration	Ratio of Tumor Concentration to that in:			
	%/g†	Blood	Muscle	Liver	Spleen
	RAT				
RFT, poorly differentiated malignancy	4.2	19.0	20.0	4.3	3.0
MMD 7794A hepatoma	3.6	13.0	130.0	4.6	3.5
MMD 7777 hepatoma	5.6	33.0	39.0	6.1	3.9
MMD 5123C hepatoma	10.0	92.0	110.0	14.0	9.2
	HAMSTER				
Adenovirus 31	2.9	28.0	83.0	2.0	2.3
SV-40	3.9	20.0	15.0	3.8	3.6
	MOUSE				
SaI, spindle cell sarcoma	8.2	3.9	20.0	1.0	1.7
Ehrlich ascites cells	3.6	1.6	2.9	1.1	1.6
C3HBA, mammary adenocarcinoma	5.4	2.0	9.2	0.8	0.9
BW10139, rhabdomyosarcoma	6.2	3.8	15.0	1.8	1.6
dbrB, anaplastic carcinoma	6.7	3.5	12.0	1.2	2.0
SaD2, fibrosarcoma	14.2	7.9	35.0	2.6	3.0
S37, pleomorphic sarcoma	5.7	3.0	14.0	1.0	1.3
HP, Harding-Passey melanoma	13.0	5.5	24.0	1.5	3.3
6C3HED, lymphosarcoma	3.8	9.8	3.3	2.0	5.1
BW7756 hepatoma	6.4	6.6	18.0	0.6	0.4

* Distribution 24 hr after iv administration, except for adenovirus 31 (66 hr).

† Percent administered dose/g normalized to body weight of 250 g for the rat, 100 g for the hamster, and 25 g for the mouse.

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reticuloendothelial (RE) cells in the liver and spleen. Marrow from the rat femur also showed focal activity corresponding to the distribution of RE cells and to the presence of much stainable iron. The kidney had a localization of gallium in the outer cortex that was less intense than that seen in mice (see page 162) and unlike the mouse the rat kidney had no stainable iron.

Of the tumors studied, the 5123C Morris minimal deviation (MMD) hepatoma transplanted in the Buffalo rat has thus far shown the greatest absolute and relative affinity for ^{67}Ga (Table 20). Histologically the MMD 7794A, as carried in our laboratory, resembles normal liver more closely than do the MMD 5123C and the MMD 7777 which grow more rapidly and are not so well differentiated. In a comparison distribution study (not shown in Table 20), where the sizes of these three tumors were controlled, there was a significantly greater concentration in one of the faster growing tumors, MMD 5123, (8.0 ± 0.4%/g) than in the other two. Despite the differences in growth rate and histology, no significant difference was found between the tumor concentration in the MMD 7794A (5.7 ± 0.5%/g) and that in the MMD 7777 (4.5 ± 0.4%/g).

These three hepatomas have also been studied autoradiographically. The distribution in normal tissues of Buffalo rats with MMD hepatoma transplants (Fig. 36) was similar to that seen in Fischer 344 rats with RFT transplants. Concentration in hepatoma cells was higher than that in most normal cells, but phagocytes in the stroma of the tumor often had an even higher concentration (Fig. 37). In tumors with necrosis or fibrosis associated with many macrophages, the total amount in such cells exceeded that in neoplastic cells.

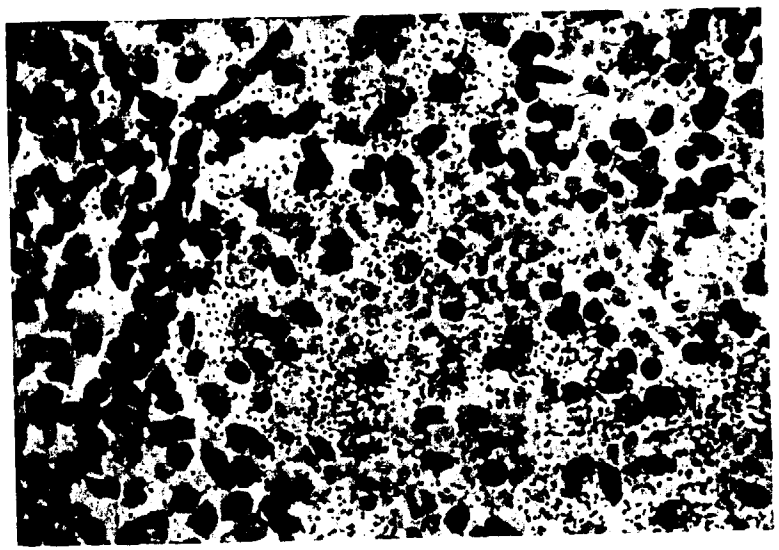


Fig. 36. Autoradiogram (ARG) of spleen of Buffalo rat given 3.5 mCi ^{67}Ga 24 hr previously showing grains in clusters corresponding to histiocytes in the red pulp (on right). Few grains are seen over the white pulp (left).



Fig. 37. ARG of transplanted Morris minimal deviation hepatoma 5123C (same rat and same exposure time as in Fig. 36, showing many grains over tumor cells, and more intense foci corresponding to benign histiocytes in the fibrous stroma (lower right).

Gallium was administered intravenously as the citrate in the original early studies of the distribution of ^{72}Ga in humans because of its stability and solubility at physiologic pH in that form (Radiology 61, 570, 1953). It was in turn convenient to use the citrate form in initial studies of the distribution of ^{67}Ga in humans, since this facilitated adjustment of pH and provided assurance that the preparation was not colloidal. An arbitrary level of 7 mg citrate/kg of body weight was initially used.

To determine whether the level of citrate administered had any effect on the localization of ^{67}Ga in tumor and normal tissues, studies were carried out with Fischer 344 rats bearing RFT tumors. No significant difference in ^{67}Ga tissue distribution was observed when the citrate dose was reduced by a factor of 100. In a second study using Buffalo rats bearing MMD 7777 hepatomas, the citrate dose was reduced by a factor of 1000 as well as by a factor of 100. When compared to the tissue distribution at the 7 mg/kg level, some significant differences in tissue concentration were obtained with lower citrate doses in the hepatoma rats (Table 21). At the 0.07 mg/kg citrate level the liver ($P<0.001$) and spleen ($P<0.02$) concentrations were increased while at the 0.007 mg/kg level, in addition to the liver ($P<0.001$) and spleen ($P<0.001$) concentrations, those of the kidney ($P<0.001$), femur ($P<0.01$) and blood ($P<0.01$) were also elevated. As with the RFT study, there was no significant difference between the tumor concentration obtained at the 7 and 0.07 mg/kg citrate levels. At the 0.007 mg/kg level, however, some significant ($P \sim 0.05$) increase in tumor concentration may have occurred.

Tumor to nontumor concentration ratios provide a means for assessing the basic utility of a radiopharmaceutical for tumor visualization by external scanning techniques.

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TABLE 21

Effect of Amount of Citrate on 24-Hour Tissue Distribution of ^{67}Ga in Buffalo Rats Bearing MMD 7777 Hepatomas.

Tissue	Amount Citrate Administered (mg/kg) 7×10^{0}	7×10^{-2}	7×10^{-3}
	% Administered ^{67}Ga/g Tissue*		
Liver	0.86 ± 0.05†	1.21 ± 0.05	1.65 ± 0.06
Spleen	1.69 ± 0.11	2.17 ± 0.11	2.71 ± 0.16
Kidney	0.79 ± 0.04	0.88 ± 0.05	1.19 ± 0.07
Lung	0.51 ± 0.10	0.36 ± 0.02	0.72 ± 0.20
Muscle	0.16 ± 0.02	0.13 ± 0.03	0.23 ± 0.03
Femur	0.88 ± 0.06	0.98 ± 0.14	1.34 ± 0.11
Femoral marrow	1.56 ± 0.12	1.46 ± 0.08	1.78 ± 0.26
Blood	0.22 ± 0.02	0.23 ± 0.02	0.31 ± 0.02
Tumor	6.52 ± 0.36	5.89 ± 0.60	8.69 ± 0.84
	Ratio Tumor Concentration to that in:		
Liver	7.7 ± 0.6	4.8 ± 0.4	5.3 ± 0.6
Spleen	3.9 ± 0.3	2.8 ± 0.3	3.3 ± 0.4
Kidney	8.3 ± 0.7	6.6 ± 0.4	7.3 ± 0.6
Lung	14.8 ± 2.9	16.6 ± 2.1	16.2 ± 3.9
Muscle	42.5 ± 3.9	48.1 ± 6.1	39.4 ± 4.0
Femur	7.6 ± 0.6	6.2 ± 0.5	6.5 ± 0.4
Femoral marrow	4.3 ± 0.5	4.1 ± 0.5	5.4 ± 0.9
Blood	29.5 ± 2.1	24.9 ± 1.0	27.8 ± 2.1

* Normalized to 250 g body weight.

† Standard deviation of the mean; 5 animals per group.

The lower portion of Table 21 shows such ratios for this study. The averages were obtained from individual ratios, each animal in effect serving as its own control. Using this treatment of the data, there is somewhat less significance attached to the differences between the 7 mg/kg and 0.07 mg/kg citrate levels for the tumor to liver and tumor to spleen concentration ratios ($P<0.01$ and $P<0.05$). At the 0.007 mg level only the tumor to liver ratio was significantly different from that at the 7 mg/kg level and at a considerably lower degree of confidence ($P<0.02$).

It thus appears from these animal studies of the effect of citrate dose on ^{67}Ga distribution that the presence of some citrate (~ 0.1 mg/kg) in the administered preparation may be desirable for the achievement of optimum ^{67}Ga tumor concentrations relative to those in the liver and spleen.

A number of factors such as age and amount of stable gallium administered have been found to influence the tissue distribution of gallium. Recently, studies in rats have shown that its distribution is also affected by sex of the animal. Groups of young males and females of equal age were employed. To compensate for differences in weight between the groups, concentrations were normalized to body weights of 250 g. Selected results for Fischer 344, COBS and Buffalo rats are shown in Fig. 38. Differences between males and females in ^{67}Ga concentration in these tissues were highly significant for the Fischer 344 and Buffalo rats ($P<0.001$) and somewhat less so for the COBS rats ($P<0.02$). Although these studies were not designed to test for differences between strains, it is apparent that such differences may exist.

When ^{67}Ga is administered to rats as citrate, the presence or absence of stable gallium carrier has a profound effect on the amount excreted and route of excretion, stable gallium favoring increased total excretion predominantly via the urine as reported by Bruner, et al (Radiology 61, 602, 1953). Excretion of ^{67}Ga in the feces has, on occasion, made difficult the interpretation of scans in patients even though they were given daily laxatives and an enema just before scans. It was felt that this undue retention in the intestinal tract might be due to ^{67}Ga binding to the surface of

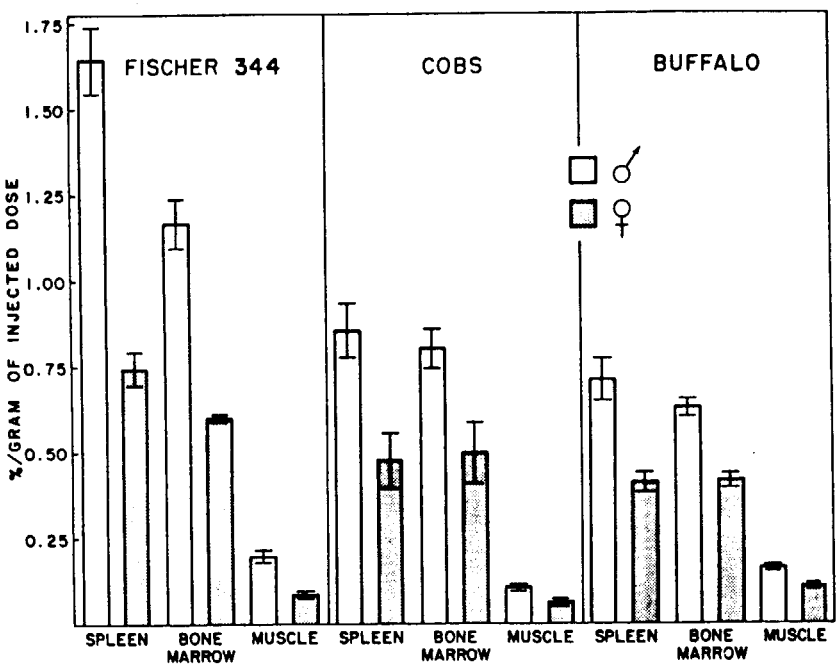


Fig. 38. Spleen, bone marrow, and muscle concentrations of ^{67}Ga (24 hr) in the male and female of Fischer 344, COBS and Buffalo rats. The ages of the male and female for each strain were the same (± 1 wk); concentrations were normalized to a body weight of 250 g.

1027935

the mucosa and that the oral administration of stable gallium (unabsorbed from the tract) might speed passage of activity through the tract. Buffalo rats were accordingly given daily oral doses of 5 mg stable gallium (as citrate) both before and for 12 days after intravenous administration of ^{67}Ga. No difference in whole-body retention or fecal excretion was detected between this treated group and control animals.

Comparison of ^{67}Ga with Other Tumor-Scanning Agents

We have expanded our initial comparison of ^{67}Ga with several other tumor-scanning agents in Fischer 344 rats bearing RFT tumors to include similar studies in the P-1798 mouse lymphosarcoma and the MMD 5123C rat hepatoma (R. L. Hayes, B. L. Byrd, and J. E. Carlton). The series includes comparisons of the affinity of tumors for ^{67}Ga with those for ^{75}Se-selenomethionine, ^{75}Se selenite, ^{131}I-(^{125}I)-albumin, ^{203}Hg-mercuric chloride, ^{206}Bi-citrate and ^{111}In(^{114m}In)-citrate. **Table 22 shows** a portion of the results. In general, except for ^{206}Bi, the concentration of ^{67}Ga was superior to that of these other tumor-scanning agents in absolute and relative (compared to other tissues) terms. In the P-1798 lymphosarcoma mice, except for the tumor to kidney ratio, somewhat higher tumor to nontumor ratios were obtained with ^{206}Bi than with ^{67}Ga; in the MMD 5123C hepatoma rats and the RFT tumor rats, the ratios were similar (kidney again excepted). The absolute tumor concentrations for ^{67}Ga and ^{206}Bi were similar in the P-1798 lymphosarcoma mice while the concentrations obtained with ^{67}Ga in the MMD 5123C and RFT rats were far superior to those obtained with ^{206}Bi. The very low tumor to kidney ratios seen with ^{206}Bi in all three types of tumor animals reflect its early preferential concentration in the kidney. Most of the gamma rays associated with the decay of ^{206}Bi are above 800 kev, while those emitted by ^{67}Ga are much lower and consequently of more utility in scanning procedures.

The Effect of Scandium on the Tissue Distribution of Gallium

The simultaneous administration of low levels of scandium (0.5 mg/kg) with ^{67}Ga results in the rapid clearance (4 hr) of ^{67}Ga from soft tissues other than kidney. This action is similar to the carrier effect of stable gallium. However, unlike stable gallium, scandium does not affect the concentration of ^{67}Ga by RFT tumor tissue. This observation has raised the interesting possibility of a method for enhancing tumor to nontumor tissue ratios and the potential use of the short half-life nuclides of gallium, i.e. ^{68}Ga (T 1/2 = 68 min) and ^{66}Ga (T 1/2 = 9.5 hr); clearance of ^{67}Ga from normal tissues sufficient for tumor scanning usually requires one day or more.

To determine whether this effect of scandium on gallium tissue distribution in the RFT tumor rat was of a general nature, rather than specific to that particular tumor animal, we studied one other tumor rat (MMD 7777 hepatoma) and three other tumor mouse strains (P-1798 lymphosarcoma, SaI spindle cell sarcoma, and SaD2 fibrosarcoma) at 24 hr as well as at 4 hr (R. L. Hayes, Bill Nelson, B. L. Byrd, and J. E. Carlton). A portion of the data is shown in Table 23. With MMD 7777 hepatoma rats at 4 hr we obtained results similar to those in the RFT tumor rat; both tumor rats showed greatly enhanced tumor-to-nontumor ratios at 24 hr. The tumor mice at 4 hr exhibited less marked effects while at 24 hr tumor-to-nontumor were not enhanced significantly. The scandium effect thus seems to be general in both rats and mice with tumors at early time periods, but not at 24 hr for mice.

With whole-body retention studies, in Fischer 344 rats (host for RFT tumor) and CD_2F_1 mice (host for P-1798 lymphosarcoma) scandium (0.5 mg Sc/kg) dramatically increases ^{67}Ga excretion during the first few hours after administration (**Fig. 39**). The differences in whole-body retention between scandium-treated animals and controls persist through 120 hr. This effect presumably accounts, at least

TABLE 22

COMPARISON OF ^{67}GA WITH OTHER TUMOR-SCANNING AGENTS

IN THREE DIFFERENT TUMOR-BEARING ANIMALS

Agent	Tumor Concentration %/g*	Ratio of Tumor Concentration to that in: Blood	Muscle	Liver	Kidney
	P-1798 MOUSE LYMPHOSARCOMA				
^{67}Ga	5.6	6.5	10.0	1.3	1.4
^{75}Se-selenite	2.9	0.9	3.9	0.3	0.2
^{75}Se-selenomethionine	3.8	1.6	2.5	0.4	0.3
^{131}I-albumin	2.7	0.4	3.4	2.1	1.3
$^{203}HgCl_2$	3.0	1.8	6.4	0.6	0.03
^{206}Bi	4.4	23.0	34.0	3.4	0.07
^{67}Ga†	7.1	39.0	75.0	1.8	1.9
^{111}In†	5.0	14.0	5.4	0.9	0.3
	MMD-5123C RAT HEPATOMA				
^{67}Ga	10.0	66.0	94.0	15.0	14.0
^{75}Se-selenite	0.8	1.0	9.6	0.7	0.2
^{131}I-albumin	0.8	0.5	8.6	3.1	1.5
$^{203}HgCl_2$	1.7	3.8	26.0	1.0	0.04
^{206}Bi	0.5	48.0	82.0	2.7	0.04
^{67}Ga	5.0	120.0	180.0	16.0	12.0
^{75}Se-selenomethionine	1.8	3.0	21.0	2.6	0.7
^{114m}In	2.2	6.4	26.0	3.3	1.2
	RFT RAT TUMOR				
^{67}Ga	4.9	16.0	24.0	4.7	4.8
^{75}Se-selenomethionine	0.6	1.2	3.4	0.5	0.2
^{125}I-albumin	0.3	0.2	3.2	1.3	0.6
^{67}Ga	2.8	19.0	20.0	3.4	4.5
^{75}Se-selenite	0.3	0.4	2.7	0.3	0.08
$^{203}HgCl_2$	0.8	3.7	13.0	1.0	0.03
^{206}Bi	0.4	47.0	55.0	2.6	0.02
^{67}Ga†	4.8	110.0	33.0	6.6	5.3
^{111}In†	0.7	3.9	3.2	0.5	0.2

* Percent administered dose/g normalized to body weight of 250 g for rat and 25 g for mouse.

† Three day studies; other studies one day.

1027936

in part, for the enhanced tumor-to-nontumor ratios obtained with scandium. Why there is little enhancement in the tumor mice at 24 hours is, however, unclear.

Scandium maintains its effect on gallium tissue distribution when administered as much as 2 hours before ^{67}Ga. Its optimum dose in the rat is approximately 0.5 mg Sc/kg. Higher doses do not produce further enhancement. At 0.25 mg Sc/kg, the effects of scandium were much less marked.

To determine whether the microscopic deposition pattern of ^{67}Ga had been affected by scandium treatment, autoradiograms of tissues from Buffalo rats bearing MMD 7777 and MMD 5123C hepatomas were prepared. Although, as expected, fewer silver grains were seen over the normal tissue sections from scandium-treated animals, compared to those seen over control tissue sections,

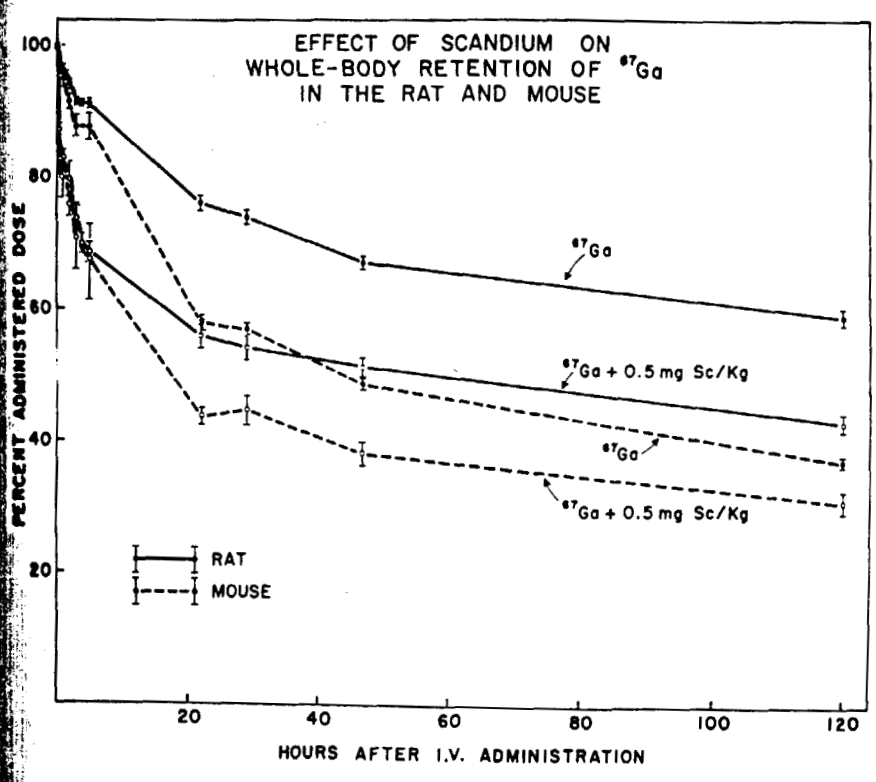


Fig. 39. The effect of scandium (as citrate) on the whole-body retention of intravenously administered ^{67}Ga in the CD_2F_1 male mouse and Fischer 344 male rat. Each point is the average of five animals.

TABLE 23

EFFECT OF SCANDIUM ON THE DISTRIBUTION OF ^{67}Ga IN TUMOR-BEARING RATS AND MICE

	Tumor Type									
	MMD 7777		RFT		P-1798		SaI		SaD2	
	Rat		Rat		Mouse		Mouse		Mouse	
Scandium (mg/kg)	0.0	0.5	0.0	0.5	0.0	0.5	0.0	0.5	0.0	0.5
					4-Hour Distribution					
Tumor Concentration (%/g)*	2.8	5.9	2.3	2.8	6.7	10.1	5.9	5.6	8.6	10.4
Ratio Tumor Concentration to:										
Liver	5.0	31.0	2.7	16.0	1.7	8.6	1.4	2.3	1.8	4.0
Spleen	3.8	36.0	2.5	23.0	2.0	11.0	1.5	3.0	1.6	4.3
Muscle	17.0	210.0	9.8	110.0	9.2	120.0	7.3	23.0	7.2	39.0
Blood	2.6	18.0	1.4	12.0	1.4	5.7	0.7	0.9	0.9	2.8
					24-Hour Distribution					
Tumor Concentration (%/g)*	5.8	7.7	3.5	3.2	6.0	4.7	8.4	6.0	13.0	12.0
Ratio Tumor Concentration to:										
Liver	7.3	34.0	3.7	11.0	1.2	2.1	1.3	1.2	2.0	2.1
Spleen	5.0	37.0	2.7	12.0	1.3	1.8	1.7	1.7	3.0	2.3
Muscle	34.0	470.0	21.0	170.0	11.0	34.0	18.0	30.0	43.0	76.0
Blood	33.0	84.0	14.0	26.0	6.6	8.4	3.6	3.3	5.8	6.4

* Percent administered dose/g normalized to body of 250 g for rat and 25 g for mouse.

the pattern of distribution over the various cell types was unaltered. The distribution of silver grains over sections of tumor from treated and control rats was similar in cellular pattern and number.

Toxicity of Scandium

We are studying the toxicity of intravenously administered scandium in the New Zealand rabbit to determine the feasibility of using it with ^{67}Ga and ^{68}Ga (T 1/2 = 68 min) in humans to improve early scans by enhancing the concentration in tumor relative to that in other tissues (R. L. Hayes, Bill Nelson, and J. J. Rafter). Sixteen rabbits were given weekly and twice weekly doses of 5 mg Sc/kg (10 times the effective dose for enhancing tumor-nontumor ratios of ^{67}Ga in the rat). One rabbit died during the treatment period at a cumulative dose of 95 mg Sc/kg. At termination of scandium treatment, the cumulative doses covered a range of 50 to 135 mg Sc/kg. Two deaths (90 and 105 mg Sc/kg) occurred approximately one month after dosing was ended; during the next two years of observation, two more deaths (50 and 90 mg Sc/kg were observed. At the end of the treatment period four rabbits were killed for histologic surveys (cumulative doses of 65, 70, 95, and 125 mg Sc/kg). The

1027937

other animals are still alive (doses of 50, 90, 90, 95, 105, and 135 mg Sc/kg) except for one rabbit (90 mg Sc/kg) lost after iliac-marrow aspiration approximately 1-1/2 years after termination of scandium administration. All the rabbits became anemic as indicated by hematocrits. Some recovery in hematocrits occurred after treatment ceased. At approximately 1-1/2 years posttreatment, platelet and white blood cell counts were normal when compared to control rabbits of the same age; red cell counts were slightly depressed. Plasma iron and iron-binding capacities and several other types of standard blood chemical tests showed no obvious abnormalities.

Splenic enlargement was consistently found at autopsy. Histologic sections of spleens showed much finely granular gray material, unstained by hemotoxylin or eosin; it distended the cytoplasm of large macrophages, and in the spleens of rabbits with larger doses many masses exceeded the size of any normal spleen cell. Some of these huge masses were associated with multinucleated macrophages. Such foreign-body giant cells also appeared in the reticuloendothelial system elsewhere, although smaller macrophages were more numerous. Even though the concentration was highest in the spleen, the largest quantity of the foreign material was undoubtedly stored in the liver. The material present in Kupffer cells bulged into the sinuses and similar material was conspicuous in macrophages of the portal connective tissue. It was also abundant in the marrow and was readily detected in occasional macrophages in the interstitial tissue of the lungs and in the medulla of the adrenals. Rare macrophages with the material were seen in other organs such as kidney.

A search for toxic lesions and for an explanation for anemia gave no definitive answers: No ulcers or other gastrointestinal lesions were found and no hemorrhage was apparent. In one rabbit (95 mg Sc/kg) patchy areas of early myocardial necrosis were associated with a slight inflammatory cell reaction. A few macrophages containing the material appeared in the interstitial tissues of the heart but no vascular lesions or other explanations for the necrosis were demonstrated histologically. Myocardial ischemia and the necrosis might have been related to the severe anemia, but the lesions could also be ascribed to an incidental myocarditis and death was not otherwise attributable to the scandium. Two of the rabbits found dead had pneumonia, a possible cause of death. Several of the dead rabbits had varying degrees of fatty change of the liver, possibly directly attributable to scandium toxicity, but also readily explained by a variety of banal derangements such as chronic anemia, malnutrition, or toxicity from infection elsewhere in the body. In one rabbit (90 mg Sc/kg; death, 1-1/2 yr) a slight fibrosis of the liver could be termed cirrhosis, and the collagenous deposition was most pronounced near the largest accumulations of the material presumed to be scandium. This rabbit had much scandium in the usual sites but we saw no fibrosis about the material in tissues other than liver. Moreover, the pattern of fibrosis in the liver was that common to portal cirrhosis from any of several causes. In this rabbit pronounced ascites, moderate pleural effusions, and considerable pulmonary edema might be attributed to cirrhosis, perhaps with hypoproteinemia from liver failure, but jaundice was not noted.

Spermiogenesis was not obviously affected histologically nor was a toxic effect evident in any other proliferating tissue. The cause for the anemia is not plainly evident histologically: It did not seem to be explained by replacement of the marrow space, although the foreign material in histiocytes did occupy as much as a fourth of the marrow. Features indicative of depression of erythropoiesis have not been detected thus far in our histologic examination, except that the persistence of the usual amounts of adiposity in the marrow was definitely abnormal for rabbits

stressed by anemia of this severity. (Severely anemic rabbits should have a more cellular marrow.)

To define the onset and extent of the anemia observed in the protracted study of the effect of scandium on New Zealand rabbits, a much more extensive hematologic and biochemical study of the early phase of the aenmia has been recently made. Two groups of young males and females matched for age were used. One group was administered weekly doses of 5 mg Sc/kg body weight; the other group was maintained as a control. On the first and sixth day following each dose a small sample of blood was taken from the ear vein for hematocrit, hemoglobin, and counts of red cells, white cells, and platelets. Controls were sampled once a week. A larger volume (10 ml) was taken from each rabbit for a battery of biochemical analyses before and at the termination of the treatment (40 mg Sc/kg cumulative dose). Figs. 40 and 41 show the hematologic findings. Differences between the hematocrits of treated and control groups (Fig. 40) was of borderline significance ($P < 0.05$) at cumulative doses of 15 and 20 mg Sc/kg (15 and 22 days after the initial scandium dose). The difference was highly significant ($P < 0.001$) at 25 mg/kg and above. Hemoglobin values and red cell counts of the treated and control animals began to diverge after a cumulative dose of 25 mg Sc/kg, the decreases in the values for the treated groups becoming highly significant at 35 and 40 mg/kg. White cell counts (Fig. 41) were unaffected by scandium. Platelet counts of the treated and control group at a cumulative dose of 40 mg Sc/kg (50 days) differed minimally. The greatly reduced platelet count in the treated females at 43 days may have been due to clumping.

Blood chemistry values for the treated animals (electrolytes, total protein, albumin, cholesterol, uric acid, creatinine, total bilirubin, alkaline phosphatase, CPK, LDH, SGOT, BUN, and glucose) showed no significant differences from those of controls at the termination of the scandium treatment. However, plasma iron levels in treated females were significantly lower ($P < 0.001$) than those of the control females (150 vs 210 $\mu g\%$); plasma irons of treated males were also lower than controls but at a lower livel of significance ($P < 0.05$). On the other hand iron-binding capacities were the same for treated and controls, both females and males. No deaths have occurred to date. Body weight gain of the treated rabbits paralleled the controls. We are observing this group of animals hematologically to determine the extent and rate of recovery from the effects of the scandium treatment.

Zonal Ultracentrifugal Studies of the Subcellular Distribution of Gallium-67

Earlier differential centrifugation experiments (18) indicated that ^{67}Ga is bound within or to subcellular particles rather than some component in the soluble phase of the cell. That ^{67}Ga is associated with some subcellular organelle is further supported by the ultrastructural evidence obtained in AKR/J leukemic mice (see page 162). Autoradiograms of tissue sections known to preferentially localize the radioisotope show the majority of the silver grains over electron-dense cytoplasmic bodies. Our immediate goal utilizing zonal centrifugation techniques and enzymatic "markers" has been to isolate and characterize these gallium-binding granules (D. H. Brown, R. L. Hayes, D. C. Swartzendruber, J. E. Carlton, and B. L. Byrd).

Our preliminary data indicate that gallium is found principally in a small granule population. The gallium-binding granules (GBG) appear to be lysosome-like according to their morphology (Fig. 46) and acid phosphatase activity (Fig. 43). However, these particles may be significantly different from the typical lysosomes usually associated with intracellular digestive processes or phagocytosis. In fact,

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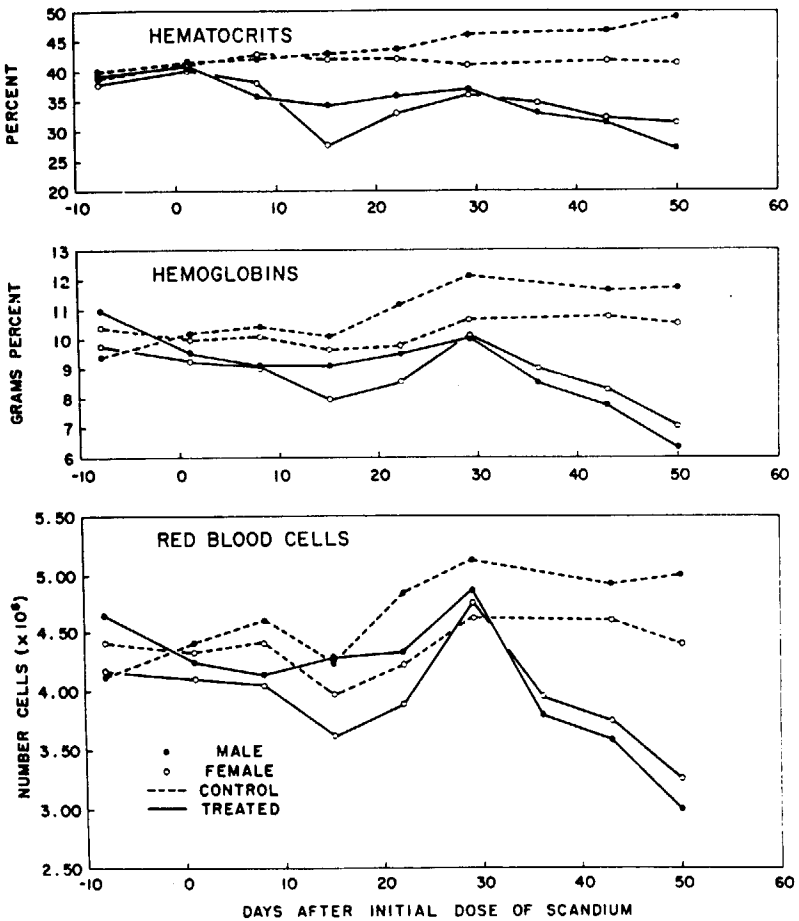


Fig. 40. Effect of prolonged intravenous administration of scandium citrate (5 mg Sc·kg at weekly intervals) on the hematocrits, hemoglobins, and red blood cell counts of male and female New Zealand rabbits. Measurements were made on the first and sixth day after each scandium dose up to a cumulative total of 40 mg Sc/kg; only the values for the measurements on the first day after each dose are plotted (day 50 is cumulative dose of 40 mg kg). The female control group had 5 animals; all others contained six animals each.

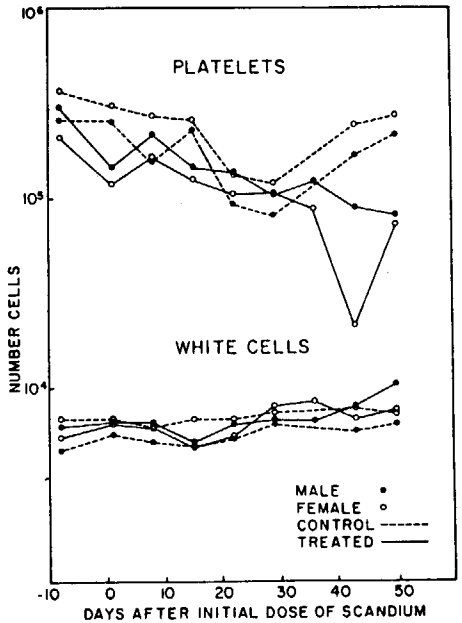


Fig. 41. Effect of prolonged intravenous administration of scandium citrate (5 mg Sc/kg at weekly intervals) on the platelet and white blood-cell counts of male and female New Zealand rabbits. Details same as in Fig. 40.

the sedimentation characteristics of the GBG suggest they are not typical lysosomes (Fig. 42).

Further purification and enzymatic characterization of the GBG should be of considerable assistance in tracing the origin and development of these organelles. The preferential uptake of gallium in malignant tissues will be of great value in studies concerned with altered metabolic processes in neoplasms.

Until recently attempts to subfractionate the granule population of cells have met with limited success. The development of the zonal centrifuge has improved the resolution and density-gradient centrifugation fractionation procedures. There is evidence for the existence of at least two and possibly three distinct lysosome populations in cells; these differ with respect to size, enzyme content, and origin. Baggiolini et al (J Cell Biol 40, 529, 1969), subfractionated leukocyte granules into two distinct lysosome populations by using a BXIV zonal rotor. We have used the recently developed BXXIX rotor (Anal Biochem 31, 255, 1969) to fractionate the organelle population from tumor homogenates. This rotor makes it possible to perform particle separations in multiple stages, since it can be loaded and unloaded at its edge or outer wall. By "sweeping" the outer edge of the gradient for particles that have collected during the interval of centrifugation, it is possible, through

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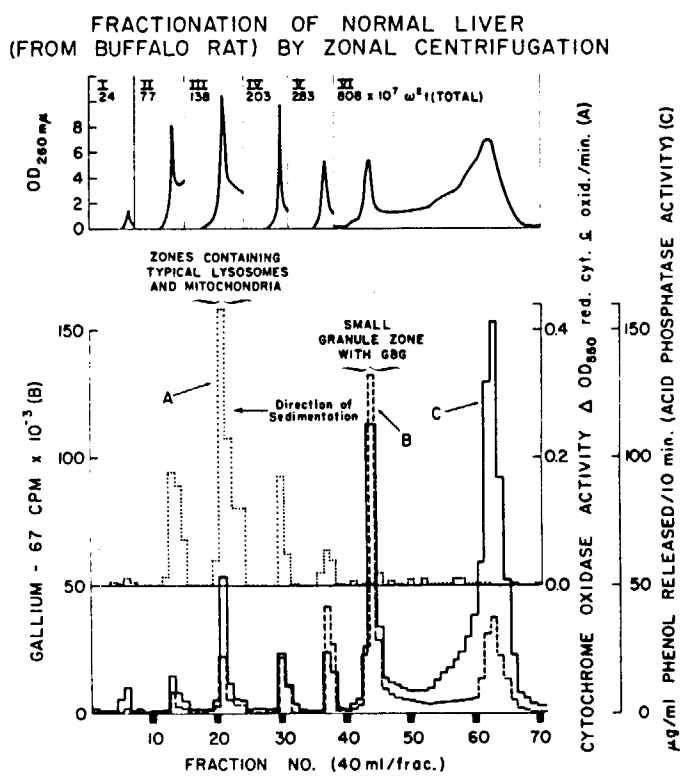


Fig. 42. Separation of subcellular particles from a homogenate of a Buffalo rat liver by zonal centrifugation. The first five stages of the zonal procedures were discontinuous rate separations; the sixth stage was an isopycnic banding of the smallest particles remaining in the gradient. The peaks at the top of the figure represent the particles that have accumulated after each period of centrifugation on a dense cushion of 45% sucrose at the edge of the rotor. The total accumulated centrifuged interval (w^2t radian sq sec) is shown at the top of each stage. The direction of centrifugation is from right to left. The cushion and accumulated particles were removed at each interval at the edge of the rotor, after which new cushion material was introduced. An estimate of particle concentration was obtained by light scatter at 260 mμ. Mitochondria are represented by cytochrome oxidase activity (B) measured spectrophotometrically at 550 mμ by the rate of reduced cytochrome c oxidation. Lysosome-like particles are characterized by their acid phosphatase content, (C) obtained by measuring colorimetrically the phenol split from substrate phenol phosphate. The localization of gallium-67 (B) was determined by counting 5-ml aliquots of the fractions in a gamma counter. It is apparent that the majority of the radioactivity is in those fractions of the sixth stage containing the particles that have been banded isopycnically. The peak area at the far right of the figure represents those components that have been released from particles and other soluble cytoplasmic constituents that have not left the starting zone where the sample was introduced into the rotor. The sample was a homogenate from 10 gm of liver from a rat given ^{67}Ga 24 hr earlier.

differences in sedimentation coefficient, to fractionate the particle population of a cell homogenate according to size.

A six-stage fractionation by this procedure of the granule population from a homogenate of the liver of a Buffalo rat is shown in Fig. 42. The rat had been given ^{67}Ga intravenously 24 hr before the study. The first five stages of the zonal procedure are discontinuous rate separations; the sixth stage is an isopycnic banding of the smallest particles remaining in the gradient. Lysosomes, determined by their acid phosphatase activity, are distributed in the fractions collected from all the stages indicating that they are present in a wide range of sizes. It is apparent that ^{67}Ga is also distributed in all stages but the greatest amount is located in the smallest granule fraction (Stage VI).

When we look at tumor homogenates by this procedure, we also find the majority of the radioactivity in the sixth-stage granules. Figure 43 shows the results obtained from an RFT tumor homogenate (18). It is apparent from Fig. 43 that a relatively higher proportion of ^{67}Ga is found in the sixth-stage tumor-granule population (GBG) than is found in the liver homogenate from a normal animal. A better comparison is shown in Figs. 44 and 45. In these two separations the GBG were isolated from the homogenate of the liver and tumor taken from the same animal, a Buffalo rat bearing a 7794-A Morris minimal deviation (MMD) hepatoma. These data show that there is a preferential uptake of ^{67}Ga in the tumor tissue, since there was a greater percentage of radioactivity in the GBG fractions obtained from the tumor homogenate than in the GBG fractions isolated from the liver homogenate. In addition there is a greater relative specific activity in the tumor GBG than in the liver gallium-binding granules. It is noteworthy that the tumor tissue used in this case is cytologically minimally different from the liver tissue from which it was derived. It thus appears, that whether the neoplasm is poorly differentiated, as in the case of the RFT tumor, or highly differentiated, as in the case of the 7794-A MMD hepatoma, tumor cells produce more than healthy cells of whatever it is that binds the gallium.

Although we have yet to determine the specific agent(s) that bind gallium, we have discovered that it is associated with a particle that has acid phosphatase activity. Electron microscopic autoradiograms of thin sections made from the particles isolated by zonal centrifugation reveal that the silver grains produced in the emulsion by the radioactivity in the sections are predominantly localized over small electron-dense single-membrane organelles (Fig. 46). These granules are morphologically similar to those observed in ^{67}Ga autoradiograms of murine tissue sections (see page 162). Tentatively, on the strength of these data, we are classifying the gallium-binding granule as a small lysosome-like organelle.

We have mainly utilized rats in this investigation; however, other animals and other types of tumors are under investigation and thus far similar observations have been made.

Concomitantly studies are in progress to determine the characteristics of the component that binds gallium. Our preliminary data indicate gallium is bound to a macromolecule that has a sedimentation coefficient of 4.6 ($S_{20,w}$). This value was obtained from an approach to equilibrium zonal centrifugation study (Fig. 47). The gallium-binding component was localized by its radioactivity and its sedimentation coefficient was obtained by computer computation using a program (Nat Cancer Inst Monograph #21, 1966, p. 175) designed to determine sedimentation coefficients of particles of known density in known sucrose gradients. An arbitrary density range of 1.3 - 1.4 was chosen for the gallium-binding macromolecule on the assumption that it is a protein similar in density to most proteins.

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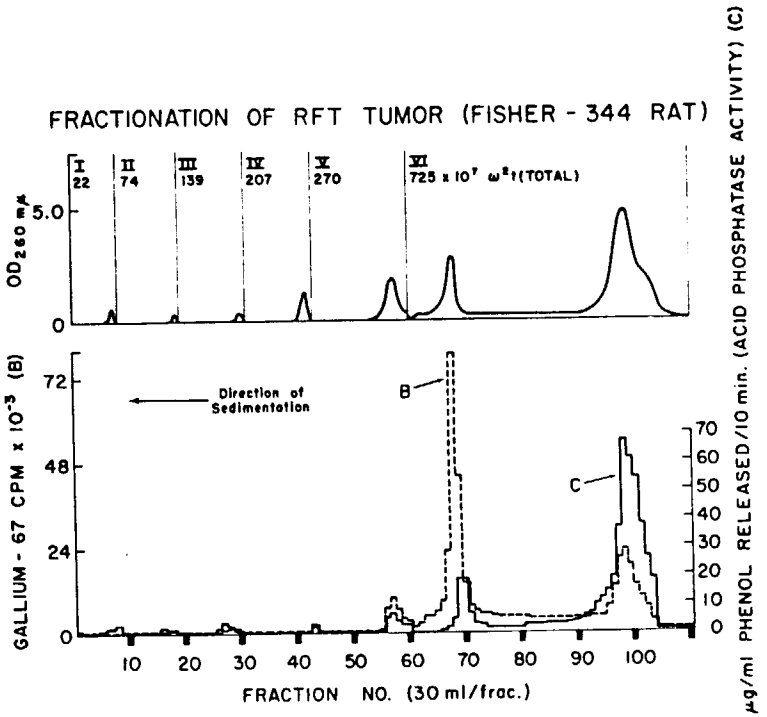


Fig. 43. Separation of subcellular particles from a homogenate of RFT tumors. For direct comparison the components in this figure were normalized to represent a 10-gm sample of tissue. Note the reduction of all components in the first five stages compared to those in the normal liver. On the other hand, the gallium-67 activity in the sixth peak is greater than that found in the homogenate of normal liver (see Figs. 42 and 44). Technique was the same as in Fig. 42.

Distribution of Gallium-67 in Tumor Tissue Homogenates

We have continued to search for the sites of ^{67}Ga uptake in the cell (R. L. Hayes and J. E. Carlton). When a 0.25 M cold sucrose homogenate of transplanted RFT tumor tissue (from a Fischer 344 rat previously given intravenously ^{67}Ga) is contrifuged at 100,000 x g for one hour, 80 to 90% of the ^{67}Ga activity in the homogenate is spun down. If the homogenate is made 5% with respect to n-butyl alcohol, 0.1% with Triton X-100, or highly hypotonic with distilled water and then centrifuged, 80 to 90% of the ^{67}Ga activity then appears in the supernatant. Sonification also releases ^{67}Ga into the supernatant. These types of treatment rupture the organelle membranes. If the subcellular structures identified as major sites of ^{67}Ga concentration by zonal ultracentrifugation and electron microscopic auto-

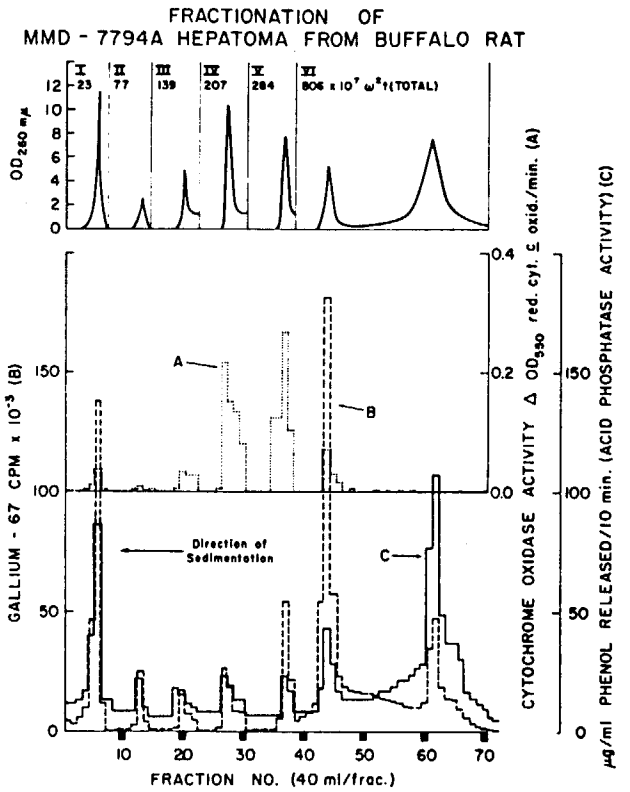


Fig. 44. Separation of subcellular particles from homogenate of livers from Buffalo rats having 7794-A Morris minimal deviation hepatoma implants. Technique was the same as in Fig. 42.

radiography (see pages 90 and 162, Brown and Swartzendruber) are indeed "lysosomes," the detection of ^{67}Ga activity in the supernatant after treatment presumably indicates that it is not bound to the organelle membrane in the untreated homogenate, although a secondary effect of the treatment on possible membrane binding cannot be disregarded. The ^{67}Ga activity in the supernatant from treated homogenates does not dialyze (24 hr) appreciably indicating that the gallium is bound to a macromolecular agent or agents. If the sucrose homogenate is treated with 5% trichloroacetic acid, ^{67}Ga is also released into the supernatant but it is then 85% dialyzable. Adjusting the pH of the sucrose homogenate to pH 5 and pH 9 does not produce any appreciable solubilization of ^{67}Ga.

The gallium-macromolecular complex in RFT tumor extracts is sensitive to

1027941

changes in temperature, as judged by dialysis experiments. On standing at room temperature for 3 hours, 15% of the ^{67}Ga activity was dialyzable (24 hr) at 5° C while only 5% dialyzed after being stored for an equal time at 5°. When the extract was warmed to 60° C for 15 minutes, 30% dialyzed.

Approximately 85% of the ^{67}Ga activity in RFT tumor extracts precipitates with proteins in the cold when the solution is made 60% saturated (or higher) with ammonium sulfate. When the precipitated proteins were redissolved in water, little ^{67}Ga activity was dialyzable, indicating that this treatment did not change the essential nature of the gallium-macromolecular complex. We could achieve no great increase in specific activity (^{67}Ga activity/mg protein) with lower concentrations of ammonium sulfate; therefore, fractionation by this method appears to be impractical. It may be of use, however, for concentrating the complex. Rivanol (6, 9-diamino-2-ethoxyacridine lactate), another protein precipitant, carried down 60% of the activity at pH 8.5.

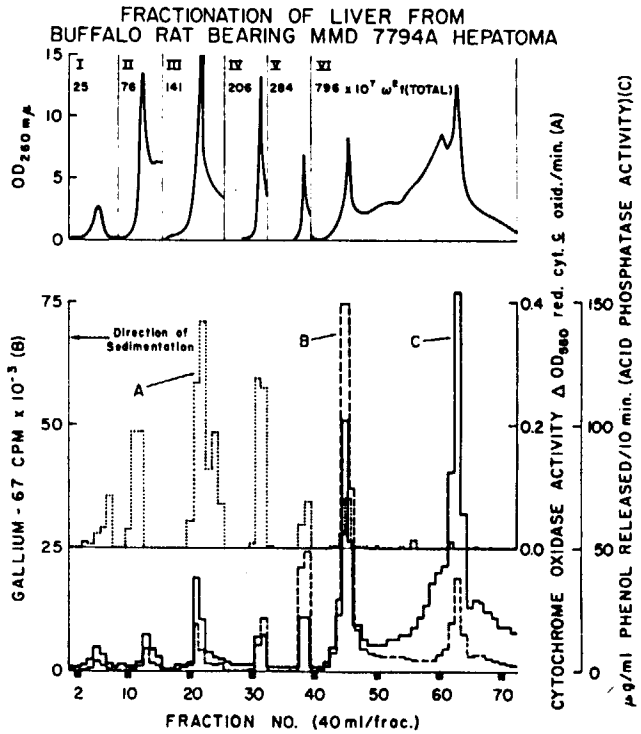


Fig. 45. Separation of subcellular particles from homogenate of 7794-A Morris minimal deviation hepatomas. Technique was the same as in Fig. 42. Data was normalized to 10 gm of tissue. The similarity of the optical trace to that of the liver particle profile (Fig. 44) is striking, but in the tumor, a larger percentage of the ^{67}Ga was found in the sixth-stage granule population.

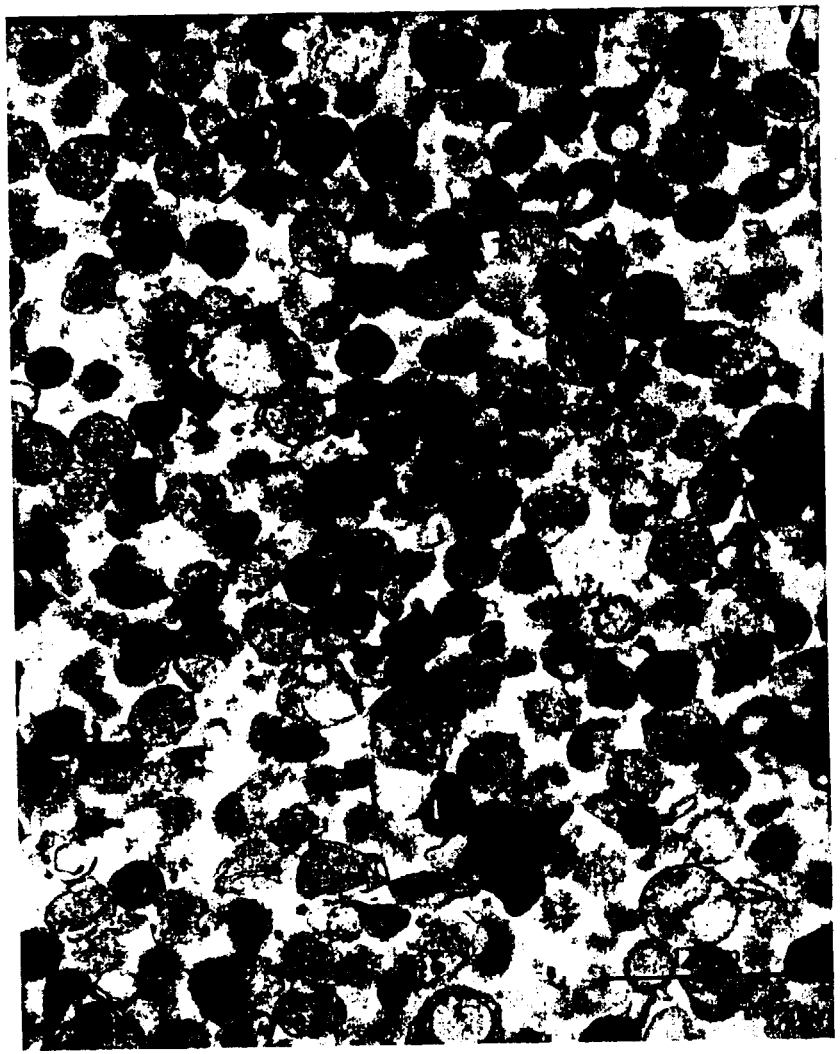


Fig. 46. Electron microscopic autoradiogram of the small granule fraction from stage six in the zonal centrifugation (RFT Tumor) shown in Fig. 43 (Magnification 36,000X).

1027942

We have subjected ultrafiltration concentrates of RFT tumor extracts to gel filtration at 5° C (required because of instability of the gallium complex at room temperature). On Sephadex G-200 (40-120μ) approximately 10% was eluted in the void volume along with the majority of the 280 mμ absorbing material present in the sample. There was very little ^{67}Ga eluted at the level of the bed volume and beyond where free ^{67}Ga would be expected. (At room temperature the majority of the activity was eluted at the level of the bed volume.) Recovery of activity was essentially complete. The major peak of ^{67}Ga activity occurred at an elution volume corresponding to a molecular weight of approximately 80,000 assuming a globular protein (column was calibrated with purified proteins). This is in general agreement with the sedimentation coefficient of 4.6 determined by zonal ultracentrifugation (see page 94). Gel filtration should provide one approach to the purification of the gallium macromolecular complex. Ion exchange chromatography is in progress.

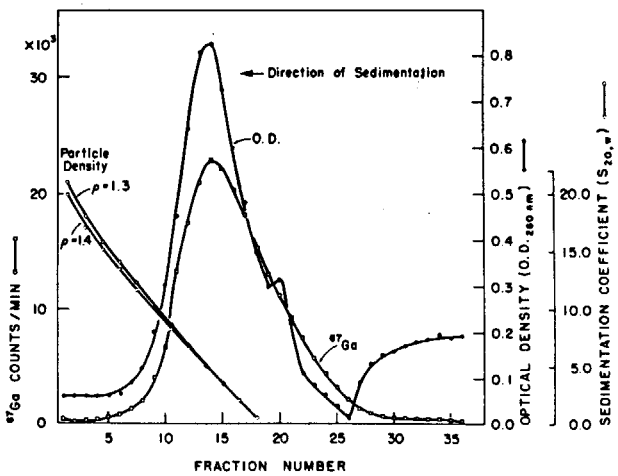


Fig. 47. Determination of the sedimentation coefficient of the gallium-binding component in tumor tissue by density-gradient zonal centrifugation. Five grams (approx. 300 mg of protein) of RFT tumor tissue from rats injected with ^{67}Ga was homogenized in 0.25 M sucrose, treated with 0.05% Triton X-100 and centrifuged at 27,000 rpm for 75 min in an SW #27 Spinco rotor. The resulting supernatant fraction was centrifuged at 25,000 rpm for 18 hr in a B-XXIX zonal rotor through a linear sucrose gradient (12-23.5%) containing Miller-Golden buffer (pH 7.5 ionic strength 0.1).

Clinical Investigations in Nuclear Medicine

The discovery in 1968 that gallium-67 given carrier-free in citrate form would localized in a variety of tumors stimulated widespread interest in this agent. We have ordered our priorities so that a significant part of our clinical effort is devoted to this project and we have accumulated important preliminary data concerning its use as a tumor-scanning material, and also on its distribution, excretion, and turnover in man. From published reports and personal communications, we note that the agent is being used clinically in a number of centers in the United States, in Japan, and in Europe. We will continue to test in humans other promising radioisotopic agents as they are brought from the laboratory to the point of clinical trial. Other clinical investigations involve the use of radioisotopes in understanding the nature and status of disease processes and in evaluating the effects of therapy; these are applied particularly to the group of diseases treated by total-body irradiation.

Cooperative Group to Study Localization of Radiopharmaceuticals

During its 1970 visit to the Medical Division, our Advisory Committee recommended that a cooperative effort with other laboratories might be undertaken to broaden the study of gallium-67 as a tool in cancer management. We were granted funds for a work meeting by the American Cancer Society and invited representatives of the Departments of Nuclear Medicine from all the Oak Ridge Associated Universities member schools to meet in Oak Ridge in June. Persons from 13 regional schools and Memorial Hospital in New York attended the meeting, as well as staff members of the Medical Division and of Oak Ridge National Laboratory. A second meeting followed in July at which time a provisional constitution and bylaws were developed. The group formally constituted itself as "The Cooperative Group to Study Localization of Radiopharmaceuticals," requesting Oak Ridge Associated Universities to provide the Secretariat. Preliminary protocols were outlined and subsequently a proposal was prepared seeking support from the American Cancer Society. Concurrently, negotiations were undertaken with the National Cancer Institute and the Atomic Energy Commission to support a project to study gallium in lymphoma and lung cancer. The immediate objectives are to answer questions about the value of this agent as a diagnostic aid for staging (a prerequisite for planning optimal therapy), for determining the effectiveness of treatment, and later for assessing the need for further treatment. These diseases were chosen because renewed hope to cure Hodgkin's disease has recently emerged as the result of new, vigorous radiotherapy protocols that require precise methods of assessing the extent of the disease, and in cancer of the lung, we see the continuing inexorable rise in incidence coupled with low cure rates and low operability rates because of late detection, with gallium scanning perhaps offering a method of earlier detection.

If the group's enthusiasm is sustained and if this kind of cooperative work proves to be an effective way of quickly broadening the data base and standardizing methods of evaluating a new agent, then activities may extend to protocols answering questions about other kinds of neoplasms, and eventually evaluating other promising radiopharmaceuticals.

We have attempted to anticipate and deal with the many difficulties and pitfalls in this kind of cooperative research. It will be important to obtain continuing financial support for the group's activities.

1027943

Tumor Scanning with Gallium-67

During 1970 tumor survey scans with carrier-free ^{67}Ga have been made on additional patients thought to have advanced malignant neoplasms. Added to our earlier experiences this represents a total of 165 intravenous doses that have been given to 116 patients without any significant reactions to the preparations (C. L. Edwards and R. L. Hayes). Thirty-four patients received more than one dose and several were scanned serially to study the effect of therapy and to look for recurrence of disease.

Table 24 lists the results of the initial scan survey in 100 patients with known viable tumor. The results are listed merely as positive or negative for concentration. A scan is regarded positive if there is evidence of gallium concentration in one or more sites of known tumor, whether or not other known lesions are positive. The high percentage of positive scans obtained with reticulum cell sarcoma, lymphoblastoma, and squamous cell carcinoma of the lung, and the frequency of negative scans with lymphocytic lymphosarcoma and multiple myeloma indicate that the histologic type is an important factor in the affinity of tumors for gallium. Thus gallium does not localize in malignancies in general, but rather in tumors of specific cellular types.

That the concentration of gallium varies from one type of tumor to another can also be seen in the assay data obtained from surgical specimens from patients who had been injected with gallium-67 (Table 25). These data also indicate that for the same histologic type of tumor the concentration of gallium may be quite different in one patient from that in another. Further, within the same patient, different lesions of the same original tumor may show a wide range of concentrations. These are important facts which affect the interpretation and usefulness of gallium tumor scanning. Unfortunately finding gallium concentrated in a single metastasis with no other focus of activity cannot be interpreted as proof of the absence of other metastases. The greatest discrepancies seem to be associated with fibrosis or necrosis of tumor.

Figure 48 shows a chest X ray and scan of a 44-year-old woman with stage III A Hodgkin's disease, nodular-sclerosing type. The scan clearly indicates tumor in the hilar nodes and mediastinum. Figure 49 is a scan of this patient's abdomen and a lymphangiogram. The lymphangiogram is abnormal and at laparotomy, tumor was found in the region of the left iliac lymph nodes. Failure to see the tumor on preoperative scan is explained by the assay of a biopsy which showed it contained less than 3% of the injected dose/kg. Although the tumor was largely fibrotic, there were small islands of viable malignant cells. Recent treatment is also an important factor affecting the gallium localization. While this may be merely a manifestation of necrosis associated with the treatment, in six patients local irradiation of a known lesion was followed by a greatly reduced gallium uptake in the radiation field. However, the gallium may then localize in tumor outside the radiation field in tumor not apparent on the preirradiation scans, as in Fig. 50. In one patient (Fig. 51) gallium localized in a bronchogenic squamous cell carcinoma that was still viable 9 months after an inadequate course of radiation treatment (1600 rads). We have had less experience with scans made after systemic chemotherapy, but in the few observations we have been able to make the scans have become negative after effective therapy. The scans in Fig. 52 confirmed the clinical impression of progression of the disease while the patient was being treated with chemotherapy. The failure of melphalan to alter the concentration of gallium in this tumor may be related to the ineffectiveness of the drug in controlling the disease. Such results suggest a possible clinical use of gallium scanning in

TABLE 24

RESULTS OF SCANS OF 100 PATIENTS WITH KNOWN TUMORS

	Positive		Negative	
Hodgkin's Disease	12		6	
Reticulum Cell Sarcoma	6		0	
Lymphosarcoma	7		9	
Lymphoblastic		4		0
Other		3		9
Myeloma	2		4	
Lymphocytic Leukemia	0		3	
Mycosis Fungoides	0		1	
Carcinoma of the Lung	10		5	
Squamous cell		8		0
Adenocarcinoma		0		1
Undifferentiated		1		3
Unspecified		1		1
Cancer of the Breast, Metastatic	6		2	
Cancer of the Thyroid	1*		1†	
Cancer of the Ovary	0		2	
Cancer of the Cervix	1		0	
Cancer of the Colon	0		1	
Cancer of the Pancreas	0		1	
Cancer of the Kidney, Metastatic	2		1	
Wilm's Tumor	1		0	
Ewing Sarcoma	1		0	
Malignant Tumor (Paraganglioma?)	1		0	
Histiocytoma	0		2	
Angiosarcoma	0		1	
Sarcoma of Soft Tissue	0		1	
Malignant Giant Cell Tumor of the Bone	0		1	
Osteogenic Sarcoma	0		1	
Glioblastoma	0		1	
Metastatic Cancer of Unknown Primary	5		2	
Adenocarcinoma		4		0
Squamous Cell Carcinoma		1		0
Undifferentiated		0		2
	55		45	

*Poorly differentiated
†Medullary

assessing the effectiveness of therapy; early testing after initiation of chemotherapy may assist in the determination of an effective agent or dose.

The size of the tumor is, of course, another important factor in whether it will be detected with scans. Even with high tumor to non-tumor ratios, small tumors may escape detection because of the low absolute amount of activity present, particularly if the tumor is deeply located and not precisely at the focal plane of the detector.

1027944

TABLE 25

GALLIUM-67 CONCENTRATION IN BIOPSIED TUMORS

Tumor	Interval (days)	Concentration (% dose/kg)	Ratio* (tumor/blood)
Hodgkin's Disease Lymphocyte depletion	1	11 - 19	22 - 38
Lymphoblastoma	1	49 - 68	25 - 34
Lymphoblastoma	4	26 - 43	87 - 143
Hodgkin's Disease Mixed	4	8.0† - 51	-
Hodgkin's Disease Nodular Sclerosing	7	2.9‡ - 16	7 - 40
Adenocarcinoma	2	11‡ - 39	15 - 55
Squamous Carcinoma	9	12 - 14	-
Histiocytoma	8	2.1 - 4.5	-
Paraganglioma (?)	2	1.6† - 6.9	-

*The range of values results from multiple varying samples of tumor.
†Tumor was largely necrotic.
‡Tumor was fibrotic.

Intralymphatic Injection of Carrier-Free ^{67}Ga

In an effort to improve the visualization of tumors in small pelvic and abdominal lymph nodes, we studied intralymphatic injection of gallium. If gallium can get into tumors via the lymphatics as well as from the blood, direct intralymphatic injection should result in a greater concentration of activity in the tumor by exposing it to a higher concentration of gallium (the dose is dissolved in only a few milliliters of lymph rather than the whole blood volume). After the intralymphatic injection of dogs (R. L. Hayes, B. L. Byrd, J. J. Rafter, and S. Ominsky) the level of gallium in the blood increased through the first hour then decreased at a rate parallel to that of an intravenous dose (Fig. 53). Necropsy at 24 hours showed a similar tissue distribution for intravenous (I.V.) and intralymphatic (I.L.) doses except in the lymph nodes directly along the lymphatic drainage. At 24 hours the amount in the popliteal node on the side of injection was 5-6 times that for I.V. injection (Table 26). The differences decreased in lymph nodes farther from the injection site.

Subsequently, in four patients gallium-67 citrate was given directly into the doral lymphatics of the feet ahead of the Ethiodol used in a standard lymphangiogram for staging lymphomas (C. L. Edwards, S. Ominsky, and R. L. Hayes). The nodes in the pelvis of the first patient were not well visualized because the gallium, given as an aqueous solution of citrate, diffused freely through the walls of the lymphatics of the legs before reaching the proximal nodes. We then returned to the

TABLE 26

^{67}Ga DISTRIBUTION IN DOGS AFTER INTRALYMPHATIC AND INTRAVENOUS INJECTION

(Percent of injected dose per gram of tissue x 10^3)

	4 Hr Il*	4 Hr Iv	24 Hr Il*	24 Hr Iv	24 Hr Il With Plasma†
Liver	11.7	10.8	20.1	25.2	12.4
Spleen	5.9	6.8	5.3	10.9	3.0
Kidney	8.6	12.4	11.5	14.9	5.2
Lung	6.9	7.5	3.8	5.4	3.2
Muscle	2.6	1.6	1.1	1.5	0.1
Rib	10.9	8.4	8.2	13.9	8.0
Lymph Nodes					
Popliteal Node (side of injection)	96.6	6.5	48.5	9.0	178.0
Popliteal Node (opposite side)	3.8	-	3.7	-	6.9
Internal Iliac (side of injection)	35.4	5.0†	9.8	6.8†	2.2
Internal Iliac (opposite side)	3.6	-	9.7‡	-	3.7
External Iliac (side of injection)	44.1	6.6	18.0	6.3	104.8
External Iliac (opposite side)	4.2	-	5.4	-	21.4
Aortic	10.4	6.0	8.6‡	6.5	45.9
Mesenteric	7.0	11.7	7.6‡	10.0	11.6
Mediastinal	5.7	7.2	6.1	4.3	5.7
Terminal Blood§	15.3	22.2	2.3	2.9	.21

*Il injection, volumes were standardized at 0.6 ml per kg body weight.
†Ga-67 citrate incubated with 6 ml of autologous plasma before injection.
‡One value, other values 2 dogs.
All doses contained 1 mg of Na citrate per kg body weight.
§Percent of injected dose in blood volume.

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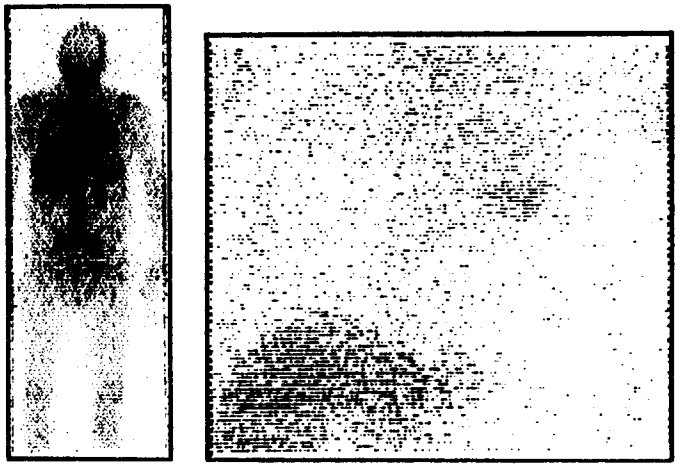


Fig. 51. A whole-body scan and an anterior chest scan with ^{67}Ga of a 66-year-old man with bronchogenic carcinoma 9 months after irradiation with 1600 rads.

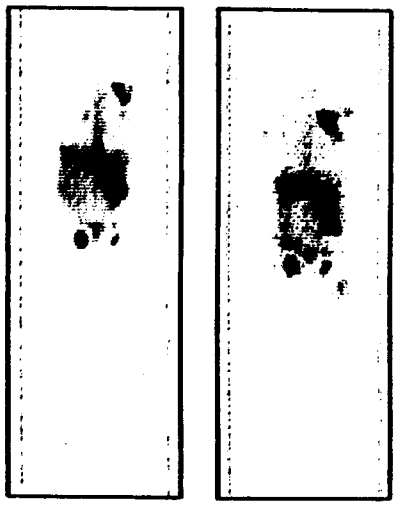


Fig. 52. Whole-body scans with ^{67}Ga of a 52-year-old man with multiple myeloma before (left) and after (right) a 6-week course of chemotherapy (melphalan) during which time there was little clinical evidence of improvement. The scans suggest progression of his disease with new and larger lesions.

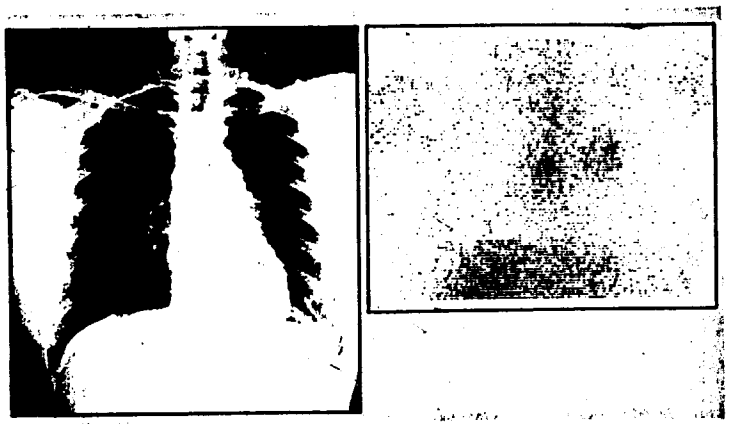


Fig. 48. Chest X ray and anterior chest scan with ^{67}Ga. Patient had sclerosing Hodgkin's disease with tumor in the left hilum and mediastinum.

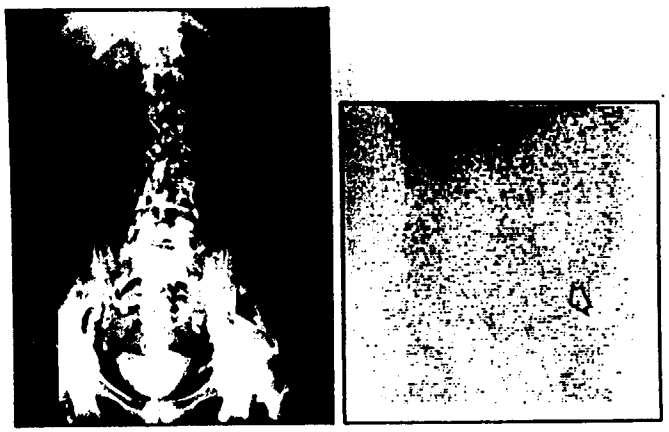


Fig. 49. Lymphangiogram and anterior abdominal scan with ^{67}Ga of the patient in Fig. 48. The lymphangiogram shows blockage of lymphatic vessels (arrow) in the region of tumor found at surgery. The scan shows no indication of the tumor.

1027946

dog model, binding the gallium to autologous plasma protein before injection to make it diffuse more slowly. It could then be seen (using a scintillation camera) filling the pelvic and para-aortic chains of lymph nodes to the upper abdomen of dogs (Table 26).

In two patients with tumors in the pelvic lymph-nodes the serum-bound gallium gradually left the pelvic nodes soon after injection while it increased in tumors not associated with the injected lymphatic chain, e.g., the axilla. Later scans showed as high a concentration in the tumors of axillary lymph nodes as those in the pelvic nodes. The gallium transported in the lymph apparently bypasses the neoplasm altogether as it passes through the node. It then gradually diffuses or flows through lymphatico-venous communications into the blood in which it is transported as if it had been injected intravenously. Because more gallium is retained by the first lymph node along the lymphatic than by other normal lymph nodes, as demonstrated in dogs, scans made as late as 24 hours after I.L. injection may be erroneously interpreted as showing abnormal localization in the pelvic lymph nodes. There appears to be no advantage to injecting the nuclide directly intralymphatically even to demonstrate tumors along the lymphatic vessels.

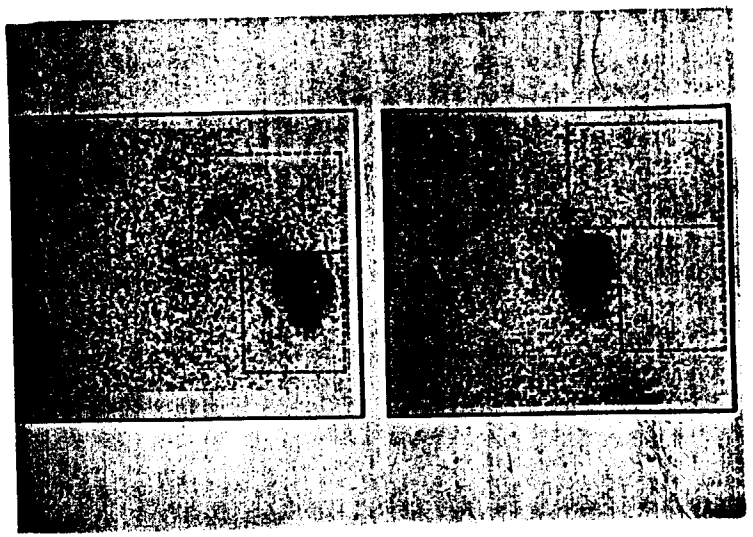


Fig. 50. Anterior chest scans with ^{67}Ga of a 52-year-old woman with reticulum-cell sarcoma before (left) and after (right) ^{60}Co teletherapy to the areas indicated by the broken line

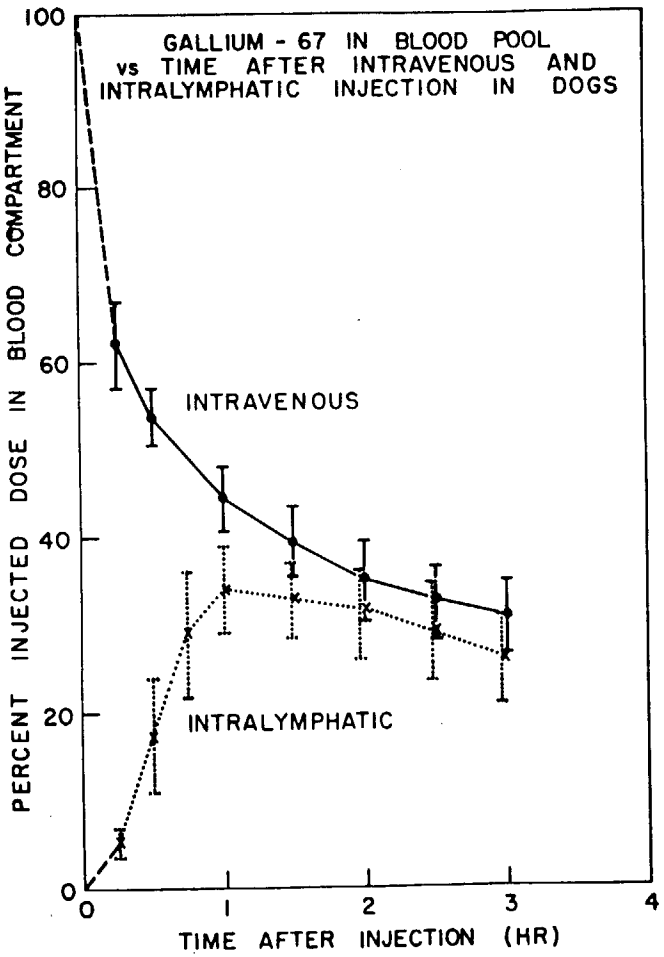


Fig. 53. Gallium-67 in the blood pool in dogs after intravenous and intralymphatic injection of the aqueous solution.

1027947

Distribution of Gallium in Human Tissues After Intravenous Administration in Low Concentration as a Citrate

To obtain data for dosimetry and to better understand the factors related to tumor scanning, we reviewed all postmortem assays after intravenous administration of ^{67}Ga to patients for scanning in our hospital (Bill Nelson, R. L. Hayes, C. L. Edwards, R. M. Kniseley, and G. A. Andrews). Our early publications on the tissue distribution of gallium (Radiology 61, 534, 1953) cannot be directly applied to the present use of ^{67}Ga, because the ^{72}Ga was given with large amounts of stable gallium, which alters the relative concentrations in various tissues. Most of the samples taken for assay were accompanied by histologic sections. From the beginning of the study, gross autoradiography was helpful in showing details of the distribution and in guiding the selection of assay samples.

Our computer group was most helpful in listing assays with pertinent data for the 1616 samples, and in making calculations and preparing graphs. For each of the patients, values representing the various tissues and fluids were obtained by averaging assays of corresponding samples which were free of tumor and of other specific abnormalities likely to affect grossly the ^{67}Ga concentration. These values for several important tissues are summarized in Figs. 54 and 55 and are representative of the trend in most tissues, a decrease in concentration over a period of 3 weeks, although the concentration rises in the spleen.

For calculating the radiation exposure to various tissues the changes in concentration with time are a minor concern in relation to the physical decay of ^{67}Ga. We found a rather wide individual variation in patients dying at nearly the same interval, as might

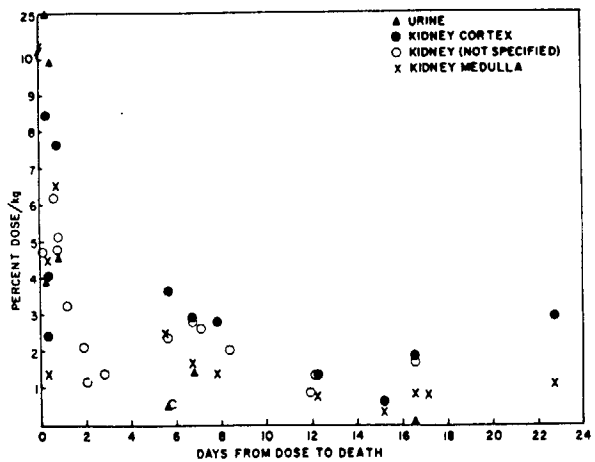


Fig. 54. The ^{67}Ga concentrations are expressed as a percent of the administered dose per kg, corrected for decay and normalized to a body weight of 70 kg. The trend toward decreasing concentration in most tissues is aptly illustrated by samples from the kidneys. This decrease is attributable to excretion.

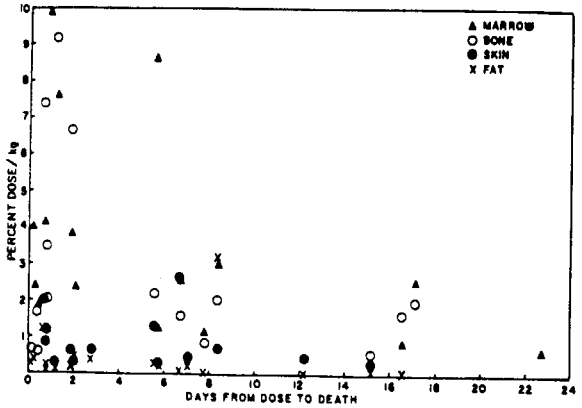


Fig. 55. Variability in assay values for bone and marrow is partly explained by the different concentrations of ^{67}Ga in the several components of these tissues. The samples of skin were also affected by varying proportions of fat, which contained less ^{67}Ga.

be expected in view of the various clinical conditions represented. Accordingly, we have listed the organs in the order of concentration expressed as averages and ranges for each organ (Table 28). Organs represented by fewer than 11 patients are not listed but some are of interest: Spinal cord samples generally contained more ^{67}Ga than samples of brain, possibly because they include more of the meninges, which were demonstrated to have a higher concentration. Cerebrospinal fluid was much less active than brain (0.05%/kg, mean of 5 patients). Ascitic and pericardial fluid had activity similar to that given for pleural fluid. Samples of diaphragm were consistently more active than other skeletal muscle and in several instances this was shown to be attributable to a considerable concentration of ^{67}Ga in the serosa. The gonads have special interest for dosimetry; the numbers of patients represented are small but permit provisional calculations: testis 0.7%/kg (mean of 5 with range 0.4 to 1.1), ovary 1.0%/kg (mean of 5 with range 0.5 to 1.3).

The variability in some tissues can readily be explained by problems of sampling when concentrations of ^{67}Ga differ from site to site (Figs. 55 and 56). However, this does not explain the wide range of values shown in some other organs, for example spleen and adrenal, with many values well supported by duplicate assay samples. To investigate possible explanations for the considerable range of values, we grouped the patients in many ways according to circumstances that have influenced gallium distribution in clinical or animal studies: time after dose, age, sex, nutritional state, tumor localization, presence of inflammation, and stable gallium carrier.

The whole-body retention data are incomplete but one example suggests that differences in renal function and circulatory status could account for some of the variability in assays. The high renal values shown in Fig. 54 for a patient who died on the 22nd day were associated with a rather high retention of ^{67}Ga (63%; Table 27, patient No. 23). Review of the records revealed no evidence of renal failure and histologic sections showed no significant renal abnormality; the retention can be attributed to unusually pronounced localization of ^{67}Ga in tumor resulting in a secondary delay in renal excretion. The case is cited to show how some of the variations in

1027948



Fig. 56. This autoradiogram (patient No. 12, Table 27) shows ^{67}Ga concentration in marrow stimulated to unusual hemopoietic activity, with little ^{67}Ga in the fatty marrow of the head of the femur, which was in a field of irradiation therapy. Scans of some patients with Hodgkin's disease show much tumor localization of ^{67}Ga, but in this case fibrotic tumor nodules have less activity than the marrow in the shaft. Cortical bone has the fine streaks of activity expected in the adult, without the intense localization in the epiphyses and periosteum characteristic of the adolescent.

concentration could be related to as yet unidentified abnormalities peculiar to individual patients, all of whom were seriously ill when given ^{67}Ga.

The mean assay values reported in Table 28 are probably reasonable approximations for dosimetric calculations, considering the wide range of values and the 3.24-day half-life of ^{67}Ga. More precise values might be obtained by regression analysis and these might be more important for the dosimetry of the other radionuclides of gallium,

TABLE 27

AUTOPSIES OF PATIENTS GIVEN ^{67}Ga DATA PERTINENT TO DISTRIBUTION

Pt. No.	Time: dose-death	Age, Sex	Body wt. (kg)	Carrier gallium (mg/kg)	Tumor: Diagnosis	Tumor: Est.wt. (kg)	Highest concentration*: Tumor (%/kg)	Highest concentration*: Other tissue (%/kg)	Highest concentration*: Other tissue: Organ	Heart* (mean %/kg)	Whole-body retention at death (%)
	Hours										
1	3	42 M	42.3	0.0	Glioblastoma multiforme	0.1	0.2	7.1	Kidney cortex	1.0	
2	6	16 F	40.9	0.0	Ewing's sarcoma	0.5	2.2	5.7	Kidney cortex	0.3	
3	9	47 F	36.4	0.0	Lymphosarcoma	1.0	9.8	2.4	Kidney cortex+	1.1	
4	10	71 M	67.0	0.0	Reticulum-cell sarcoma	3.0	9.4	4.6	Kidney medulla	0.7	
5	16	24 F	54.3	0.2	Hodgkin's granuloma	1.0	2.7	7.4	Bone	0.4	
6	19	16 F	40.5	0.0	Osteosarcoma	1.0	22.0	11.1	Adrenal	0.9	96
7	20	45 F	51.3	0.0	Adenocarcinoma, breast	1.0	19.9	5.1	Kidney	0.7	
	Days										
8	1.2	15 M	38.6	0.2	Osteosarcoma	1.5	14.9	9.2	Bone	0.2	69
9	1.9	14 F	42.3	0.2	Osteosarcoma	0.02	11.0	6.6	Bone	0.4	
10	2.1	23 M	40.0	0.2	Osteosarcoma	5.0	18.07	3.9	Adrenal	0.4	<80
11	2.8	80 M	51.9	0.2	Adenocarcinoma, prostate	1.0	8.8	6.3	Spleen	0.8	<79
12	5.6	31 F	47.3	0.0	Hodgkin's granuloma	0.2	7.2	11.5	Adrenal	0.6	67‡
13	5.8	46 F	40.0	0.2	Adenocarcinoma, breast	1.0	1.9	1.3	Lymph node	0.3	<85
14	6.8	60 M	64.3	0.0	Medullary carcinoma, thyroid	1.2	5.4	8.6	Spleen	0.4	
15	7.1	41 F	46.1	0.2	Adenocarcinoma, breast	2.0	41.0	6.3	Spleen‖	0.6	<67
16	7.8	58 F	42.3	0.0	Adenocarcinoma, lung	2.0	2.9	2.8	Kidney cortex	0.2	<65
17	8.4	55 M	37.8	0.2	Adenocarcinoma, lung	1.0	9.1	9.2	Spleen	0.4	
18	11.9	27 M	42.7	0.2	Fibrosarcoma	1.0	4.6	4.3	Liver	0.1	<73
19	12.2	53 F	46.6	0.0	Lymphoma, mixed lymphocytic and histiocytic	1.0	16.2	8.4	Spleen	0.3	
20	15.2	45 F	53.0	0.0	Reticulum-cell sarcoma	1.0	4.4	2.2	Liver	0.1	54‡
21	16.5	59 M	88.6	0.0	Small-cell carcinoma, lung	2.0	13.7	10.2	Spleen	0.2	52‡
22	17.1	56 M	46.0	0.0	Squamous-cell carcinoma, lung	0.2	3.5	3.0	Spleen‖	---	
23	22.7	58 M	44.1	0.0	Plasmacytoma, atypical (reticulum-cell sarcoma)	2.0	16.8	5.3	Spleen	0.4	63‡

* All assay values are expressed as a percent of the administered dose corrected for physical decay to the time of the dose and normalized to a body weight of 70 kg; except for tumor, values are means of all available samples considered normal histologically.

\+ A lymph node had the same value, 2.4% dose/kg.

‡ Percent of dose measured by whole-body count, corrected for decay; other values in this column were determined by assay of urine and feces, with incomplete collections except for Patients No. 6 and 8.

‖ Two other values are probably spurious: lymph node (13.0%/kg) and pituitary (7.8 %/kg).

‖ A lymph node with 6.6 %/kg had pronounced histiocytic hyperplasia.

1027949

TABLE 28

CONCENTRATION OF ^{67}Ga IN TISSUES AND FLUIDS

VALUES REPRESENTING 23 AUTOPSIES

	No. of Patients	Assays (% administered dose/kg)*: Mean	Assays (% administered dose/kg)*: Range
Spleen	20	4.1	0.5 - 10.2
Kidney cortex	12	3.8	0.7 - 8.4
Adrenal	16 (8+)	3.8 (5.1+)	0.6 - 11.5
Marrow	16	3.6	0.7 - 9.9
Liver	19	2.8	0.6 - 5.2
Kidney	16 (7+)	2.7 (3.3+)	0.6 - 6.2 (5.1+)
Bone	16 (12+)	2.6 (1.4+)	0.04 - 9.2 (3.5+)
Lymph nodes	16 (15‡)	2.2 (1.5‡)	0.2 - 13.0 (6.6‡)
Kidney medulla	11	2.0	0.4 - 6.5
Jejunum	12 (4+)	1.9 (2.1+)	0.6 - 4.5
Colon	13 (4+)	1.6 (2.0+)	0.3 - 5.2
Lung	15	1.3	0.3 - 3.0
Duodenum	12	1.3	0.3 - 4.4
Bile	19	1.0	0.04 - 3.4
Stomach	15	0.9	0.2 - 1.7
Thyroid	14 (6+)	0.9 (1.2+)	0.1 - 2.2
Skin	14 (6+)	0.9 (1.1+)	0.3 - 2.6
Pancreas	15	0.8	0.3 - 1.3
Heart	22	0.5	0.1 - 1.1
Blood	12	0.5	0.01 - 1.8
Pleural fluid	15	0.4	0.1 - 1.2
Fat	18 (17‡)	0.4 (0.3‡)	0.04 - 3.2 (1.2‡)
Plasma	11	0.3	0.02 - 1.8
Muscle	21 (20‡)	0.2	0.03 - 1.1 (0.6‡)
Brain	14 (13‡)	0.1	0.00 - 0.7 (0.1‡)

*Corrected to the time of dose and normalized to a body weight of 70 kg.

+After omission of patients with gallium carrier; mean not given unless differing more than 20% from mean of whole series.

‡After omission of possibly spurious high value.



Fig. 57. Autoradiogram from a slice of kidney (patient No. 14, Table 27) showing higher concentration of ^{67}Ga in the cortex, with a complex, delicate pattern of distribution. The "hot spot" over the hilar aspect of the cortex was also seen on an adjacent slice, but histologic examination failed to provide an explanation (lesion lost in processing?). In another patient (No. 3 in Table 27) such a finding led to the discovery of a 2-mm renal metastasis (56).

all with shorter half lives, but we can expect the best values to be within the ranges given in **Table 28.** In this study the factors other than time that are known to affect the distribution of gallium are not clearly manifest and do not seem to cause substantial alterations of the values. Only two patients with much localization of ^{67}Ga in tumor had generally low assay values that might be ascribed to diversion of ^{67}Ga to neoplasm (patients No. 8 and 10, **Table 27**); in other cases the presence of tumor undoubtedly had some effect but it was not clearly apparent. Bones of adolescents tended to have higher concentrations than those of adults. The few effects that might be attributed to the

1027950

addition of stable gallium to the ^{67}Ga doses are minor at the carrier level given to most patients in the 1951-1953 series (0.2 mg/kg body weight); the advantage of a larger number of patients for analysis warrants the inclusion of 11 autopsies in the early series with the 12 autopsies in the current series.

More important from the standpoint of irradiation dosimetry is the demonstration of the considerable variation from patient to patient, and possibly most important, the different concentrations within different portions of the same organ. For example, as shown in Figs. 54 and 57, renal cortex has a higher ^{67}Ga concentration than the renal medulla. Microscopic autoradiograms in rats and mice have shown that much of the activity in the renal cortex is in convoluted tubules, and that much of the activity in the liver, spleen, and marrow is in phagocytes and related histiocytic cells. Because the irradiation from internal conversion and Auger electrons is high focally, permitting detailed autoradiography, dosimetric calculations may need to be elaborated on a comparable anatomic scale.

Organ Monitoring in Erythropoietic Disorders

In the 1969 Research Report (ORAU-110, pp. 103-106) we pointed out the value of the day 1 index of ^{59}Fe uptake over liver or spleen compared to heart as one of three radioisotopic findings useful in the diagnosis of primary myelofibrosis. During the past year we studied one additional patient with myelofibrosis secondary to acute granulocytic leukemia and found a day 1 index of 2.82, a value which progressively decreased during the next five days (Francis A. Goswitz, Helen Vodopick, and Martha L. Clevenger). Necropsy later confirmed extramedullary erythropoiesis. In our experience with 6 cases of extramedullary erythropoiesis studied during the past two years, all had liver or spleen to heart ratios on day 1 greater than 1.75. Except for two patients who had increased iron stored in the liver and spleen, the ratio decreased after day 1. This ratio is based on the principle that ^{59}Fe activity will reach its peak on day 1 since iron must subsequently be incorporated into hemoglobin and released with red cells.

Several studies on patients with bone marrow as the main site of erythropoiesis showed that none had a day 1 index greater than 1.75. Table 29 compares values of our index with that of Gardner's index for the spleen (Gardner and Nathan, New Engl J Med 274, 420, 1966), and supplements the data on extramedullary hematopoiesis given in the Research Report last year. Data for the two different indices correlated well, as might be expected, since the bone marrow in these patients is the principal organ of erythropoiesis. With the sacrum index, only two of the six patients had values greater than two which indicates that normal erythropoiesis can exist when Gardner's indices are below 2.

We find our index useful in detecting early the transition of one phase of a myeloproliferative disorder to another; it substitutes for splenic aspirations or liver biopsies that have their limitations.

Protocol for Optimal Use of $^{*}CoB_{12}$ Tests

To avoid errors in the diagnosis of anemias a modified protocol has been developed for study of vitamin B_{12} metabolism (Francis A. Goswitz and Helen Vodopick).

(1) Collect serum for determination of B_{12} level. Obtain background count in

TABLE 29

EXTERNAL (BODY-SURFACE) MONITORING IN MEDULLARY ERYTHROPOIESIS

Patient	Disorder	Day 1 Index Organ†/Heart	Gardner's Indexes* Spleen	Sacrum
I	Aplastic Anemia	0.76	0.70	1.63
II	Rheumatoid Disease, Iron-Def.	1.19	0.90	2.51
III	AGL, Tuberculosis	0.76	0.71	1.63
IV	Secondary Polycythemia	0.88	1.04	1.41
V	Polycythemia Rubra Vera	1.24	0.86	2.05
VI	Hodgkin's Disease	1.16	0.91	1.62
	Index for Erythropoiesis	>1.75	>2	>2

*Ratio of counts on day 1: day 5 over same organ.

†Either spleen or liver, which ever had the highest count rate.

low-level whole-body counter. (2) Perform the *CoB_{12} absorption test without hog intrinsic factor, assay an 8-hr plasma sample for *Co activity, and obtain reticulocyte counts daily for 3 to 10 days. Count patient in whole-body counter immediately after the dose and subsequently on days 1 and 2. (3) Collect urine for at least 2 days. In chronic renal disease the first 24-hr sample may show misleadingly low excretion. (4) If the blood and urine data indicate decreased absorption, complete the test with at least 60 mg of hog intrinsic factor. (5) If results are still abnormal, repeat the absorption test with 100 ml of normal human gastric juice. (6) If these values are also decreased, determine cause of the malabsorption and treat patients accordingly. (7) If the patient is unconscious and cannot cooperate or is suspected of intrinsic-factor deficiency coexistent with malabsorption, aspirate gastric juice after histamine stimulation; then give the gastric juice with *CoB_{12} to one who has pernicious anemia to determine whether the aspirated gastric juice contains intrinsic factor.

This method differs from standard practices in that we obtain whole-body counts before, immediately after, and daily after ingestion of the *CoB_{12} dose. Such counts are important to obtain information about total-body retention if the patient vomits, has diarrhea, impaired bowel motility, or fails in urine collections. The 8-hr plasma radioassay for cobalt indicates whether there has been B_{12} absorption and confirms urine results. A third addition in our protocol is the performance of the reticulocyte count which should rise within 10 days if an anemic patient is vitamin B_{12} deficient and receives exogenous vitamin B_{12} parenterally. Step 5 is needed to discover patients with antibodies to hog intrinsic factor.

* ^{57}Co, ^{58}Co, or ^{60}Co.

1027951

Whole-Body Counting

Identifying the Organs Comprising the Turnover Compartments of the Whole-Body Retention of Cobalt in Guinea Pigs

Work on the turnover of cobalt metal has continued this year (W. D. Gibbs, J. K. Turner*, J. R. Pringle, Jr.†, Harold D. Hodges, and C. C. Lushbaugh). In studying trace metal and mineral metabolism, whole-body counting can be used to define the total-body turnover kinetics if a suitable radioisotope of the element is available. The whole-body retention curve obtained is commonly analyzed with a multicompartmental model in which the kinetics of the tracer in the various compartments or metabolic pools are considered to be exponential functions. These compartments often have biologic meanings but not uncommonly they are mathematic simplications. If the identities of these mathematically derived compartments can be described in morphologic or physiologic terms, alterations in the whole-body retention curves can then be used to point out how compartmental size and turnover rates are altered in certain pathologic states. An excellent example of how this approach can be applied to pathophysiology is seen in the relation of normal and pathologic thyroid gland morphology with whole-body retention curves of radioactive iodine. Last year we first reported on another example, the use of whole-body counting to define the total metabolic kinetics of cobalt chloride in four animal species. The guinea pig, known to be more sensitive than the mouse, rat, and rabbit to the toxic action of this metal, was shown to have the largest (third) compartment (B^{11}) where cobalt was bound and turned over slowly. On a standard dose the guinea pig could have, at the time of environmental equilibration, as much as 3.6 times his daily cobalt-intake level retained in this metabolic pool, as opposed to only 1.5 times in the mouse.

We have now extended these observations on the metabolism of cobalt in guinea pigs and attempted to identify the three metabolic pools morphologically and functionally to find the target organ(s) in cobalt toxicity.

Sixty guinea pigs were injected intraperitoneally with 0.0035 to 0.0075 millicuries ^{60}Co-labeled cobalt chloride, carrier-free, in saline. There were 12 groups of five animals each: a group was killed at 1, 2, 3, 4, and 7 days and then weekly for 8 weeks after injection. Daily whole-body counts were made on all animals up to the time of necropsy, when the following organs were removed and assayed for ^{60}Co: liver, cecum, lungs, spleen, adrenal, kidney, heart, brain, thyroid, muscle, bone (containing marrow), skin, and blood.

The concentrations in most of these organs showed two definite maxima while only an initial one was found in blood. A second maximum in many organs while the whole-body retention was decreasing occurred because of reabsorption and distribution of a significant fraction of the dose that had been excreted into the intestine. The first maximum was reached within one day in blood and within 2 days in the other organs, followed by a rapid decline to a nadir between 4 and 7 days. The second maximum, in terms of % dose/g, was reached on about the 14th day for all assayed organs except the cecum. The relative amounts and the rate of loss from these two organ-concentration

* Research Participant from Western Illinois University, Macomb, Illinois.

† Summer Student from the University of North Carolina, Chapel Hill, N.C.

TABLE 30

Rankings of Guinea Pig Organs at Time of Their Cobalt Concentration Maxima on Days 2 and 14 after Intraperitoneal Injection

	Day 2		Day 14	
Rank	Organ	%/gm	Organ	%/gm
1	Liver	0.300	Liver	0.035
2	Kidney	0.110	Adrenal	0.014
3	Cecum	0.062	Kidney	0.012
4	Blood	0.052	Cecum	0.011
5	Spleen	0.050	Spleen	0.010
6	Heart	0.046	Heart	0.009
7	Lung	0.045	Bone	0.007
8	Adrenal	0.045	Lung	0.006
9	Thyroid	0.031	Thyroid	0.004
10	Bone	0.022	Muscle	0.002
11	Muscle	0.009	Brain	0.002
12	Brain	0.004	Blood	<0.001

maxima are summarized in Tables 30 and 31, where organs have been ranked according to the second maximum (% dose/g on day 14). In Table 31 we show the rapidity of turnover of the cobalt present on day 14 and the theoretical size (B_o^{11}) of this compartment determined by extrapolation to zero time. Another column indicates the specific organs we think are involved in the four metabolic pools that are defined by this study.

In Table 30 one sees that the liver, kidney, and cecum have the highest initial concentration; blood and other organs have about equal concentrations while striated muscle and brain have the lowest. These findings indicate that the initial blood cobalt level is in equilibrium with the cobalt-ion concentration in interstitial water of all organs except the brain; liver and kidney (and cecum?) actively absorb and clear cobalt from the blood, while the blood-brain barrier retards but does not prevent diffusion of cobalt ion into the brain. The initial decrease in blood and renal concentration is compatible with our previous observation of a half-time of about 12 hr for the first whole-body retention compartment (U_o) which is reduced by urinary excretion (see Fig. 58).

The comparatively large initial uptake and the subsequent ten-fold decrease in

1027952

hepatic concentration of cobalt indicate that biliary-fecal excretion occurs. The subsequent rise to the second organ concentration maximum at 14 days requires the presence of some reabsorption mechansim. Although, according to the literature, the guinea pig does not have cecal-bacterial binding of cobalt or intestinal synthesis of vitamin B_{12}, these data suggest that the cecum is somehow involved in recycling this metal. The hepatic-excreted cobalt unabsorbed by the cecum is lost in the feces and is identified then as our first "bound" cobalt compartment (B'_o) with a 2-day half-time quite consistent with other fecal transit time measurements. The third compartment, the second "bound" cobalt compartment (B''_o), then must represent the cobalt actively bound or reabsorbed in the cecum, redistributed and metabolized with a half-time of about 20 days. In Table 31 the bound (B_o'') cobalt turnovers are shown to be 18 to 25 days for all organs except liver, cecum, thyroid, and brain. The correspondence of hepatic and cecal-bound cobalt turnovers, 9 and 10 days respectively, suggests their mutual involvement in the same metabolic process.

The finding of about 0.2 to 0.5 percent of the initial dose of cobalt/g of brain and thyroid with half-times of 42 and 41 days has potential significance for this

TABLE 31

GUINEA PIG ORGANS RANKED BY THEORETICALLY MAXIMUM SIZE (B_o'') OF THEIR BOUND-COBALT CONTENT FROM CECAL REABSORPTION.

(Their Possible Metabolic Pool Involvements and Turnover Rates are Shown)

		Bound Cobalt Turnover		
Rank	Organ	B_o''	$B''T_{50}$ (days)	Possible Metabolic Pool Involvement (See Figure 1)
1	Cecum	0.110	9	3
2	Liver	0.100	10	2, 3
3	Lung	0.010	18	3
4	Spleen	0.017	18	3
5	Muscle	0.005	18	3
6	Bone	0.017	19	3
7	Kidney	0.018	22	1, 3
8	Adrenal	0.021	23	3
9	Heart	0.013	25	3
10	Thyroid	0.005	41	3, 4
11	Brain	0.002	42	3, 4
12	Blood	0.001	> 400	1, 3, 4

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1027953

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problem. There are several possible ways to explain logically these and our previous findings in relation to the susceptibility of the guinea pig to cobalt-ion toxicity. Our failure to find this binding previously demonstrates that the truly minute trace amounts involved, combined with a long half time, may be undetectable and prevent definition of this metabolic compartment by whole-body counting. Such slowly turning over traces may be seen only as an increase in the size of the compartment that has the next more rapid turnover time. The last definable compartment could be partitioned further after a prolonged observation of whole-body retention if we could increase either the sensitivity of the counter or the amount of residual radioactivity. The size of a long-lived residuum might be increased biologically by feeding the isotope chronically rather than giving a single injection of a large dose. Such experiments will be required to evaluate our present hypothesis which is that toxicity is due to an initial binding of inorganic cobalt by the thyroid and brain during blood-interstitital space equilibration as suggested by these findings and shown in Fig. 58.

Our working hypothesis of possible routes of cobalt metabolism in the guinea pig (Fig. 58) would seem to fit also the whole-body retention models for the mouse, rat, and rabbit reported last year. The rabbit is unique in that it manufactures vitamin B_{12} in its cecum and obtains it for absorption in the upper small intestine by coprophagy. Our previous measurements in the rabbit show the increase in size (34 vs 18%) of the

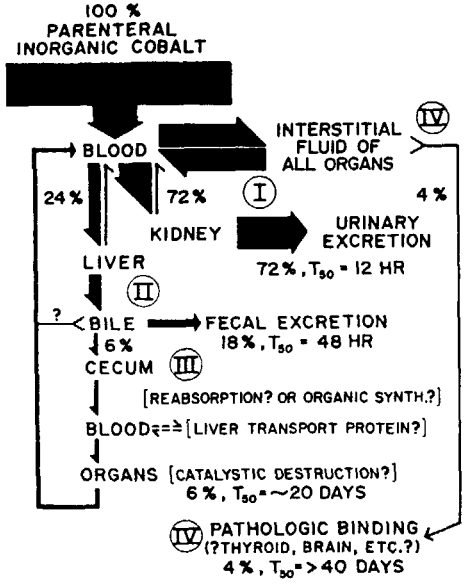


Fig. 58. Tentative compartmental model of metabolism of parenterally acquired inorganic cobalt in guinea pigs. The encircled roman numerals identify major metabolic pools indicated in Table 30. See text for further explanation. The question marks emphasize some of the more obvious areas of conjecture and uncertainty.

1027954

hepatic-cecum-fecal excretion route (Bo[1]) that this physiologic process would require. The short half-time in the third compartment for the rabbit (13 vs 21 days) requires further explanation but fortifies our conclusion that this compartment, as it is now defined by us, may be comprised of several different metabolic pools, not necessarily physiologic.

Instrumentation and Computer Technology

Improving Our Electronic Tools

Electronic interface equipment has recently been developed to control and transmit data from a four-probe counter to the IBM-1800 computer (A. C. Morris, Jr., T. R. Barclay, and Paulette Aaron) (Fig. 59). Through this interface the system can rapidly perform and analyze many dynamic function studies on patients or large animals where external monitoring data from several sites is required. Studies in ferrokinetics, erythrokinetics, granulocyte survival after splenic irradiation, and blood lymphocyte survival will now be possible with continuous counts being received from detectors placed over four organs (e.g., heart, liver, spleen, bone marrow). These counts acquired from a specific organ will help us to understand the kinetics for certain radionuclides as they are distributed and redistributed throughout the body. Other more standard studies will be possible with this system, such as measurements of renal and lung function, cardiac output, circulation time, hepatic blood flow, evaluation of extramedullary erythropoiesis, and erythrocyte sequestration. Before a clinical experiment, the four crystal probes are first equalized for counting rate and positioned over the patient sites. The physician requests program time from the computer. The isotope is injected into the patient and simultaneously computer operation is initiated. The pulse data are received and converted into counting rates. From these, voltages are generated in proportion to the



Fig. 59. Technician operating interface controls during a patient study using the four-probe counter. Signal lines run from this equipment to a computer, located 450-feet distant, that records and analyzes the counting-rate data.

rate received and transmitted from the analog output section of the computer back to the experiment site, where they are graphed by a four-pen recorder. The physician can thereby watch progress and make adjustments as required. This system is able to change the parameters of an experiment as it proceeds. For example, the physician might want to sample rapidly during the initial stages, and later, when the isotope becomes more generally distributed, change to a much slower sampling rate. Our programs call for the computer to store all the counting rates received during an experiment. When the procedure is completed, the computer will automatically subtract appropriate background rates and generate the required counting profiles which can be graphed on the computer plotter and can also be assembled in tabular form on a printer. We also require the computer to make basic calculations and do smoothing operations on the received data. Possible calculations include percentage uptake versus time after injection, time to maximum counting rate, time from maximum counting rate to a return to half-maximum value, first and second derivatives of the curve, and the integration of all counts under the curve. This interface system gives the experimenter a great deal of flexibility in operating the four-probe system, plus the capability for analysis by the IBM 1800 computer. Construction of the interface has been completed this year and we are in the midst of programming for initial clinical use.

Over the past four years we have been using a physiologic system in our low-exposure total-body irradiator (LETBI) to monitor patients receiving radiation for changes in EKG waveform, heart rate, pulmonary waveform, respiration rate, and rectal temperature. Signals are transmitted on-line to the IBM-1800 computer for analysis. More recently need has arisen to monitor patients receiving treatments in the medium-exposure total-body irradiator (METBI) and at the Variable Dose Rate Irradiation Facility (VDRIF) (located at the UT-AEC Agricultural Research Laboratory). Moreover, we need to monitor patients in hospital rooms pre- and postirradiation, and in the laminar-air-flow (LAF) clean room. To fulfill these needs we obtained a commercial roll-around physiologic monitor and have wired and modified it to fit our requirements (A. C. Morris, Jr. and T. R. Barclay). The mobile system has one more signal channel than its stationary counterpart which we expect to use to measure galvanic skin resistance (GSR). Figure 60 shows this new equipment being used to compile baseline data on a patient's response to heavy bicycle exercise. For such applications we have incorporated two subminiature NASA preamplifiers into the signal lines attached to the front of the patient's belt. This arrangement reduces interference in the physiologic signals when the subject is in a remote location (ours are less remote than NASA's). At the VDRIF, with the use of this preamplifier connection, noise-free signals have been obtained over an 82-ft distance. Among several research instruments that have been interfaced to our IBM-1800 digital computer over the past few years are the Packard "Tri-Carb" liquid scintillation counters, located at the Lipid Laboratory four miles from the computer site. The on-line instrument requires initial input of information identifying the experiment. Because of difficulties and limitations in the original equipment for entering these data, a new device (Fig. 61) has been built for this purpose and is now operating successfully (T. R. Barclay and A. C. Morris, Jr.). It contains several thumb-wheel switches, each corresponding to a column of the preliminary data format. A selection circuit is controlled by the computer to interrogate each switch setting. Position-indicating lights and a manual stepping switch are also built into the selection control circuits. Information received by the computer is printed on a typewriter at the computer site. Then the experimental data are serially transmitted on-line to the computer. A switch and eight test jacks have been incorporated in the data-entry module to facilitate rapid troubleshooting of the telephone-line circuits. Also included are zener diodes connected between the incoming telephone lines and ground to protect the system against damage from lightning or other high-voltage transients. Future plans include incorporation of a teleprinter terminal at

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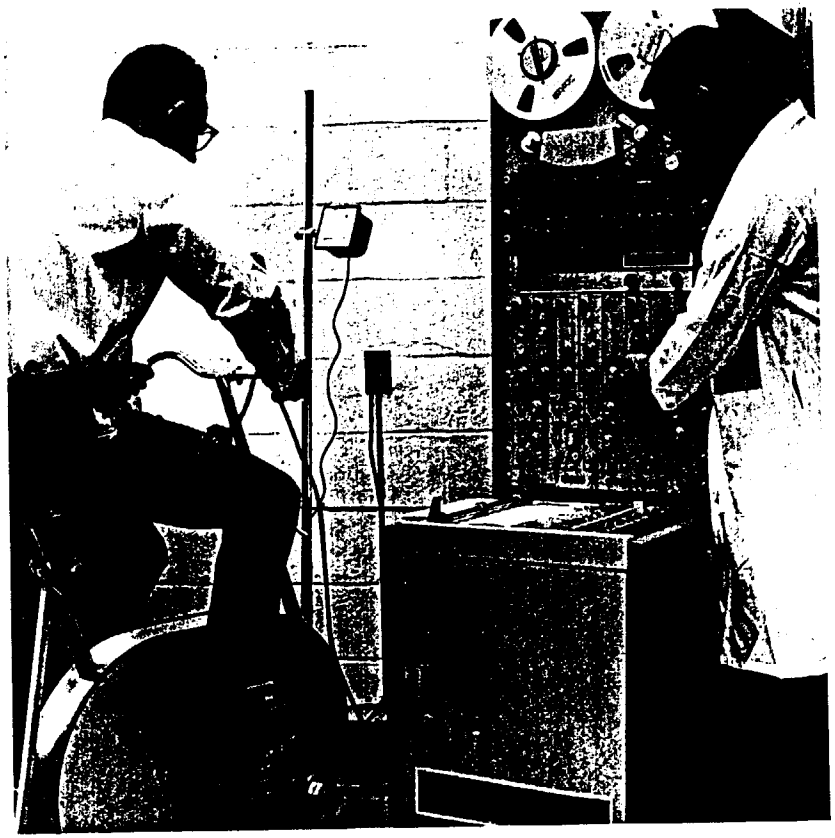


Fig. 60. Mobile physiologic monitor being used to obtain baseline data from a patient undergoing periods of constant exercise stress. Both the degree of stress and the duration of exercise are varied in these studies. Cables from this monitoring equipment can be connected to an on-line computer for rapid analysis of the patient's data.

the Tri-Carb laboratory counting room that will print the results of the computer's analysis.

As an aid to personnel during scanning procedures, we have interfaced a variable-persistence storage oscilloscope (Hewlett-Packard Model 1201A) to our scintillation camera (T. R. Barclay and A. C. Morris, Jr.). The same X and Y positioning signals used for the standard image display are routed to the new storage oscilloscope. It has the major advantage of holding in view on the screen the image of the gamma-ray emitting source and the viewer does not have to wait for a Polaroid film to integrate spots. A variety of scan images can therefore be generated, depending upon intensity,

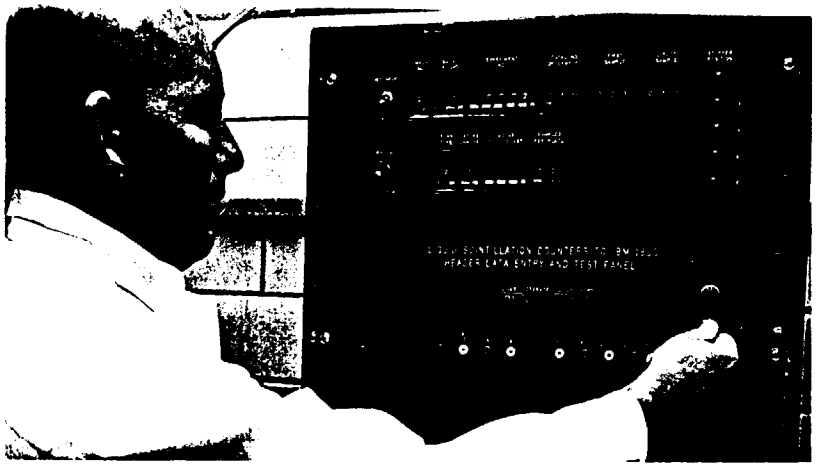


Fig. 61. Remote identification interface for Tri-Carb counters in operation.



Fig. 62. Oscilloscope presentation from a clinical study using the storage interface connection to the gamma-camera.

1027956

persistence, storage time, and gain settings. During any stage of a scan the build-up of the dots on the screen can be halted and the image displayed in the "store" mode (Fig. 62). The image construction can also be faded to a variable degree or erased at any time during a procedure. A Polaroid camera is available that adapts to the screen face for recording any desired display. This storage-oscilloscope interface will prove an invaluable tool when dynamic functions are to be studied, or when some small defect in the source distribution must be located, clarified, and photographed.

Building Our Computer Resource

During our work with the Radiation Effects Data Bank, which now includes an unparalled reservoir of information on effects of total-body irradiation in the human being, we developed a system to process, retrieve, and graph the data (Paulette Aaron, Mona Smith, Earl McDow, Jr., and C. C. Lushbaugh). This system is presently used for retrospective studies but could be used in daily clinical data banking.

In order to process any clinical data, it is first encoded onto one of the 22 digital formats, such as those for hematology, clinical chemistry, or medications. Fig. 63 shows as an example, the format for hematologic results. After being punched on cards, the data are placed on magnetic tape and subsequently sorted onto the proper format tape by means of the IBM Sort/Merge package and an IBM S/360 Assembler Program.

All retrieval programs for the data bank are based on clinical case summaries that are recorded on tapes; retrieval involves a sequential search of the tapes for the selected formats to extract the data required for specific analyses. The case summaries are divided into five radiation-exposure categories of which the first four deal with total-body exposures: (1) greater than 8 days fractionated exposures, (2) less than 8 days fractionated exposures, (3) single exposures that are free of any other radiation therapy within a 12-week period, (4) continuous or protracted exposures of the Heublein type, and (5) portal thoracic and abdominal fractional exposures. As data are added to a data bank, audits can be done to list its current contents. Such an audit will show the number of males and females, classification in the five graphs first listed, radiation dose, dose distribution, diagnosis, and whether there is information on formats for nurses' notes, general hematology, and leukocyte differential counts. The instant availability of the audit allows a check to be made on the amount of information in the data bank before valuable computer and programmer time is used to search formats that may lack adequate data for analysis.

Data-bank housekeeping and auditing have identified many errors in the data itself. One of the most important programs for data maintenance is a record correction program developed for use on any of various formats in the data bank. The program has been most instrumental in organizing the bank in its present, workable form.

The next step is data retrieval; this has been completed for the nurses' notes format and hematology format. The nurses' notes were analyzed by a data reduction program that determined the incidence of nausea, anorexia, and vomiting with respect to time of radiation exposure.

Data reduction and retrieval of hematologic information have been completed for the first four categories of radiation exposure. We have a table with the values for hemoglobin, hematocrit, white blood cells, and platelets for each case during exposure and for 42 days before and after. These data are being studied by probit and multiple regression analysis. Another program computes the regression line for each set of

Fig. 63.

HEMATOLOGY
(RBC)

Card No. H O (1 2)

Institution No. (3 4)

Hospital (Patient) No. (5 6 7 8 9 10)

Date (Month, Day, Year) (11 12 / 13 14 / 15 16)

Hour (24:00) (17 18 : 19 20)

Red Blood Cell Count (RBC) (21 22 . 23) Million/cu mm

Hemoglobin (24 25 . 26) G/100

Hematocrit (27 28 . 29) %

White Blood Cell Count (WBC) (30 31 32 , 33 34 35)/cu mm

Sedimentation Rate (36 37) mm/hr

1. Corrected
2. Uncorrected
9. Not stated

(38)

Sedimentation Method (39)

1. Wintrobe
2. Cutler
3. Westergren
4. Landsberg
8. Other (specify)_____
9. Not stated

Volume Packed Buffy Coat (40 41 . 42) %

Platelet Count (43 , 44 45 46 , 47 48 49)/cu mm

Platelet Method (50)

1. Phase (Brecher-Cronkite)
2. Rees and Ecker
3. Smear count related to red cells
8. Other (specify)_____
9. Not stated

Reticulocyte Count (51 52 . 53) %

Other counts related to RBC
(Write out name and number/unit)_____

Platelet count (No. 3 method) (54 55 56)/1000 RBC

1027957

HEMATOLOGY

Total Blood Volume (57 , 58 59 60) ml

Plasma Volume (61 , 62 63 64) ml

Red cell mass (65 , 66 67 68) ml

Red cell Survival (Cr^{51} Study) (69 70) T/2 (Days)

Eosinophil Count (71 72 73 74)

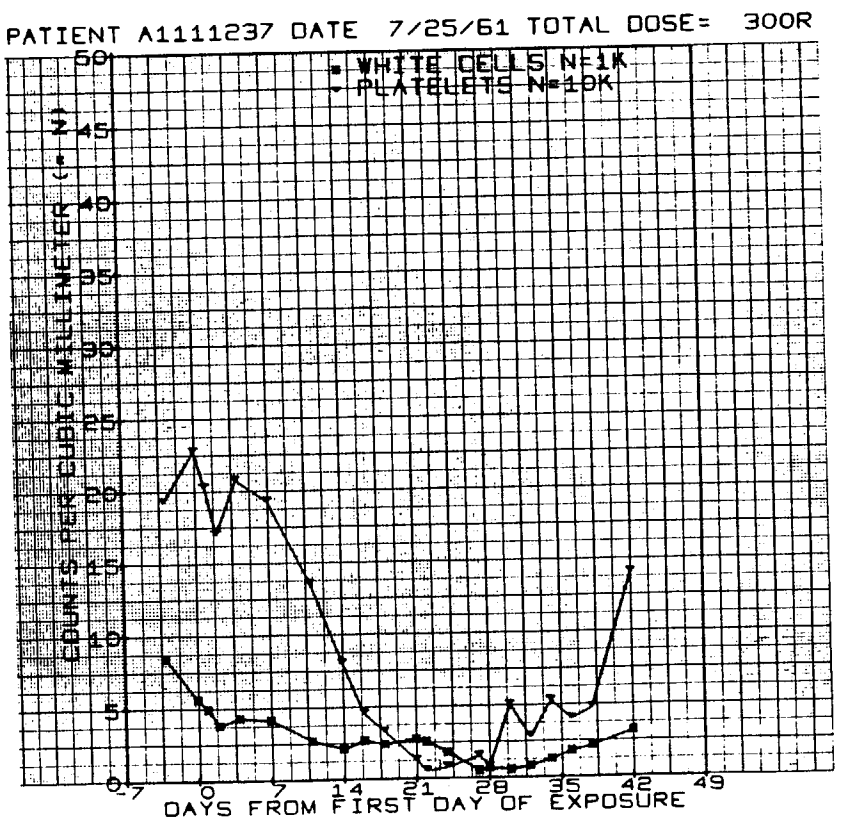


Fig. 64.

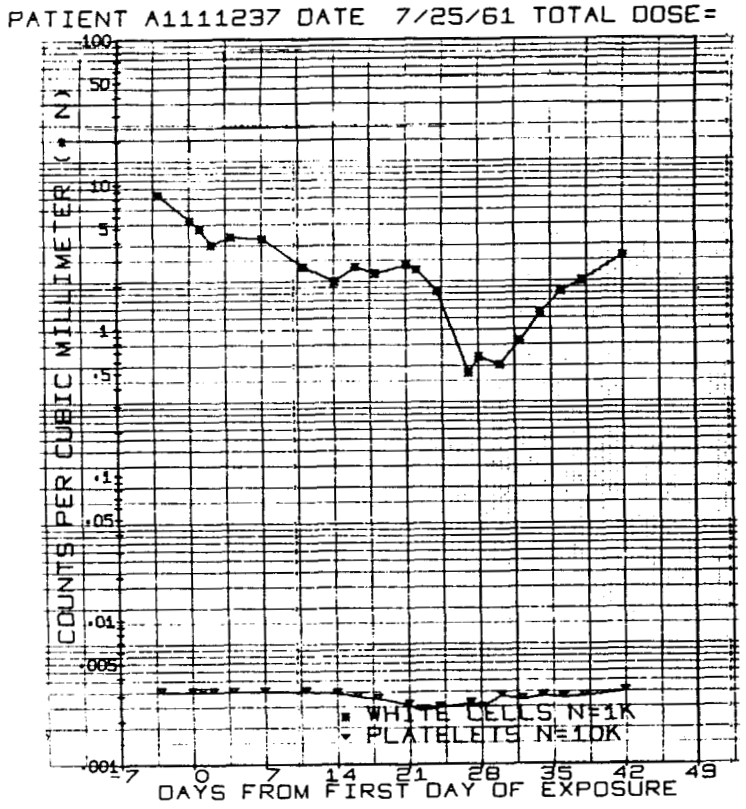


Fig. 65.

responses and its 95% confidence interval; a plotting program for the IBM 1800 1627 plotter graphs these data automatically. Examples of a linear and a logarithmic plot of hematologic values are shown in Figures 64 and 65, respectively. It is now apparent that data retrieval and subsequent reduction could be done more expediently and more cheaply if the bank were stored on two disk packs rather than 28 tapes. One of the greatest advantages of using a disk-oriented system is that data access can be random rather than sequential. We hope to make the transfer to disks as soon as feasible. With or without this improvement, it is apparent that with these techniques many additional types of data can be processed, retrieved, and graphed in a way that will distinctly aid the research program.

Last year we reported the development of a system using digital computer programming to make power spectral estimates of 4 min segments of impedance pneumograph analog traces. We have recently modified the program to afford greater data reduction and a less complicated measurement (Robert C. Ricks,

1027958

Ann Sipe, Earl McDow, Jr., Edward Frome*, and C. C. Lushbaugh). The original system reduced the mass of analog data to a two-dimensional graphic display defined as the power spectrum (variance) of pulmonary impedance changes at the respiratory frequencies that occurred. The initial application of this physiologic monitoring system was in studying gastrointestinal distress in irradiated patients. We showed that the method can be used to detect and measure the effect of any stress which directly or indirectly alters respiratory efforts. The modification is based on the fact that the area under a pulmonary impedance power spectrum is the total variance in terms of amplitude and frequency of respiration. This single number can be computed and used, therefore, as a one-dimensional expression of the level of respiratory effort.

When this number is plotted as it changes with time, one gets a continuous display system of respiratory effort. This new system requires approximately one half the previous computing time; the output consists of an index (the mean and variance of the power spectrum) that can be used without graphing the individual power spectrum. In this case, the mean is the average transthoracic voltage as measured by the impedance pneumograph coupler, while the variance is directly proportional to the area under the power spectral curve. The actual power spectrum is not graphed in this new system unless it is requested by the investigator or clinician. In addition this new method provides separate analysis of each of 4 consecutive minutes of pulmonary impedance data instead of one combined analysis of 4 minutes of data. An average mean and variance (sum of four 1-minute data periods/4) also is provided. We have defined the minute-by-minute variance as the continuous variance and their average over 4 minutes as the average variance. Fig. 66 shows a typical output obtained by the old method in a volunteer who had ingested an emetic. Shifts in the pulmonary impedance power spectra occurred between the 17th to 20th minute and the 49th to 52nd and 57th to 60th minutes. These shifts and large increases in the pulmonary impedance power spectra correspond with periods of nausea and vomiting. This same data analyzed by the new system is shown in Fig. 67. Increased continuous variance corresponds exactly to the minute with the occurrence of severe nausea (18th minute) and emesis (50th and 59th minute). These changes in respiration are also well portrayed by the average variance which tends to smooth the data without losing fidelity. Comparison of Figs. 66 and 67 shows that the inferences drawn from the results would be the same except that the data are reduced to a more easily comprehended form in Fig. 67.

An automatic graphic plot-routine program adds to the data reduction capabilities of this new method. It draws and letters the graphs of the pulmonary impedance analysis program saving considerable time of the graphic artist and investigator. The plot routine is flexible and can be altered easily to accommodate a variety of different time scales (minutes, hours, minutes/day(s), etc.). We anticipate that this new approach will greatly enhance our capabilities for quantitating radiation-induced gastrointestinal distress and performance decrement in man.

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* Emory University (PHS Fellow).

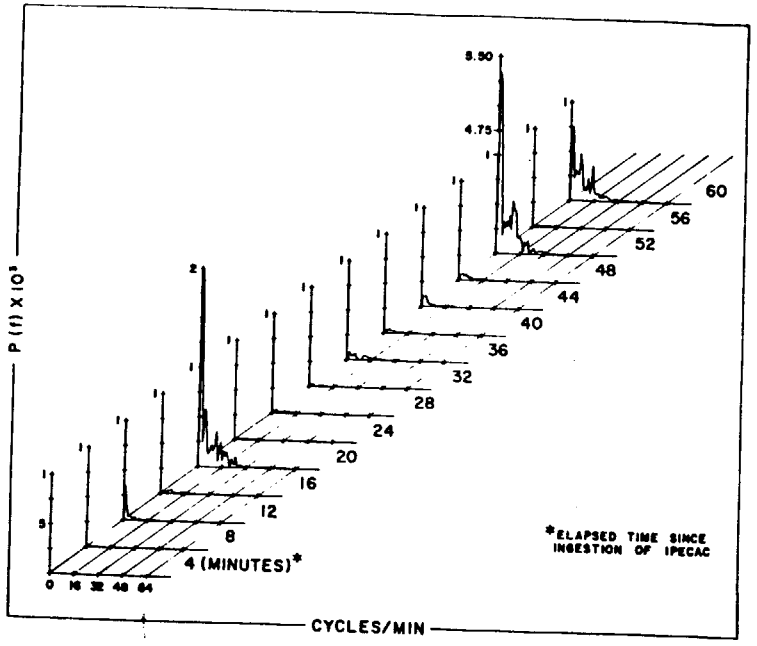


Fig. 66. Pulmonary impedance power spectra obtained from a volunteer following ingestion of an emetic.

The main function of our computer system (IBM 1800) is as a tool for research. To be effective, the hardware and software components must function together in an orderly fashion. The operating system as supplied by IBM is maintained and modified where necessary by ORAU to provide a basis for programs tailored to meet our needs. While each computer program must meet the individual investigator's requirements, it must also operate smoothly with all other system components. With these objectives in mind, we are seeking new areas of computer application and ways of improving existing services (Earl McDow, Jr.). Some of the accomplishments are explained below.

To provide the flexibility necessary for cable connections between the experiment interface and the computer, we installed a plugboard patch panel as the terminating point for all computer interface cabling. It replaces a hardwired system and now a computer interface can be added, deleted, or changed easily and efficiently.

A video output device has been installed to extend the output capabilities of the computer. It is in operation at the computer site but can be expanded to include remote locations. Several demonstrated uses include display of clinical laboratory results and scanning data.

1027959

We now use two general purpose subroutines to give easier access to the plotter capabilities. These programs provide scaling, axis generation, and labeling for linear and logarithmic plots. These routines have been used extensively for both NASA and AEC research projects.

In addition to the uses of the paper-tape reader in the whole-body counter and cell sizing programs, it is being used to read spectral data from various multichannel analyzers. The program currently supports the code from the multichannel analyzer of the Radiation Safety Office and the code generated by the Special Training Division's four thousand channel analyzer. The data are plotted logarithmically on the digital plotter and linearly on the line printer, with channel counts and summations. Data formats different from the above can be incorporated easily.

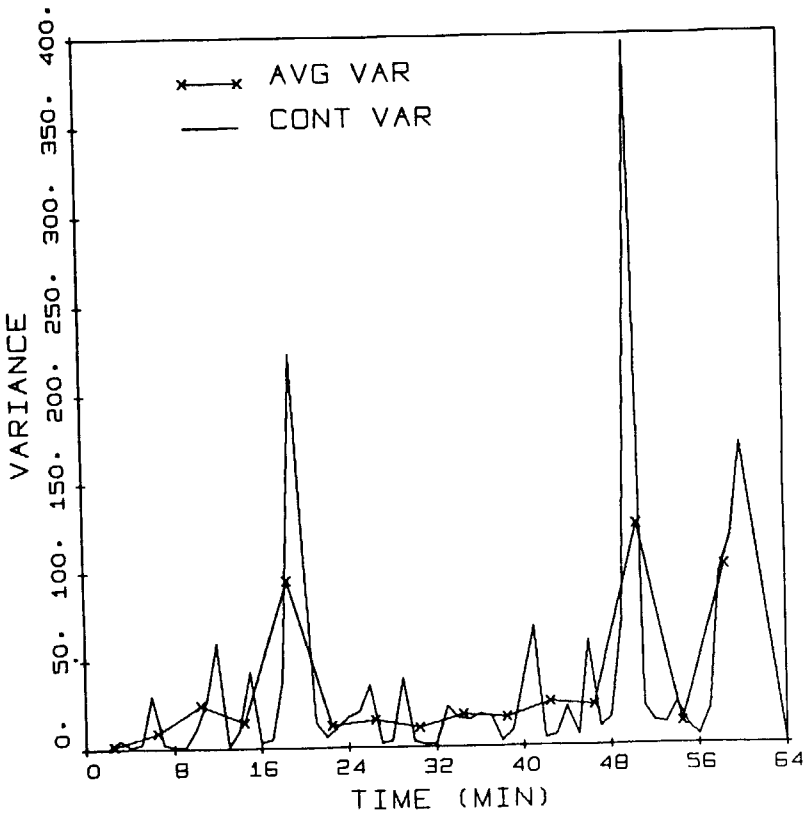


Fig. 67. Graphs of continuous and average variance of pulmonary function in a volunteer after ingestion of an emetic.

The computer has become a significant tool in reducing the man-hours of work on routine mathematical problems relating to research.

The computer section studied extensively the procedures used in analyzing data from the Division's human and animal whole-body counters and formulated a computerized system for routine storage on magnetic tape of the data as it is collected (Mona Smith and W. D. Gibbs). Data can now be retrieved and analyzed in a variety of ways. The most demanding effort, and first in importance, was to provide analytical procedures for the studies of whole-body retention. This involves making certain adjustments in the raw data: transferring data from tape or cards to magnetic tape, selecting the appropriate energy region for the particular nuclide, comparing with a reference source, and correcting for radioactive decay. The resulting curve shows retention as percent of administered dose and allows calculations of the slopes of the curve. A program was constructed to perform these functions on a routine basis; it was field-tested and modified so that it would require less operator time and intervention. The time of operation was cut by 75% and a method was developed to run analyses without an operator during off-shift hours. A special program permits the Radiation Safety Office to use the same data bank for an accurate report of whole-body counting of employees.

While writing the programs to create the data banks and to allow for their analysis, we encountered other problems that required numerous additional computer programs. One major one was the necessity of correcting error in records caused by encoding mistakes, oversight, etc. Consequently we developed a new updating program that can correct any conceivable mistake. Also, to manipulate magnetic tapes, we wrote several special purpose programs that will (1) duplicate tapes, (2) test for tape errors using various methods and messages, (3) place end-of-file marks where desired, and (4) perform merging operations. The new system, with a minimum of programmer effort, allows for a rapid, accurate analysis of data with varied options and numerous opportunities for computer assistance to research studies.

The experience with the computer in dealing with specific types of research data has encouraged us to broaden the scope of information on our research patients that will be handled by the computer. It is expected that we will increase the accessibility and shorten the retrieval time for much information of research value. For some time now the reports of bone-marrow examinations and certain other types of laboratory reports have been handled in this way.

We have begun by surveying the types of additional data to be handled. Generally, programs will be written in three steps: one for receiving data and storing on magnetic tape, another for selecting desired data from tape, sorting, and storing on disks, and a third for retrieval, which will direct the data to optional output units (printers or optical scope output). Ultimately, the physicians will be able to retrieve easily and promptly many of the kinds of information now recorded in a patient's chart; for example, medications, fever, fecal and urinary excretions, and chemical and hematologic values. Eventually we will use the data bank for research statistical analyses.

Large quantities of data are involved in a comprehensive study of distribution of radionuclides in tissue; hence a set of computer programs were written to aid the researcher (Earl McDow, Jr., Bill Nelson, and Brenda Pritchard). The initial application was limited to data from patients who had received gallium; however, the program can handle any combination of radionuclides.

The first step generates a data file from radioassay data sheets and then verifies the data. An update program provides the ability to add, delete, and correct

1027960

data. Five additional programs give the researcher the flexibility of sorting and listing the data in any desired order.

Three other programs provide a first stage in data analysis. When multiple samples are available, the programs calculate a mean concentration value for each organ of each patient. Based on the criteria selected by the researcher, the patient organ data are grouped into categories for comparisons and for statistical analyses which the computer is set up to provide.

The computer program designed for on-line acquisition and analysis of data on a single isotope has been modified to process data from experiments with double isotope labels (^{3}H, ^{14}C) (R. F. Queener* and Earl McDow, Jr.) For the lipid project the current program will acquire and process, in real-time, the area and zonal scans of single and double labeled thin-layer chromatograms. The area scan program provides the net count, percent of total for each sample, and the total recovery. The zonal scan program, in addition, plots for each sample the disintegrations per minute for double-isotope studies and counts per minute for single-isotope studies.

The current program enters as program constants the efficiencies for each channel; however, plans are being made to modify the program so that these efficiencies will be calculated automatically with each experiment. The output format of the double-label zonal scan also shows a ratio of ^{3}H to ^{14}C under each peak.

A cooperative study with Oak Ridge National Laboratory and Baptist Hospital, Knoxville, is in progress to evaluate a currently available computer electrocardiogram (EKG) analysis program of the Mayo Clinic and to develop a new analysis procedure based on Fourier analysis (Earl McDow, Jr., M. R. Patterson,† and Frank London‡). We received daily from Baptist Hospital, Knoxville, an analog tape containing EKG data that was played back on a marquette computer interface terminal and digitalized by our computer. The digital data was then processed by the Mayo Clinic EKG program which generated a printed diagnosis for Baptist Hospital and a digital tape for Fourier analysis at ORNL. The first phase of this project involved first the collection of 500 EKG's (Frank Lead) and their analysis by the Mayo Computer Program.

The second phase, Fourier analysis, involves determining the amplitudes of the component frequencies of the X, Y, and Z Frank lead waveforms. After normal frequency distributions are established, the frequency distribution of an EKG under study will be compared with the normals. It is expected that deviations from the normal will be useful in spotting an abnormal EKG and in understanding the nature of its abnormality.

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† Oak Ridge National Laboratory.
‡ East Tennessee Baptist Hospital.

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM

Summary of Accomplishments

In the biological chemistry program, the biosynthesis of O-alkyl and O-alk-1-enyl glycerolipids was elucidated through enzymic studies; the specific sequence of steps in formation of the former was established and a collaborative study with the University of North Carolina led to proof of the identity of O-alkyl ketone intermediates. A cell-free system that synthesizes plasmalogens was reported and described. Other significant progress in biochemistry is reported in a summary at the beginning of that section *(see page 135).*

In the marmoset program, a red cell antigen was defined. It is useful as a marker to show chimerism of the erythropoietic series. It was shown to be inherited as a Mendelian dominant. Marmoset hematopoietic cells derived from marrow and from peripheral blood were successfully cultured in vitro and sometimes continued to proliferate for many weeks, thus opening a new technique for study of chimerism and immune compatibility of chimeric co-twins. A further summary of recent research is given at the beginning of the section on marmosets *(see page 153).*

Electron microscopic studies of ^{67}Ga in the thymus of AKR mice showed that there is a pronounced concentration of this nuclide in phagocytic cells, and these cells are more common in the thymus of leukemic mice than in the thymus of normal mice. Whether there is also a greater uptake of the radioisotope in leukemic than in nonleukemic thymocytes is not yet clear. In both normal and abnormal cells of various types the ^{67}Ga appears to be localized mainly in dense bodies of the cytoplasm believed to be lysosomes. In a separate investigation it was demonstrated, by means of colloidal lanthanum nitrate added to the fixative, that the electron-dense material previously seen in association with large germinal center cells is clearly extracellular. Other ^{67}Ga studies are reported in the second section of this report.

In the cytogenetics program the latest assessment of cumulative data on oral contraceptives indicates that these drugs do cause a small but distinct increase in chromosomal abnormalities in lymphocytes. The very extensive project has yielded interesting information on incidence and seasonal variations in abnormal chromosomes in control subjects. Two patients with acute leukemia had chromosomal markers in the leukemic clones. One of these patients was the one who received a high dose of total-body irradiation and a marrow graft; in him the leukemic marker disappeared after the special treatment. In this patient after radiation, chromosomes were unusually loosely coiled and it was possible with light microscopy to see the subchromatids and to count the number of loops resulting from their crossing.

A special study of two patients with spontaneous cyclic leukocytosis and thrombocytosis in the course of chronic granulocytic leukemia helped to clarify some aspects of the cycling phenomenon.

1027961

An earlier study on an obscure thyroid disorder in marmosets gave rise to an investigation of the effect of DDT on the pigeon thyroid. The DDT caused decreased or absent binding of radioiodine in the thyroid and hastening of whole-body radioiodine turnover with resulting hypothyroidism; these effects were dose-dependent. The damaged thyroid glands were hyperplastic and had increased weight.

Biological Chemistry

Introduction

A goal of the biological chemistry unit during the past several years has been to elucidate certain aspects of the complex chemistry of the cancer cell and its molecular derangements. Our work has dealt with the metabolism of membranes (microsomes) isolated from the endoplasmic reticulum of a diverse group of normal and neoplastic cells of varying growth rates. The microsomal fraction of all cells consists of three major components in varying proportions: the free ribosomes, rough-surfaced membranes with ribosomes attached to their outer surfaces, and smooth-surfaced membranes that are devoid of ribosomes. The attached ribosomes are responsible for the synthesis of secretory proteins, whereas the membranous tubules segregate and transport the newly synthesized proteins. Of the many functions associated with the endoplasmic reticulum, we have been specifically interested in a number of its catalytic properties and its biochemical organization.

During the course of our research with the cancer cell, we have discovered several new metabolic reactions (Figs. 68-70) that occur in the endoplasmic reticulum. These pathways involve lipids that are prevalent in cancer cells. Some of the reactions involve hydrogen transfers that apparently reflect the high reductive state of the cancer cell.

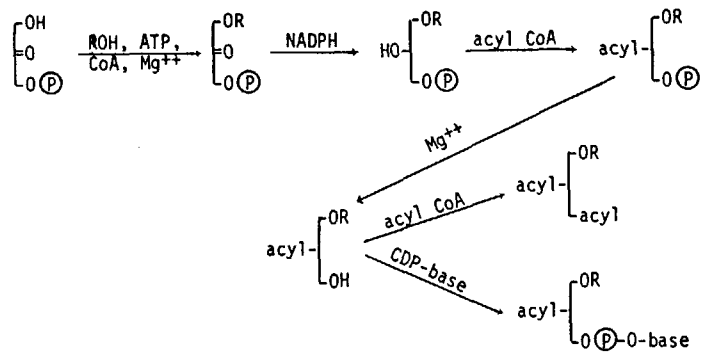
The most significant result accomplished in 1970 was the isolation of an enzymic system from Ehrlich ascites cells that synthesizes plasmalogens (O-alk-1-enyl glycerolipids). It is the first cell-free system known to make plasmalogens, a group of compounds that were first detected by a group of German scientists in 1924 (Feulgen and Voit). Nervous tissue, neoplasms, bone marrow, and heart are rich sources of plasmalogens: their chemical structure was determined only 12 years ago by Rapport and his coworkers. Although the exact reaction mechanism for the biosynthesis of plasmalogens is not known, we have been able to elucidate the steps in the biosynthesis of O-alkyl glycerolipids (Fig. 68).

The sequence of reactions involved in the pathway for O-alkyl glycerolipids, including the ketone intermediates, was postulated by us in 1968 on the basis of the reaction occurring between long chain fatty alcohols and glyceraldehyde-3-P. The entire sequence has now been firmly established by us in a series of five papers that were published during 1970 (23, 24, 26, 29, 43). The O-alkyl ketone intermediates, characterized in the enzymic experiments, have been synthesized (Fig. 71) and verified in the organic chemistry laboratory in collaboration with Dr. Claude Piantadosi* and Dr. K. S. Ishaq*.

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* Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill.

A. BIOSYNTHESIS OF *O*-ALKYL GLYCEROLIPIDS



B. BIOSYNTHESIS OF *O*-ALK-1-ENYL GLYCEROLIPIDS

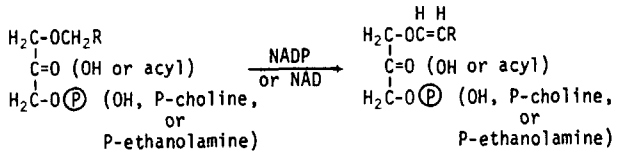


Fig. 68. Enzymic pathways involved in the biosynthesis of ether-linked glycerolipids.

The complete metabolic pathway for O-alkyl lipids consists of the following steps: (1) Alkylation of dihydroxyacetone-P, (2) Reduction of the ketone by an NADPH-linked reductase, (3) Acylation to form the phosphatidic analogue, (4) Dephosphorylation to form the diglyceride O-alkyl analogue, and (5) Acylation to form the triglyceride O-alkyl analogue or (6) Transfer of phosphorylcholine or phosphorylethanolamine via cytidine diphosphate to the diglyceride O-alkyl analogue. Once the phosphatidic O-alkyl analogue is synthesized, the reactions that occur are identical to those that are well known in the synthesis of normal acylated glycerolipids, and in fact the same enzymes probably utilize the O-alkyl analogues as substrates.

We have also examined the specificities of acid and alkaline phosphatase for key intermediates involved in the biosynthesis of complex phospholipids. Alkaline phosphatase is highly specific since it only removes free phosphate groups from glycerolipids when the 2-position possesses a hydroxyl or ketone moiety. In contrast, acid phosphatase is active independently of the nature of the 2-position (Fig. 70). The significance of these findings to diseases involving abnormal phosphatase activities remains to be investigated.

During the last year, insights on the nature of the mechanism responsible for the cleavage of the alkyl ether bond have emerged from studies of a liver enzyme system (a tetrahydropteridine-requiring hydroxylase) that utilizes O-alkylglycerols as a

1027962

substrate. New requirements of ammonia ions and sulfhydryl compounds have been established for optimal activity of this enzyme (Fig. 70). Activities of the cleavage enzyme are absent or depressed in the cancer cell. Data suggest that the cleavage enzyme is an important regulator of ether lipid levels in cells.

Specific enzymes and mechanisms involved in the complex assembly of fatty acids from acetate and malonate by two separate cellular systems have been known for some time. In contrast, until this past year essentially nothing has been known about the biosynthesis of long chain fatty aldehydes and long chain fatty alcohols except as a by-product of several degradative reactions involving complex molecules, e.g., O-alkylglycerols, sphingosine, or dihydrosphingosine. Because of the precursor role of fatty alcohols in the biosynthesis of ether-linked lipids, it became necessary to explore their origin in mammalian tissue. Our experiments have demonstrated that a microsomal NADPH-NADH-linked reductase can reduce fatty acids to the corresponding fatty alcohols and that the reverse reaction can be mediated by an NAD-linked enzyme (Fig. 69). These enzymes appear to be of special importance in the

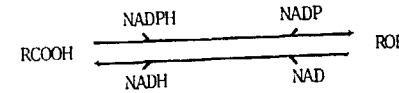


Fig. 69. Enzymic interconversions of long-chain fatty alcohols and fatty acids.

cancer cell since the reduction of fatty acids to fatty alcohols provides the precursor that leads to the abnormal production of ether-linked lipids in neoplasms. Currently emphasized in this project are experiments dealing with the possible involvement of an aldehyde as an intermediate and with the control mechanisms involved in this pathway and their role in the cancer cell. We have found long chain fatty alcohols present in free and bound forms in most mammalian cells. Levels are higher in neoplastic cells, and their chain length and degree of unsaturation mirror that of the O-alkyl and O-alk-1-enyl moieties of glycerolipids.

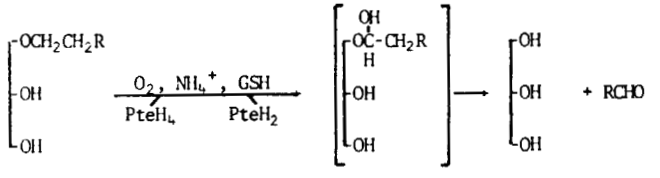
A significant segment of the long-range biomembrane project was completed in 1970; these experiments were on the turnover and composition of lipids in membranes and on enzymic activities associated with the smooth and rough endoplasmic reticulum. This work has shown that the proportion of free ribosomes to membrane-bound ribosomes is higher in neoplastic tissues than in normal tissues. Also, the amount of microsomal membranes, especially the smooth-surfaced fraction, is much lower in tumors than in normal rat liver. The data suggest that the neoplastic cell has been transformed to a state that increases its potential for synthesizing proteins that are used for cell growth and proliferation and not for export. In addition, the results obtained in the metabolic studies are consistent with the concept that the endoplasmic reticulum is a mosaic array of structural lipoproteins and enzymic proteins in a dynamic state of heterogeneous turnover, but it appears that there is a delicate balance of synthetic and degradative enzymes between the two microsomal subfractions.

Several miscellaneous items are also worth noting. A new sensitive technique for quantifying sulfhydryl moieties was developed. It is based on a color quenching principle that we have previously used for other types of mass measurements.

Abstracts of the work completed during 1970 summarize only published findings. Long-range experimental projects or the total research program are not necessarily

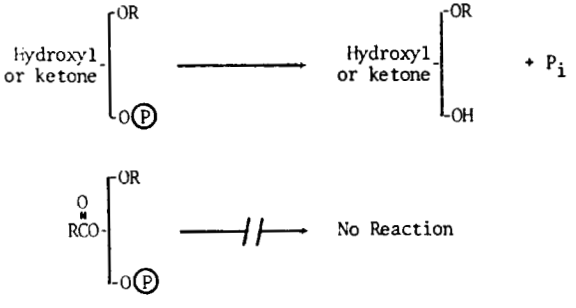
reflected in the data reported. Moreover, new studies that we have initiated during the past year with nervous tissue, mutant mice possessing neurological disorders, tissue culture systems, and harderian glands are not discussed.

A. CLEAVAGE OF *O*-ALKYLGLYCEROLS BY LIVER ETHERASE



(GSH = reduced glutathione; Pte = pteridine)

B. DEPHOSPHORYLATION OF *O*-ALKYL LIPIDS BY ALKALINE PHOSPHATASE



[1]Acid phosphatase is less specific; it hydrolyzes the phosphate moiety regardless of the substituent in the 2-position.

Fig. 70. Degradation of O-alkyl glycerolipids.

Biosynthesis and Degradation of O-Alkylglycerols

We have found that O-alkyl bonds in glycerolipids are synthesized by microsomal enzymes of preputial gland tumors when dihydroxyacetone-P or glyceraldehyde-3-P and 1-^{14}C long chain fatty alcohols are used as substrates; ATP, coenzyme A, and Mg^{++} are essential cofactors (Fred Snyder, Boyd Malone, and M. L. Blank). The O-alkyl moieties formed from the 1-^{14}C- and 9,10-^{3}H-labeled long chain fatty alcohols show that the fatty alcohol chains are directly incorporated. Dihydroxyacetone-P is an obligate source of the glycerol portion of glyceryl ethers, since only the dihydroxyacetone-P served as a precursor when 1-hydroxy-3-chloro-2-propanone phosphate was used as a specific inhibitor of triose-P isomerase, which is present in the microsomal preparation (Table 32). Kinetic data on the following parameters were obtained: time, CoA, Mg^{++}, ATP, protein, pH, and inhibitors (phosphate and 1-hydroxy-3-chloro-2-propanone phosphate). We documented the chromatographic behavior of the intermediates (O-alkyldihydroxyacetone and O-alkyldihydroxyacetone-P) and identified

1027963

the O-alkyl linkage by thin-layer and gas-liquid chromatography of the isopropylidene derivatives of the O-alkylglycerols formed by $LiAlH_4$ reduction. Only the 1-isomeric O-alkylglycerols could be isolated after $LiAlH_4$ reduction. Although glutathione or cysteine could not replace the CoA requirement, other nucleotides (ADP and ITP) substituted for ATP in this system. Metals other than Mg^{++} did not provide optimal conditions, and copper ions inhibited the biosynthesis of O-alkyl bonds. To some extent, dihydroxyacetone was able to replace the dihydroxyacetone-P requirement, but it is possible that phosphorylation occurred in the system. The enzymic synthesis of the O-alkyl linkage is localized in microsomes and appears to decrease as the tumor ages. The preputial gland tumors contain significant quantities of O-alkyl- and O-alk-1-enyl-linked lipid classes, but the enzyme system described was unable to make O-alk-1-enyl bonds under our experimental conditions. However, the similar composition of the O-alkyl and O-alk-1-enyl moieties (16:0, 18:0, and 18:1) suggests that

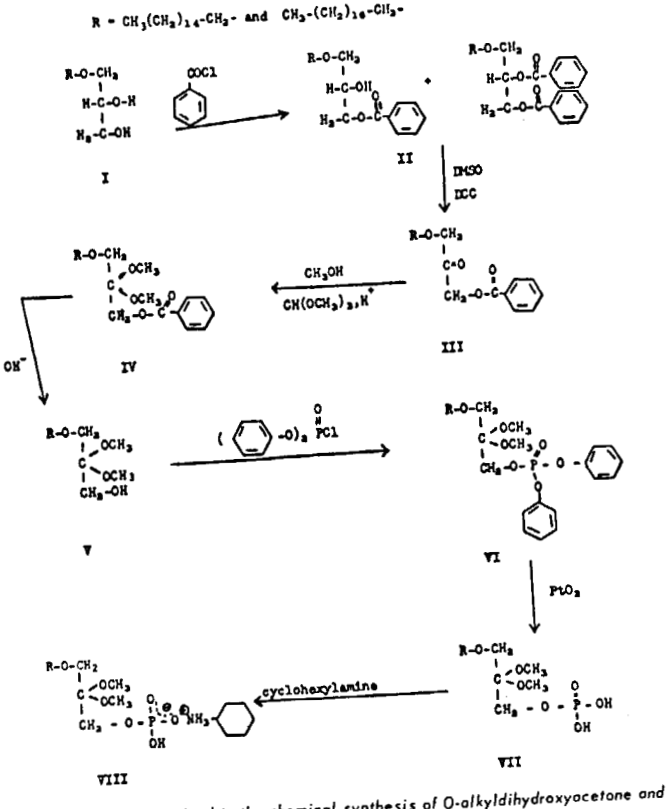


Fig. 71. Reactions involved in the chemical synthesis of O-alkyldihydroxyacetone and O-alkyldihydroxyacetone-P.

TABLE 32

Distribution of Glyceryl Ether-Synthesizing Enzymes in Organelles of Preputial Gland Tumor

Cell fraction	Triose-P isomerase inhibitor (0.133 mM) (1-hydroxy-3-chloro-2-propanone-P)	Triose-P isomerase	Alkyl glyceryl ethers
		nmoles NADH → NAD/min/mg protein	*nmoles formed/mg protein*
Mitochondria	-	0	0.86
	+	268	0.92
Microsomes	-	0	8.2
	+	213	8.8
Soluble	-	0	0.28
	+	17,104	1.7
Microsomes prepared by zonal centrifugation	-	---	9.6

interconversions between these two lipid classes or their precursors may occur (26).

O-Alkyldihydroxyacetone-P and O-alkyldihydroxyacetone were identified as intermediates in the biosynthesis of O-alkyl-linked lipids. The substrates for the initial reaction are dihydroxyacetone-P and fatty alcohols, and the cofactor requirements are coenzyme A, ATP, and Mg^{++}. The enzyme complex that carries out this reaction was found in microsomes of preputial gland tumors. The keto-intermediates were identified by thin-layer and gas-liquid chromatography of derivatives and standards prepared by the following techniques (Fig. 72): $LiAlH_4$ reduction, periodate oxidation before and after $LiAlH_4$ reduction, acid and alkaline hydrolysis, and removal of the phosphate moiety by bacterial alkaline phosphatase. We analyzed the fatty alcohols and O-alkyl ethylene glycols resulting from these treatments as the acetates; the O-alkylglycerols were identified as the isopropylidene derivatives. Both keto-intermediates can be enzymically reduced with NADPH. After reduction of the ketone group of the phosphorylated intermediate, acylation can result in the formation of diacyl glyceryl ethers (24).

Microsomal enzymes from Ehrlich ascites tumors synthesized O-alkyl glyceryl ethers from hexadecanol and dihydroxyacetone-P in the presence of ATP, CoA, and Mg^{++} (Robert L. Wykle and Fred Snyder). Glyceryl ether synthesis occurred via the formation of O-alkyldihydroxyacetone-P and O-alkyldihydroxyacetone. Although NADPH inhibited the initial reaction of dihydroxyacetone-P with fatty alcohol, it was required at a later stage for reduction of the ketone intermediates to 1-O-alkylglycerophosphate and 1-O-alkyl glycerol. 1-O-Alkylglycerophosphate was acylated by endogenous fatty acids in the Ehrlich ascites microsomes to form 1-O-alkyl-2-acyl glycerophosphate.

1027964

In contrast, no acylation of 1-O-alkylglycerol occurred. The formation of an O-alkyl analogue of phosphatidic acid suggests that O-alkyl glycerolipids are synthesized by the well-known cytidine pathway. The O-alkylglycerols were identified as isopropylidene derivatives and as O-alkyl glycolic aldehydes after periodate oxidation. The biosynthesis of O-alkyl ether bonds was measured as a function of time, pH, and the concentration of protein, 1-^{14}C-hexadecanol, dihydroxyacetone-P, Mg^{++}, ATP, CoA, and NADPH. The Ehrlich ascites microsomes contained a triose phosphate isomerase, which we were unable to remove. We showed that dihydroxyacetone-P is the obligate precursor of glyceryl ethers in this system by inhibiting the isomerase with 1-hydroxy-3-chloro-2-propanone phosphate. O-Alkyldihydroxyacetone was identified by the following methods: periodate oxidation to O-alkyl glycolic acid; reduction to O-alkyl glyceryl ethers by $LiAlH_4$, hydrogenation, $NaBH_4$, and NADPH (enzymatically); reduction by $LiAlH_4$ of the O-alkyl glycolic acid (derived from O-alkyldihydroxyacetone) to O-alkyl ethylene glycol; and identification of the acetate derivative of the O-alkyl glycol. 1-O-Alkyl-2-acyl glycerophosphate was identified by the following methods: mild saponification to 1-O-alkylglycerophosphate, more drastic saponification to alkyl glyceryl ethers, treatment by $LiAlH_4$ to yield alkyl glyceryl ether, hydrolysis with phospholipase A to yield 1-O-alkylglycerophosphate, and thin-layer chromatography in acidic and basic solvent systems. O-Alkyldihydroxyacetone-P was identified by the following methods: removal of the phosphate by bacterial alkaline phosphatase to yield O-alkyldihydroxyacetone and subsequent identification of the O-alkyldihydroxyacetone, reduction by $LiAlH_4$ to alkyl glyceryl ether, thin-layer chromatography in acidic and basic solvent systems, and enzymatic reduction by NADPH to 1-O-alkylglycerophosphate. An equimolar incorporation of hexadecanol and dihydroxyacetone-P into the ether-containing intermediates and glyceryl ethers was shown with ^{14}C-, ^{3}H-, and ^{32}P-labeled substrates (Tables 33 and 34). Data obtained with 1-^{14}C,1-^{3}H-hexadecanol showed that no oxidation of the alcohol occurred during formation of ether bonds (Table 35) (43).

Modified fibroblasts (L-M cells), grown in suspension cultures, contain a microsomal enzyme system that can synthesize alkyl ether bonds from long chain fatty alcohols and glyceraldehyde-3-phosphate or dihydroxyacetone-P (Fred Snyder,

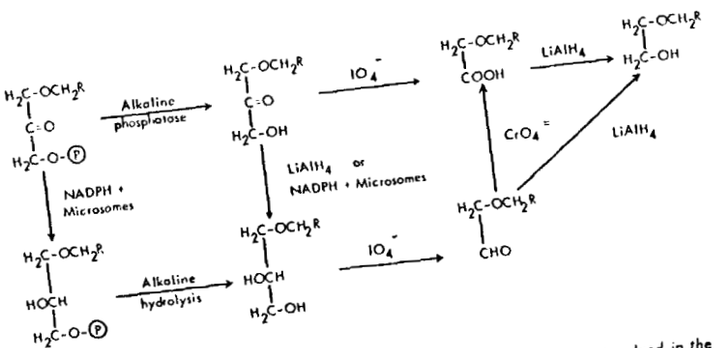


Fig. 72. Chemical and enzymic reactions used to identify keto-intermediates involved in the biosynthesis of O-alkyl-containing lipids by microsomal enzymes.

TABLE 33

Incorporation of labeled precursors into *O*-Alkyl Lipids

O-Alkyl lipid*	Molar ratio based on isotope incorporation: 1-^{14}C-hexadecanol / ^{32}P-DHAP	9,10-^{3}H-hexadecanol / (U)-^{14}C-DHAP
I. *O*-Alkyldihydroxyacetone-phosphate	1.1	1.1
II. 1-*O*-Alkyl-2-acyl glycerophosphate	1.1	1.2
III. *O*-Alkyldihydroxyacetone	---	0.9
IV. *O*-Alkylglycerol produced by $LiAlH_4$ reduction of *O*-alkyldihydroxyacetone	---	0.9

*The labeled compounds were prepared in incubations that contained ATP, (10 mM), CoA (0.1 mM), Mg^{++} (4 mM), 1-^{14}C- or 9,10-^{3}H-hexadecanol (33 uM), microsomes (approximately 2 mg protein), and ^{14}C- or ^{32}P-triose phosphate.

Boyd Malone, and R. B. Cumming*). Essential cofactors for the formation of alkyl ether bonds are ATP, CoA, and Mg^{++} (27).

Cytidyl transferases that synthesize cytidine derivatives of choline or ethanolamine are widely distributed in nature. Kennedy and coworkers established the requirement for cytidine diphosphate choline and cytidine diphosphate ethanolamine in the biosynthesis of phosphatidylcholine and phosphatidylethanolamine from diacylglycerols. Our studies on the enzymic synthesis of O-alkyl bonds in glycerolipids led to the isolation of O-alkylacylglycerophosphate, and the suggestion was made that this O-alkyl analogue of phosphatidic acid mimics the central role of phosphatidic acid in the biosynthesis of glycerides and phosphoglycerides. We have now obtained data (Table 36) documenting that cytidine diphosphate choline or cytidine diphosphate ethanolamine, O-alkylacylglycerol, and Mg^{++} are essential for the biosynthesis of O-alkyl phospholipids (Fred Snyder, M. L. Blank, and Boyd Malone). The phosphorylcholine- or phosphorylethanolamine-O-alkylacylglycerol transferase activities occur in microsomes of preputial gland tumors, and they may be identical with the enzyme(s) that catalyze the analogous steps in the biosynthesis of the corresponding diacylglycerophosphatides (23).

* Biology Division, Oak Ridge National Laboratory.

1027965

The reaction, dihydroxyacetone-P + ROH $\xrightarrow{\text{CoA, ATP, } Mg^{++}}$ O-alkyldihydroxyacetone-P + HOH, is catalyzed by microsomal enzymes of preputial gland tumors. Studies with ^{18}O demonstrate that the oxygen of ^{18}O-labeled hexadecanol is incorporated into O-alkyl lipids in vitro and in vivo (Fred Snyder, W. T. Rainey, Jr.*, M. L. Blank, and W. H. Christie*). The in vivo experiment with Ehrlich ascites cells also revealed that the oxygen of the O-alk-1-enyl bond originates from the fatty alcohol. The most prominent peak showing the presence of ^{18}O in the mass spectra of the acetates of the ^{18}O-hexadecanol (94% ^{18}O) used as the precursor was at m/e 61 $\left(CH_3C^{+}(OH)OH\right)$. The isopropylidene derivatives of the biosynthetic ^{18}O-hexadecylglycerol had a molecular peak at m/e 358 of low intensity, but removal of a methyl group by fragmentation produced resonance-stabilized oxonium ions containing ^{18}O at m/e 343 of high intensity. Except for relative intensities, the hydrogenated ^{18}O-hexadec-1-enylglycerol synthesized in vivo had a mass spectrum identical to that of the ^{18}O-hexadecylglycerol produced in vitro or in vivo. The ^{18}O content of the O-alkylglycerols biosynthesized was 79% (in vitro) and 34% (in vivo) and that of the O-alk-1-enylglycerol was 16% (in vivo) (29).

Cell-free homogenates from several neoplastic tissues and normal rat liver contain enzymes that acylate O-alkylglycerols (Table 37). Acylation of rac-1-O-alkylglycerols produced only 1-alkyl-3-acylglycerols, whereas the acylation of 2-alkylglycerols produced 1,3-diacyl-2-alkylglycerols; neither of the isomeric forms of the O-alkylglycerols was phosphorylated. ATP, CoA, and Mg^{++} were required as cofactors for acylation (Fred Snyder, C. Piantadosi†, and Boyd Malone) (28).

* From the Analytical Chemistry Division, ORNL, Oak Ridge, Tenn.
† From the University of North Carolina, Chapel Hill, N. C.

TABLE 34

Incorporation of Labeled Precursors into *O*-Alkyl Lipids

O-Alkyl lipid*	Molar ratio based on isotope incorporation: ROH (1-^{14}C-hexadecanol) / PO_4* (^{32}P-DHAP)	ROH (9,10-^{3}H-hexadecanol) / Triose [(U)-^{14}C-DHAP, (U)-^{14}C-GA-3-P]
I. *O*-Alkyldihydroxyacetone phosphate	1.1	1.1
II. 1-*O*-Alkyl-2-acyl glycerophosphate	1.1	1.2
III. *O*-Alkyldihydroxyacetone	---	0.9
IV. *O*-Alkylglycerol produced by $LiAlH_4$ reduction of *O*-alkyldihydroxyacetone	---	0.9

*The labeled compounds were prepared in incubations that contained ATP (10 mM), CoA (0.1 mM), Mg^{++} (4 mM), 1-^{14}C- or 9,10-^{3}H-hexadecanol (33 uM), microsomes (approximately 2 mg protein), and ^{14}C- or ^{32}P-triose phosphate.

We completed experiments that compare the ether-cleaving activities of normal rat liver homogenate with various transplantable tumor homogenates; the substrate used was O-hexadecylglycerol (chimyl alcohol) (James F. Soodsma, C. Piantadosi†, and Fred Snyder). The results show that the cleavage system is the most active in normal rat liver and Morris hepatoma 7794A, much less active in hepatoma 7777, and very low or absent in the other tumors tested (Table 38). These data demonstrate that neoplasms that contain high levels of O-alkyldiacylglycerols do not have an active alkyl ether cleavage system (30).

During the course of our work with O-alkyldihydroxyacetone-P, we studied the specificities of alkaline (EC 3.1.3.1) and acid (EC 3.1.3.2) phosphatases for lipid substrates that have free phosphate moieties, i.e., 1-acylglycerol-3-P, diacylglycerol-3-P, 1-O-alkylglycerol-3-P, 1-O-alkyl-2-acylglycerol-3-P, and 1-O-alkyldihydroxyacetone-P (Table 39). (M. L. Blank and Fred Snyder). Bacterial alkaline phosphatase removed the phosphate moiety from the acyl and O-alkyl substrates that do not contain aliphatic moieties in the 2-position (Fig. 70). In contrast, the acid phosphatase from wheat germ showed no specificity in the removal of free phosphate groups from the

TABLE 35

Incorporation of 1-^{14}C,1-^{3}H-Hexadecanol into *O*-Alkyl-Linked Lipids by Microsomes from Ehrlich Ascites Cells

Labeled lipid	^{3}H dpm / ^{14}C dpm
Hexadecanol (substrate)	6.7
O-Alkyldihydroxyacetone phosphate	6.4
O-Alkyldihydroxyacetone	6.8
1-*O*-Alkyl-2-acyl glycerophosphate	6.5
1-*O*-Alkylglycerol	6.7

lipid substrates. Substrates containing ether bonds reacted to a lesser extent than the corresponding analogues containing ester bonds. We have shown that alkaline phosphatase is an effective analytical tool in the lipid field (4). It is also apparent from these data that alkaline and acid phosphatases may play a role in lipid metabolism (4).

Biosynthesis of Plasmalogens (O-Alk-1-enyl Glycerolipids)

The role of O-alkyl lipids as precursors for plasmalogens has been investigated in vivo (M. L. Blank, Robert L. Wykle, C. Piantadosi*, and Fred Snyder). We have

* University of North Carolina, Chapel Hill.

1027966

TABLE 36

Requirement of CDP-Choline and CDP-Ethanolamine in Biosynthesis of *O*-Alkyl Phospholipids

The ^{14}C-labeled *O*-alkylacylglycerophosphate (170,340 dpm, 11.9 nmoles sonically dispersed for 1 min in phosphate buffer containing Mg^{++} and dithiothreitol) was used as the source of *O*-alkylacylglycerol since the microsomes contain a phosphatase for this conversion. It was incubated at 37°C for 90 min in a vial containing fresh microsomes (2.8 mg of protein), 6.67 mM dithiothreitol, 3.33 or 6.67 mM CDP-choline or 3.33 mM CDP-ethanolamine, and 10 mM Mg^{++} in 0.05 M phosphate buffer at pH 7.1; the final volume was 3.0 ml. All values were obtained with microsomes that were washed three times except for those values in parentheses. The parenthetical values were obtained with 6.67 mM CDP-choline and unwashed microsomes. (CDP = cytidine diphosphate)

Additions	Lipid class: *O*-Alkylacylglycerophosphate	*O*-Alkylacylglycerylphosphorylcholine	*O*-Alkylacylglycerylphosphorylethanolamine	*O*-Alkylacylglycerol
		% ^{14}C in *total lipids*		
CDP-choline + Mg^{++}	29 (18)	13 (17)	2.9 (3.1)	36 (49)
CDP-choline	57 --*	0.8 ---	2.0 ---	31 ---
Mg^{++}	29 (17)	2.2 (5.9)	3.2 (3.3)	50 (59)
CDP-ethanolamine + Mg^{++}	30	3.8	6.6	37
CDP-ethanolamine	57	0.8	2.0	31
Mg^{++}	31	1.6	2.9	53
CDP-choline + CDP-ethanolamine + Mg^{++} + boiled microsomes	97	0.3	0.4	---

*The dashes (---) indicate that no measurement was determined.

demonstrated that rac-1-^{14}C-O-hexadecyl-2-^{3}H-glycerol (chimyl alcohol) is incorporated into O-alkyl lipids by intact Ehrlich ascites cells and is subsequently converted to plasmalogens having essentially the same ratio of tritium to carbon-14 as the chimyl alcohol administered (Table 40). A labeled O-alkylglycerol intermediate with a polar group in the O-alkyl moiety was found in these studies and it had the same ^{3}H/^{14}C ratio as the O-alkyl and O-alk-1-enyl lipids. The unidentified O-alkyl lipid has a number of chromatographic properties that are similar to those of synthetic 1-O-(β-hydroxy)-hexadecyl glycerol. Our data indicate that O-alkyl lipids are converted to O-alk-1-enyl lipids via a substitution reaction on the O-alkyl moiety (5).

Until our recent work, the biosynthesis of plasmalogens had not been demonstrated in a cell-free system. In our earlier studies on the enzymic synthesis of O-alkyl bonds in glycerolipids, we suggested that the formation of the O-alkyl linkage from fatty alcohols and dihydroxyacetone-P might be the initial step in plasmalogen biosynthesis. In vivo data obtained with O-alkylglycerols and fatty alcohols labeled with ^{14}C, ^{3}H, and ^{18}O indicate that the O-alkyl glycerolipids are precursors of O-alk-1-enyl glycerolipids. Recent experiments in our laboratory have demonstrated the biosynthesis of plasmalogens (O-alk-1-enyl glycerolipids) in homogenates and mitochondrial supernatants prepared from Ehrlich ascites cells (Fig. 73) (Robert L. Wykle, M. L. Blank, and Fred Snyder). Substrates and cofactors (dihydroxyacetone-P, fatty alcohols, CoA, ATP, and Mg^{++}) required for the biosynthesis of O-alkyl lipids, plus $NADP^{+}$, were used for optimal incorporation of radioactivity from 1-14C-hexadecanol into the O-alk-1-enyl moiety. Almost all the radioactivity in the

TABLE 37

Acylation of 1-*O*-Hexadecylglycerol by Tissue Homogenates

Tissue	Cofactors*	pmoles of 1-*O*-alkylglycerol converted/mg protein per min	
		Glyceryl-1-ether-3-monoesters	Glyceryl-1-ether-2,3-diesters
Preputial tumor	+	286	14
	-	14	0
Hepatoma 7777	+	178	18
	-	9	1
Hepatoma 7794A	+	88	9
	-	9	0
Preputial gland	+	459	13
	-	29	2
Liver	+	33	0
	-	9	1

*ATP (10 μmoles), CoA (0.3 μmole), and $MgCl_2$ (3 μmoles); other components of the system were cysteine (25 μmoles), NaF (125 μmoles), KCl (78 μmoles), 0.3 μCi of the 1- or 2-isomers of hexadecyl glyceryl ether (200 mμmoles in 10 μl 1% Tween-80), and homogenate (10 mg) in phosphate buffer at pH 7.0. Sufficient endogenous fatty acids were available in the homogenates to make it unnecessary to add additional quantities of fatty acids.

plasmalogens of the phospholipids was present in the ethanolamine-containing fraction. Identification of plasmalogens was based on the isolation of various derivatives obtained from the total phospholipids or the purified ethanolamine-phospholipid fraction. These derivatives included dimethylacetals (HCl-methanol treatment), O-alk-1-enylglycerols ($LiAlH_4$ reduction), free fatty aldehydes (released from O-alk-1-enylglycerols with concentrated HCl), hydrogenated O-alk-1-enyl glycerols (H_2 over Adams catalyst), and the isopropylidenes of the hydrogenated O-alk-1-enylglycerols (acetone-$HClO_4$). The purified O-alk-1-enylglycerols used for hydrogenation were contaminated with less than 3% ^{14}C-O-alkylglycerols. The dimethylacetals, the isopropylidenes, and the free fatty aldehydes were each analyzed by thin-layer and gas-liquid chromatography. The reaction(s) involved in the formation of O-alk-1-enyl lipids and the role of $NADP^+$ in plasmalogen biosynthesis are not known. It is possible that $NADP^+$ is needed for the reduction-oxidation of O-alkyldihydroxyacetone-P or that it serves as an acceptor of hydrogens from the α,β carbon atoms of the O-alkyl moiety. The cell-free system and experimental approach described in this report provide the necessary tools to investigate the specific reactions responsible for the biosynthesis of plasmalogens (42).

1027967

TABLE 38

Cleavage of *O*-Hexadecylglycerol by Normal Liver and Neoplastic Tissues

Tissues	Host animals	Cleavage enzyme activity*
Rat liver	Buffalo†	7.3
Host rat liver†	Buffalo†	5.8
Morris hepatoma 7794A	Buffalo†	5.8
Morris hepatoma 7777	Buffalo†	1.4
Preputial gland tumor ESR-586	C57BL/6§	1.0
Sarcoma 180	HA/ICR§	0.42
Friend virus leukemia	DBA/2§	0.40
Melanoma B-16	C57BL/6§	0.31
Pituitary tumor	Wistar/Fu (females)†	0.30
Sarcoma T-241	C57BL/6§	0.30
Carcinoma E-0771	C57BL/6§	0.24
Fischer R-3259	Fischer 334†	0.19
Taper liver tumor	HA/ICR§	0.15
Ehrlich ascites carcinoma	HA/ICR§	0.14
KHZ Mammary tumor	C3H§	0.11
Walker 256	Carworth Farms Nelson†	0.10

* Expressed as mμmoles *O*-hexadecylglycerol cleaved/20 min/mg protein. Each value is the average of duplicate assays on a single sample.
† Rat strains.
‡ Host rat liver refers to pooled samples of livers of rats bearing 7794A and 7777 hepatomas.
§ Mouse strains.

TABLE 39

Dephosphorylation of Phospholipids by Alkaline and Acid Phosphatases

Substrate	Alkaline Phosphatase	Acid Phosphatase
	% Dephosphorylated in 1 hr.	
Diacylglycerol-3-P (20 μg P)*	0	35
1-Alkyl-2-acylglycerol-3-P† (15 nmoles)	1.2	11
1-Acylglycerol-3-P (30 μg P)	87	65
1-Alkylglycerol-3-P† (15 nmoles)	63	47
1-Alkyldihydroxyacetone-P† (30 nmoles)	49	12
Phosphatidylcholine (40 μg P)	0	0

*The values in parentheses represent the approximate quantities of substrates added to each incubation vial. The nanomoles of ^{14}C-labeled compounds were calculated from data obtained from the enzymic systems used to synthesize these substrates.

†The number 1 carbon atom of the *O*-alkyl moiety was labeled with carbon-14.

TABLE 40

Direct Conversion of Chimyl Alcohol into Plasmalogens

Labeled chimyl alcohol injected			% of Injected ^{14}C incorporated into total lipids	*O*-Alkyl-glycerols		*O*-Alk-1-enyl-glycerols		Unidentified polar lipid		$^{3}H/^{14}C$ Ratio in fatty aldehydes liberated from *O*-alk-1-enyl-glycerols by acid hydrolysis
Location of label	$^{3}H/^{14}C$	Hrs after injection		% ^{14}C of total lipid ^{14}C	$^{3}H/^{14}C$	% ^{14}C of total lipid ^{14}C	$^{3}H/^{14}C$	% ^{14}C of total lipid ^{14}C	$^{3}H/^{14}C$	
1-^{14}C-*O*-alkyl-glycerol	----	48	19.7	51.4	----	5.6	----	19.6	----	----
1-^{14}C-*O*-alkyl-2-^{3}H-glycerol	0.56	36	25.6	41.2	0.44	4.5	0.45	32.0	0.44	0.017
	0.56	68	25.3	40.4	0.49	5.9	0.49	22.4	*	0.014

*Not determined.

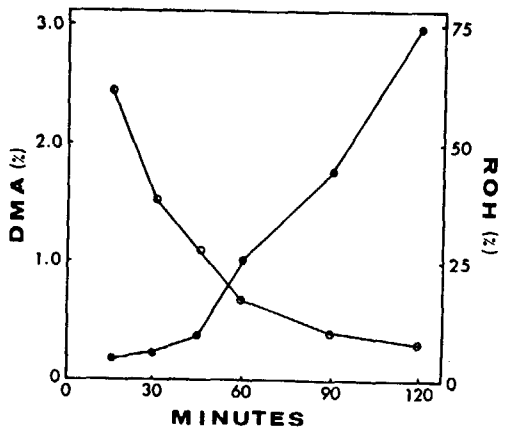


Fig. 73. The biosynthesis of plasmalogens ●——● and the disappearance of 1-^{14}C-hexadecanol (ROH) ○——○ as a function of time. DMA refers to dimethylacetals formed by treatment of total lipids with methanol-HCl. The complete incubation system is the same as that described in reference (42).

Occurrence and Biosynthesis of Fatty Alcohols in Mammalian Cells

Small quantities of long chain fatty alcohols (esterified or free or both) were found in four normal tissues (about 0.01% of total neutral lipids) and three neoplasms (about 0.3% of total neutral lipids (M. L. Blank and Fred Snyder). The major chain lengths (16:0, 18:0, and 18:1) of the fatty alcohols in both normal and neoplastic cells qualitatively resemble the O-alkyl chain lengths of glyceryl ethers. Our data showing that long chain fatty alcohols occur in vivo support the biological significance

1027968

of the metabolic pathway that uses fatty alcohols as a substrate for the alkyl chain in the biosynthesis of glyceryl ether (3).

Fatty alcohols are precursors of the ether bond in glycerolipids and they have been detected as normal constituents in mammalian cells. Yet, surprisingly little is known about the biosynthesis of fatty alcohols. The liver contains enzymes that degrade O-alkylglycerols and sphingosine or dihydrosphingosine to fatty aldehydes, fatty acids, and fatty alcohols under certain conditions, but the conversion of fatty acids to fatty alcohols has not been studied independently of the degradative enzymes. We have been able to demonstrate the enzymic reduction of 1-14C-palmitic acid to hexadecanol (Table 41) and the enzymic oxidation of 1-14C-hexadecanol to palmitic acid (Table 42) in unwashed microsomes from mouse preputial gland tumors (Fred Snyder and Boyd Malone). NAD is required for oxidation and NADPH is required for reduction. ATP, CoA, and Mg^{++} are also required for the reduction of fatty acids (presumably for activation) but they are not necessary for the oxidation of fatty alcohols. The nucleotides may play an important role in the regulatory

TABLE 41

Enzymic Reduction of 1-^{14}C-Palmitic Acid* to Hexadecanol

Cofactors	Fatty alcohol formed (nanomoles per hour) Expt. I	Expt. II	Expt. III
1. NADPH, ATP, CoA, Mg^{++}	10, 10	8.4, 7.6	12, 12
2. NADPH	1.8, 2.3	1.4, 1.3	--
3. NADH	2.8, 3.1	0.9, 1.1	--
4. NADH, ATP, CoA, Mg^{++}	4.2, 4.2	4.3, 2.6	--
5. NADPH, NADH, ATP, CoA, Mg^{++}	13, 11	--	19, 17
6. ATP, CoA, Mg^{++}	--	--	8.6, 8.0
7. NADPH, NADH, ATP, CoA, Mg^{++} (boiled microsomes)	2.0, 3.0	--	--

*1-^{14}C-Palmitic acid was incubated with the cofactors listed and unwashed microsomes from preputial gland tumors.

mechanisms that determine the different levels of ester- and ether-linked glycerolipids in normal and neoplastic cells (25).

Biomembranes

In an attempt to determine the possible correlation between the growth rate of different neoplastic tissues and the proliferation of their endoplasmic reticulum, we have applied a technique developed for separating rat liver microsomes into smooth- and rough-surfaced membrane fractions by zonal centrifugation (Biochem Biophys Res Commun 36, 748, 1969). This technique has been applied to study the microsomal fraction from different tissues as a continuous spectrum of subcellular particles (Ten-ching Lee, D. C. Swartzendruber, H. P. Morris*, and Fred Snyder).

* Department of Biochemistry, Howard University, Washington, D. C.

The absorbancy (280 mμ) zonal profile of the postmitochondrial fractions from normal rat livers occurred primarily as three peaks: (1) soluble phase, (2) smooth-surfaced microsomes, and (3) rough-surfaced microsomes. However, the zonal profiles of the postmitochondrial fractions obtained from Morris hepatomas (5123C, 7777, and 7787) and preputial tumors differed from that of normal liver under standardized experimental conditions. The peak representing smooth-surfaced microsomes was not seen in the zonal profiles of the neoplastic tissues. However, the quantity of endoplasmic reticulum does not seem to be correlated with the growth rate of the hepatomas. These findings indicate that the tumors have an increased potential for synthesizing proteins that are used for cell growth instead of for export elsewhere. Our results were presented at the Tenth International Cancer Congress, Houston, Texas, May, 1970 (88).

TABLE 42

Enzymic Oxidation of 1-^{14}C-Hexadecanol* to Palmitic Acid

Cofactors	Fatty acid formed (nanomoles per hour) Expt. I	Expt. II	Expt. III
1. NAD, ATP, CoA, Mg^{++}	15, 15	15, 16	--
2. NAD	38, 35	41, 42	28, 26
3. NADP, ATP, CoA, Mg^{++}	0.74, 1.1	1.7, 1.8	--
4. NADP	--	3.4, 2.4	--
5. NAD, NADP	--	--	28, 27
6. ATP, CoA, Mg^{++}	--	--	0.7, 0.7
7. NAD, NADP, ATP, CoA, Mg^{++} (boiled microsomes)	0.73, 0.85	1.6, 1.8	--

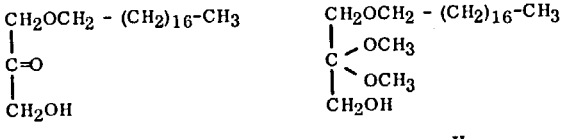
*1-^{14}C-Hexadecanol was incubated with the cofactors listed and unwashed microsomes from preputial gland tumors.

The metabolism of lipids in smooth- and rough-surfaced membranes of rat liver microsomes has been investigated in an effort to understand the functional activities of cellular membranes and to determine the intracellular distribution of enzymic pathways involved in lipid metabolism (Ten-ching Lee). The smooth and rough microsomes, isolated by zonal centrifugation, were incubated with 1-^{14}C acyl CoA and 1-acylglycerol-phosphate. The activities of acyl CoA hydrolase and 1-acylglycerol-phosphate acyltransferase were determined by radioassay of the products resolved by thin-layer chromatography. We found that specific activities of acyl CoA hydrolase and 1-acylglycerol-phosphate acyltransferase were higher in smooth microsomes than in rough microsomes. The data support the theme suggested by Glaumann and Dallner (J Lipid Res 9, 720, 1968) that indicates a difference in the biosynthetic capacities of lipids within the membranes of the endoplasmic reticulum. Each patch or tessera of the endoplasmic reticulum appears to consist of a specific group of enzymes that are not biochemically equivalent to one another (87).

1027969

The Chemical Synthesis of Keto-Glycerolipids that Contain Ether Bonds

We (C. Piantadosi*, K. S. Ishaq*, and Fred Snyder) have prepared structures with the following general formulas:



I

II

Two synthetic routes were investigated in the synthesis of 1-O-octadecyl-2,2-dimethoxy-3-hydroxypropane (II). In the first procedure, acetyl dihydroxyacetone dimethyl ketal was synthesized and then converted to 1-O-trityl-2,2-dimethoxy-3-hydroxypropane (III). Compound III was reacted with octadecyl bromide in the presence of powdered KOH in benzene, resulting in 1-O-octadecyl-2,2-dimethoxy-3-O-trityl propane (IV). In the second synthetic scheme, batyl alcohol was benzoylated in the presence of pyridine and the crude mono derivative was oxidized with dimethyl sulfoxide and dicyclohexylcarbodiimide in the presence of trifluoroacetic acid. This produced 1-O-octadecyl-3-O-benzoyl-2-propanone (V). Compound V was then ketalized to 1-O-octadecyl-2,2-dimethoxy-3-O-benzoyl-propane (VI). Compound VI was then hydrolyzed with base to give 1-O-octadecyl-2,2-dimethoxy-3-hydroxypropane (II). The two synthetic preparations of O-alkyldihydroxyacetone were reduced with $LiAlH_4$ to the corresponding O-alkylglycerol, and GLC of the isopropylidene derivatives demonstrated that they had the same retention time as the isopropylidene derivative of authentic batyl alcohol. Acid hydrolysis of the ketone-containing lipids produced octadecanol; periodate oxidation produced O-alkylglycolic acids, and they could be reduced with $LiAlH_4$ to O-alkylethyleneglycols (20).

We have also described the chemical synthesis of the cyclohexylammonium salt of 1-O-hexadecyl-2,2-dimethoxypropane-3-phosphate and its octadecyl analogue (C. Piantadosi*, K. S. Ishaq*, Robert L. Wykle, and Fred Snyder). The key reaction in the synthetic route is oxidation of benzoylated chimyl or batyl alcohol with methyl sulfoxide in the presence of dicyclohexylcarbodiimide and trifluoroacetic acid to the corresponding keto intermediate; the latter compound is subsequently phosphorylated and isolated as the cyclohexylammonium salt (Fig. 71). The chemical and physical properties characterized are similar to those described for O-alkyldihydroxyacetone. The organically synthesized O-alkyldihydroxyacetone phosphates and those synthesized by microsomal enzymes of mammalian cells were found to have identical properties. The O-alkyldihydroxyacetone phosphates are central intermediates in the pathway that produces diacyl-O-alkylglycerol, O-alkylacylglycerylphosphorylcholine, and O-alkylacylglycerylphosphorylethanolamine (49).

Technology

We have developed a method for use with liquid scintillation instrumentation that quantitates sulfhydryl-containing compounds by color quenching (Fred

* University of North Carolina, Chapel Hill.

Snyder and Anita Moehl). Color produced by a reaction between sulfhydryl groups and 5,5'-dithio-bis-2-nitrobenzoic acid to form 3-carboxy-4-nitrothiophenol quenches fluorescence of a scintillation source; the colored compound absorbs maximally at 412 nanometers. The scintillation source consists of either a plastic or liquid scintillator mixed with a beta emitter (^{14}C) in a glass tube that

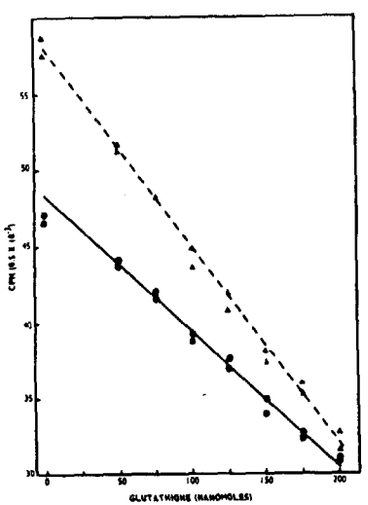


Fig. 74. The measurement of glutathione by color quenching in a liquid scintillation spectrometer. The color was produced by a reaction between the sulfhydryl group of glutathione and 5,5'-dithio-bis-2-nitrobenzoic acid. The two slopes reflect the light source used; ▲-----▲ plastic scintillator plus ^{14}C-triolein and o———o PPO-POPOP-toluene plus ^{14}C-tripalmitin.

is inserted in the center of a scintillation vial containing the colored solution. Quenching is linear over a range of 50-200 nanomoles of glutathione (Fig. 74). This quenching technique could be modified for the rapid automated analysis of large numbers of samples (67).

1027970

Marmoset Research Project

The Medical Division maintains one of the few research colonies of marmosets in the United States. These small New World primates are unique in that they have a high frequency of fraternal twinning and placental vascular anastomoses between the fetuses. The hemopoietic chimerism resulting from the interaction of these two features of their reproductive physiology makes these animals ideally suited for studies on immunologic tolerance, particularly as related to tissue transplantation. In a continuing effort to further define this animal for our transplant program, we have this past year emphasized the following areas of study: (1) evaluation of the in vitro growth potential of blood-forming cells, (2) development of an in vitro test for immunologic histoincompatibility, (3) further kidney transplants between related and unrelated marmosets, and (4) the response of neonates to foreign leukocyte antigens. In addition to these efforts, one of our primary goals, the definition of a red cell antigen suitable for demonstration of red cell chimerism, was realized this past year. This was achieved through use of an isoimmune reagent developed in one of the subspecies of the S. fuscicollis, S. f. illigeri. In addition to being a phenotypic marker, family studies have shown the antigen detected to be inherited as a simple Mendelian dominant factor.

Since our continuing studies of blood-cell chimerism have shown a relatively stable population of the mixed hemic cells, the in vitro studies with bone marrow and peripheral blood leukocytes were undertaken to ascertain the stability of these cell populations in an artificial environment. This attempt led to the surprising observation that marmoset blood-forming cells can be cultivated rather easily for extended periods of time, far beyond the anticipated 2 or 3 weeks. Both myelocyte and megakaryocyte cell lines could be propagated from either marrow or blood leukocyte cultures for periods ranging up to 120 days. The need for more sensitive indicators of histoincompatibility among related and unrelated marmosets prompted another in vitro study, the mixed culture (MLC) reaction. For this purpose, lymph node cells were treated with mitomycin C to prevent DNA synthesis; use of these "stimulating" cells with other lymphocytes capable of undergoing a mitogenic response, i.e., responding cells, yielded cultures for demonstration of in vitro histoincompatibility. As anticipated, MLC reactions between lymph node cells obtained from chimeric co-twins showed no uptake or very low uptake of the DNA label (^{14}C-thymidine), indicating absence of any incompatibility. This supported our original observations on the state of specific immunologic tolerance existing between co-twins following reciprocal exchange of skin allografts.

Kidney transplant studies, initiated last year to further demonstrate allograft tolerance between co-twins, were extended this year. Although additional information was obtained on the rejection of kidney grafts exchanged between unrelated marmosets, the three chimera co-twin animals died within the first postoperative week; kidney malfunction apparently was due to nonimmunologic factors. Based on information obtained in our initial kidney transplant attempts, however, a tentative conclusion is that immunologic tolerance also extends to this organ. In neonatal marmosets attempts to include tolerance to cellular antigens of another closely related marmoset failed; however, two animals receiving the foreign spleen-cell inoculum showed severe signs of runting, suggestive of a graft-versus-host reaction. This interpretation was tempered by the finding of T. cruzi-like trypanosome forms in the recipients and in the donor of the spleen cells, and subsequent histological studies confirmed the pathogenicity of these blood parasites in the young marmosets.

The work reported in this section is closely related to the earlier part of this report dealing with marrow transplantation and radiation immunology.

An Isoimmune Reagent Showing Red Cell Chimerism

The natural hemopoietic chimerism of the marmoset results from the high frequency of fraternal twinning and the consistent development of vascular anastomoses between the two placentae. Definitive evidence for chimerism has been limited to distinguishing sex characters of male and female cells: sex chromosomes in metaphase plates from cells following tissue culture and the sex-chromatin (drumstick) marker of the blood neutrophil. In male-male and female-female co-twins chimerism cannot be discerned. Furthermore, these indices reveal chimerism for lymphopoietic and myelopoietic elements, but not erythropoietic, although the latter is presumed to exist. Our initial attempts to demonstrate red cell chimerism were concerned first with developing suitable antisera reagents capable of distinguishing red cell antigenic differences among various species and subspecies of marmosets. Although the isoimmunizations were successful and the antisera did distinguish certain animals by reacting to varying degrees with their red cells, the strict monospecificity necessary to detect red cell chimerism was not evident with these reagents. Attainment of this specificity was made more difficult by the prevailing red cell chimerism of both the immunized animal and the cell donor. Subsequent attempts were confined to immunizations within one subspecies of Saguinus fuscicollis, S.f. illigeri. For the most part, these immunizations were unsuccessful, but ultimately we obtained an S.f. illigeri antiserum which reacted with varying degrees of intensity with cells of animals of this subspecies. Monospecificity was still not evident, but based on these reactions, additional isoimmunizations were performed. One of these yielded an agglutinin (incomplete antibody detected with a rabbit antimarmoset globulin reagent) which, when tested against a random panel of cells, reacted strongly with certain cells and not at all with others (N. Gengozian). That is, a clear-cut distinction was made between the positive reactors and the nonreactors. Further testing of additional panels of cells revealed some cells which gave only partial agglutination, even with the reagent undiluted; this latter observation suggested the detection of two cell populations, those bearing the specific antigen and those not. This interpretation was confirmed when the red cells of known lymphocyte chimeras (by sex chromosome analysis) were tested; some of these showed a partial agglutination reaction, the intensity, i.e., 1+, 2+ (on a 0 to 4+ grading system), approximating the degree of white cell chimerism. Representative reactions are shown in Table 43.

Since this was noted all animals in our colony have been tested with this reagent. The primary observations are: (1) the antigen appears in approximately 29% of our imported animals, S.f. illigeri; and (2) it appears to be specific for the S.f. illigeri subspecies, not reacting with other subspecies of Saguinus fuscicollis and other Saguinus species present in our colony. As anticipated also, we can now demonstrate cell chimerism among isosexual co-twin pairs.

We have tentatively designated phenotypically positive animals as Ka+ and those negative as Ka-. That this is also a genetically inherited antigen, however, has also been revealed upon testing of families in our breeding colony. Thus, analyses of red cells of offspring have shown that Ka- x Ka- pairs never produce Ka+ young; Ka+ x Ka- matings may produce either Ka+ or Ka- young; and Ka+ x Ka+ pairs may also produce Ka+ or Ka- young. Therefore, it appears to be inherited in a simple Mendelian dominant fashion; genotypically, a Ka- individual may be designated as Ka-/Ka-, while a phenotypically Ka+ individual may be either Ka+/Ka+ or Ka+/Ka-.

1027971

Studies now in progress are aimed at making quantitative comparisons between lymphocyte chimerism, as determined by sex chromosome analyses, and red cell chimerism, as determined by the mixtures of Ka+ and Ka- cells. The stability of white cell chimerism versus that of red cell chimerism in individual animals can now be investigated.

TABLE 43

Monospecificity demonstrated by *S.f. illigeri* isoimmune reagent, $^{S.f.}K_{i}a^{*}$

S.f. illigeri red cell (animal no.)	Log_2 antiserum dilution								Red cell phenotype designation
	2	3	4	5	6	7	8	9	
1	4+†	4+	4+	4+	4+	3+	3+	1+	Ka+
2	-	-	-	-	-	-	-		Ka-
3	4+	4+	4+	4+	4+	3+	3+	1±	Ka+
4	4+	4+	4+	4+	3+	3+	3±	1±	Ka+
5	-	-	-	-	-	-			Ka-
6‡	2+	2+	2+	2±	1+	1±	-		Mixed, Ka+, Ka-
7‡	4±	4±	4±	3+	3±	2+	1+	-	"
8‡	3+	3+	3+	3±	3±	2±	1±		"

* Tentative designation of isoimmune reagent, superscript *S.f.* = *Saguinus fuscicollis*, subscript *i* = *illigeri*.

† Reaction detected with Coombs reagent, rabbit-anti marmoset globulin antiserum.

‡ Animals known to be white cell chimeras by sex chromosome analyses. Degree of reaction with reagent through Log_2 4 dilution approximates percentage white cell chimerism (see text).

Cultures of Hematopoietic Tissue

The study of immunologic tolerance of marmoset blood has been expanded during the past year to include an in vitro culture system. These studies were carried out because we wanted to know how a genetically-mixed population of hematopoietic or lymphopoietic cells behaved in the absence of the in vivo controls that regulate their homeostasis. Ongoing in vivo experiments have demonstrated the stability of the ratio of male to female cells in numerous heterosexual blood cell chimeras. These data have shown that over a 5-year period mixed hemic cells of the marmoset are relatively stable with respect to the percentage of cells from its twin; in the changes observed, the donor cell population may shift slowly upward or downward from its first assayed value (N. Gengozian and J. Batson). We therefore investigated the possibility of culturing bone marrow and nucleated blood cells in vitro with the intent

of determining the stability of the mixed population of cells under conditions removed from in vivo homeostatic control (Raymond P. Porter and N. Gengozian).

Although marrow cells from other laboratory animals and from man have resisted efforts of tissue culturists, suspensions of cells from the femoral marrow of marmosets were found to grow easily in a liquid medium designed to propagate human lymphoid cells. Marmoset marrow cultures grew as nonadherent and adherent populations. Although these cultures were not perpetual cell lines, they proliferated extensively; one grew well up to 120 days, producing many times the initial number of cells. As an example of the extent of the increase in cellularity of these cultures, one containing 10 x 106 cells at day 20 contained 500 x 10^6 at day 60. Experience with more than 50 nonadherent marrow cultures grown in bottles has shown that myelopoietic proliferation predominates during most of the life of all cultures. We identified promyelocytes as being the most common dividing cell; these were most prominent during a burst of accelerated growth that usually occurred during the first 2 or 3 weeks. The maturation of the cells of the myelocytic series into polymorphonuclear (PMN) leukocytes designated the end of this burst of proliferation, and differentiating cells then predominated (Fig. 75). This was followed by slowly diminishing cellularity and ultimate self-termination of the culture. Cells identified as megakaryocytes went through the same sequence in their development; however, their burst of proliferative activity occurred either early or late during the total life of the culture. Erythroid elements were seen for a few weeks but did not continue to proliferate possibly because of lack of humoral factors (erythropoietin).

Preliminary data on the analysis of the stability of chimerism in vitro indicate

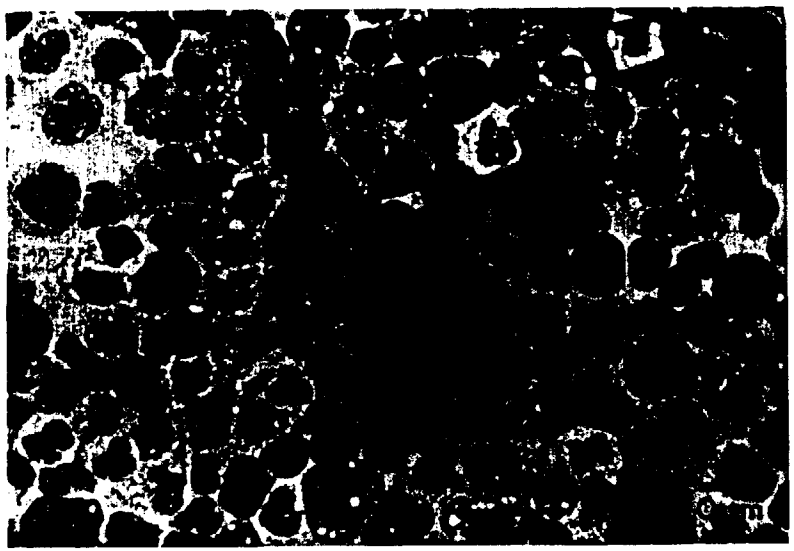


Fig. 75. Smear of marmoset bone marrow after 7 weeks of in vitro culture. Many differentiated cells of the myelaid series and large cells with multilobed nuclei and acidophilic cytoplasm, typical of the mature megakaryocytes, are prominent features (See text.).

1027972

that the ratio of the mixture of male-female cells in some marrow cultures was stable, while in others it varied slowly with time (Table 44).

Peripheral nucleated blood cells did not produce long-term lymphoid cultures as was expected from data available for man. However, to our surprise, some marmosets' peripheral blood cells developed into cultures with qualitative characteristics much the same as those of cultures derived from marrow. We do not yet know which

TABLE 44

ANALYSIS OF CULTURED BONE MARROW CELLS FOR CHIMERISM

Culture designation	#BM - 37		#BM - 45	
Sex of Marrow Donor	Male		Male	
Peripheral Blood Chimerism*	36%† (36/100)		69% (69/100)	
	Day of Culture	Chimerism	Day of Culture	Chimerism
In Vitro Bone Marrow Chimerism	6	22% (4/18)	2	78% (78/100)
	14	29% (29/100)	17	65% (15/23)
	20	23% (13/56)	25	61% (33/54)
	29	23% (15/66)	31	66% (66/100)
	38	18% (6/34)	35	65% (37/57)
	59	100% (16/16)	50	72% (36/50)

*Base line three-day PHA stimulated peripheral blood cultures, determined previous to the culture of marrow.

†Percentage chimerism = Number of metaphases scored as the opposite sex of the tissue donor/total number of metaphases scored.

cells of the blood have the capacity to serve as precursor forms. Marmoset lymph node, spleen, and thymus lymphocytes proliferated for up to 3 weeks when cultured in the same medium; however, these cultured cells did not differentiate into marrow-like elements.

The Response of Neonatal Marmosets to Foreign Leukocyte Antigens

The phenomenon of naturally occurring hemopoietic chimerism in marmosets and its corollary of specific tolerance between fraternal co-twins, unique in primates, dictates the potential usefulness of marmosets in transplantation immunology. Previous studies demonstrated that the adult marmoset expresses immunologic competence by rejecting skin allo- and xenografts, second- and third-set grafts showing progressively decreasing survival times. The rate of graft rejection, however, was dependent upon the genetic relationship between host and donor. For example, intrasubspecies grafts among the S.f. illigeri showed a mean graft survival time of 38 days, rejections occurring from 13 to a maximum of 64 days posttransplant; however, intersubspecies grafts, i.e., between S.f. illigeri and S.f. lagonotus,

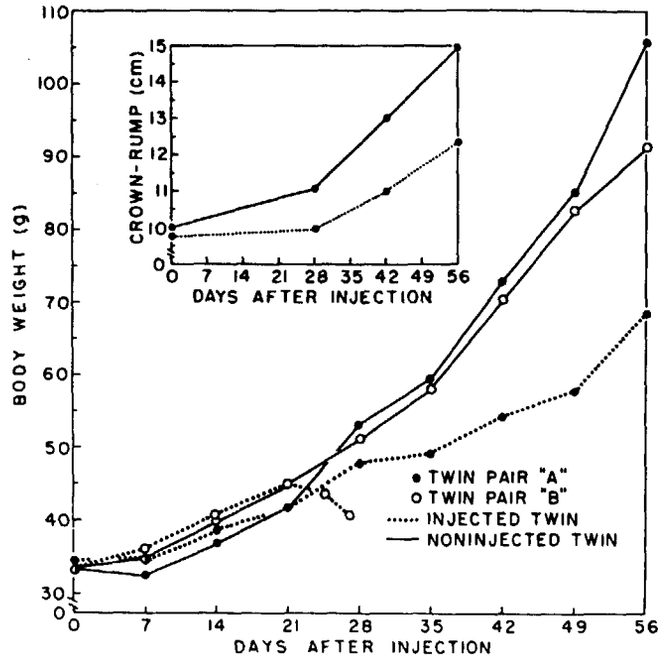


Fig. 76. Growth of S.f. illigeri tamarins injected with S.f. lagonotus spleen cells in comparison to their noninjected co-twins, •, twin pair "A," experimental twin injected within 18 hours after birth; o, twin pair "B," experimental twin injected at 7 days of age.

showed a primary mean survival time (MST) of only 20 days, with the rejection times ranging from 14 to 29 days.

In view of the existing immunologic tolerance of almost all marmosets to two "sets" of transplantation antigens, their own and that of their co-twin, we wanted to evaluate the possibility of inducing cell chimerism and subsequently tolerance to a third "set" of antigens by the inoculation of neonates with hemopoietic cells from an unrelated donor (Gengozian and Porter). With the intent, also, of evaluating the susceptibility of the young marmoset to a graft-versus-host (GvH) reaction, spleen cells in an intersubspecies grafting combination were used to treat the neonate. Since previous skin grafting and red cell immunization studies showed a fairly strong immunogenetic difference between S.f. illigeri (host) and S.f. lagonotus (donor), these two subspecies were used for this study. Spleen cells from an adult S.f. lagonotus marmoset were injected into newborn or 7-day-old S.f. illigeri marmosets. One member of a neonatal twin pair received either intravenously or intraperitoneally 72 x 10^6 to 105 x 10^6 nucleated spleen cells in one ml. Three months later the survivors and their co-twin controls were grafted with the skin from S.f. lagonotus to test their immunologic competence.

The first experiment involved four pairs of neonatal S. f. illigeri marmosets,

1027973

two pairs newborn and two pairs 7-day-old. One animal of each pair was injected with spleen cells from an adult S.f. lagonotus. As evidenced by body-weight gain, size, and gross appearance, no adverse effect attributable to the spleen cell inoculum was noted over a period of 100 days when compared with the noninjected co-twins. Tests for lymphocyte chimerism resulting from the injected spleen cells were negative on the 35th day posttreatment. All animals received skin transplants from the spleen cell donor when they were approximately 3 months old. In the four noninjected co-twins, the MST of the allografts was 22 days (range 18 to 35 days). In three experimental animals (one graft lost due to technical failure), one animal rejected its graft in 40 days, and the other two 50 days postgrafting.

In the second experiment, one newborn and one 7-day-old marmoset were injected with cells from another adult S.f. lagonotus. The twin inoculated on day 7 died 25 days later, weighing approximately 11 grams less than its co-twin control at that time (Fig. 76). The injected newborn failed to gain weight relative to its co-twin control up to day 28 and in the subsequent 4 weeks its growth was markedly reduced (Fig. 76 and inset; Fig. 77). Tests for chimerism as a result of the spleen cell injection were negative in both of these animals. The mortality and runting observed might suggest induction of a GvH reaction by the foreign spleen cells, but this interpretation is thrown in doubt by the lack of evidence for transplanted spleen cells and also by the detection of T. cruzi-like forms in the tissue of the dying animal and in the blood of the surviving yet physically runted marmoset. Similar forms were subsequently identified in the spleen (imprints) and blood of the S.f. lagonotus donor.

Our attempts to show any tolerance induction conferred by the foreign spleen cell inocula were essentially negative, although the donor skin allografts showed slightly



Fig. 77. S.f. illigeri co-twins (Pair "A" of Fig. 76). Animal on right had been injected within 18 hours after birth with S.f. lagonotus spleen cells and was inadvertently infected with T. cruzi; on left is noninjected co-twin. Photograph taken 47 days after injection.

longer survival times in the experimental animals of the first group. In any event, the animals were not sensitized by the foreign spleen cells, suggesting that newborn and 7-day-old marmosets are immunologically deficient with respect to sensitization by allogeneic leukocytes.

Marmoset Kidney Allografts

We had previously reported (ORAU Research Report, 1969) on the survival of kidney allografts exchanged between unrelated and co-twin marmosets. In that study, three chimera co-twins were bilaterally nephrectomized and then received from their co-twin a single kidney, the survival and function of which would reflect the degree of immunologic tolerance existing between these animals. As a control, one marmoset, also bilaterally nephrectomized, received a kidney from an unrelated donor. This animal died 9 days posttransplant with an elevated blood urea nitrogen (BUN); histologically, the donor kidney showed a florid host-versus-graft lymphoid reaction. As originally reported, of the three co-twins grafted, one died on day 60. Since that time, the remaining two also died, one on day 169, and the other on day 302. All animals showed normal kidney function as gauged by their BUN levels until shortly before death; histologically, only one (day 60 death) showed a slight lymphoid cell infiltration suggesting a HvG reaction. Thus, although renal failure was documented in these animals shortly before death (elevated BUN noted from 12 days to one day before expiration) it could not be stated unequivocally that the kidney malfunction was due primarily to immunologic incompatibility. To obtain a better understanding of kidney acceptance and rejection among marmosets additional co-twin chimera grafts and grafts between unrelated animals were performed this year (Gengozian, Lee, Porter). In addition, unilateral nephrectomies were performed on three marmosets to evaluate the functional capabilities of a single kidney in these animals, simulating the situation in our experimental graft animals.

Three marmosets that had received a single kidney from an unrelated donor immediately after bilateral nephrectomy died on days 12, 23, and 28. All three showed normal kidney function after one week as gauged by the BUN level. However, by the second week all three showed an elevated BUN, ranging from 125 mg/100 ml to 337 mg/100 ml. The two animals that survived to day 23 and day 28 showed a continued increase in their BUN. Histologically all donor kidneys showed varying degrees of lymphoid infiltration, suggestive of a strong HvG reaction. Unfortunately, the three co-twins that had received kidneys from their fraternal co-twins all died within the first week posttransplant due to postoperative complications. Two died on day 4, the BUN of both measuring approximately 200 mg/100 ml (serum obtained after death). The animal surviving for 7 days showed no significant urination during this period, indicating lack of any kidney function, and the BUN at death was 462 mg/100 ml. On autopsy, all animals showed an intussusception of the lower intestine. The three marmosets on which unilateral nephrectomy was performed are still alive with apparently normal renal function, the BUN on all within the normal range approximately 210 days posttransplant.

The failure of our three experimental animals, i.e., the co-twin kidney exchanges, to survive the immediate postoperative period still leaves unanswered the question of immunologic tolerance with respect to this organ among chimeric co-twins. Although the three previous co-twin kidney allograft recipients died, their times of death and the absence of any definitive evidence for host lymphoid cell infiltration suggests host-donor immunologic incompatibility was not a factor. Tests for blood chimerism during the posttransplant period and at time of death showed no change in their chimeric status; skin grafts from their co-twins, received some time before the kidney graft, also appeared to be in good condition at time of death. These observations, however,

1027974

still do not rule out the possibility of the existence of a state of split tolerance in these animals, similar to that reported by Stone et al. for chimeric cattle twins (Science 148, 1335, 1965). In their studies, chronic rejection of a co-twin skin graft occurred in the face of a stable population of chimeric blood-forming cells. Immunofluorescent examination of the kidney tissues involved in both the co-twin (60-169 day survivors) and unrelated transplant studies are planned in an attempt to clarify further the occurrence of any immune mediated reaction.

The Mixed Lymphocyte Culture as an Index of Histocompatibility

This past year we investigated the possibility of utilizing the in vitro mixed lymphocyte culture (MLC) test to augment our capabilities in the detection of histocompatibility reactions between marmoset co-twins and between unrelated animals (Porter and Gengozian). Mesenteric lymph node cells were obtained from the donors of our kidney transplant experiments, S. fuscicollis, and used in combination with lymphoid cells obtained from other marmoset species, e.g., S. oedipus. The MLCs were set up in a unidirectional manner, i.e., the cells designated as the stimulating population were treated with mitomycin C to prevent them for incorporating the ^{14}C-thymidine used to measure DNA synthesis of the responding cells. The results of the experiments performed thus far are similar to those reported by others for human MLC tests. For example, S. fuscicollis lymphocytes did not respond, or responded only weakly, to autologous mitomycin C-treated lymphocytes. Combining cells of varying genetic disparity with S.f. illigeri cells in the MLC test produced the following order of responses: xenogeneic (S. oedipus, S. mystax) $\geq$ allogeneic (S. f. illigeri) >> autologous. MLC tests set up between co-twin cells indicated that lymph node cells from a co-twin chimera were not recognized as foreign, their responses paralleling the autologous cell combinations (see Fig. 78).

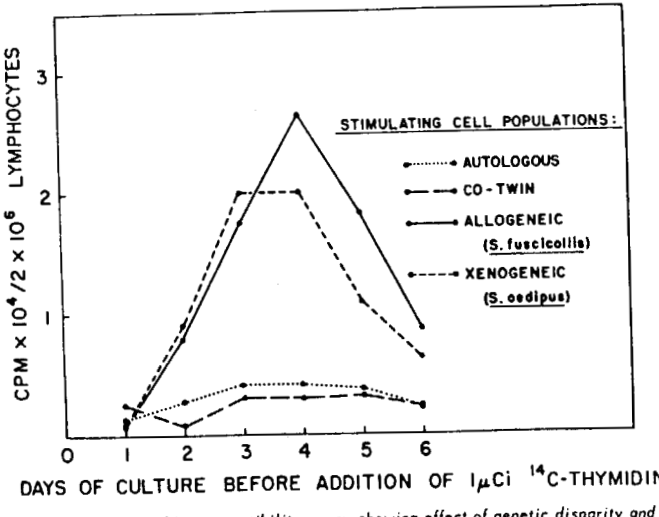


Fig. 78. Marmoset in vitro histocompatibility assay showing effect of genetic disparity and co-twin tolerance by the mixed lymphocyte culture (MLC). Points on curve represent 24-hour thymidine uptake by S. fuscicollis responding lymph code cells in a unidirectional assay. (See text.)

Since our kidney transplant studies necessitated the killing of the kidney donor, its lymphoid cells were stored in liquid nitrogen (slow-rate freezing in a dimethylsulfoxide medium) for future retrieval in tests for putative sensitization of the kidney co-twin recipient. This opportunity presented itself upon the death of our three original chimera co-twin kidney transplants. When they died, lymphocytes of the kidney donor were retrieved from liquid nitrogen storage, treated with mitomycin C, and used as the stimulating cells in the MLC test. The fresh lymph node cells taken from the recipient co-twins did not show any mitogenic response such as would be expected if any sensitization had occurred during the kidney transplant period. As positive controls for these experiments, frozen and fresh allogeneic and xenogeneic cells were mixed with the same tested populations of lymphocytes from the kidney transplant recipient; these combinations gave the anticipated high incorporation of the ^{14}C-thymidine label. Thus, renal transplantation between co-twins did not appear to induce any sensitization to co-twin antigens as measured by the in vitro MLC test.

The MLC tests are now being extended to use of blood lymphocytes. Results of these tests are not clear-cut since the peripheral blood from a single marmoset may proliferate into hemic elements, incorporating high values of ^{14}C-thymidine without any stimulation by exogenous histoantigens. We are now trying to determine a way to control this high "self-responding" activity to a level where the responses obtained in the mixed cultures can be meaningful.

Ultrastructural Studies of Tumors

The electron microscope team is an important Division-wide resource and performs important services for all of the various groups. Primarily it conducts research in cell biology of the reticuloendothelial and hemopoietic tissue associated with virus infection, malignant transformation, and immunologic responses. Collaborative research has been directed toward subcellular localization of gallium in an effort to understand the mechanisms by which it localizes. Other aspects of the gallium work are covered in the second section of this report.

Gallium-67 Localization in AKR Tissues by Light and Electron Microscopic Autoradiography

A major portion of the work in the electron microscopy laboratory during the past year has been on the localization of gallium-67 (^{67}Ga) in various tissues of AKR mice by light and electron microscopic autoradiography (D. C. Swartzendruber and Gary Wolfe). These studies grew out of collaborative work done with the radiopharmaceutical development group on the localization of ^{67}Ga in various experimental animal tumors (18). We are undertaking the use of ^{67}Ga as a tool to study leukemogenesis in the AKR model (31), basing the rationale on the findings that significantly more ^{67}Ga localizes in the leukemic AKR thymus and mesenteric lymph node than in the corresponding nonleukemic tissues (Table 45). It is known from work by Furth and colleagues that "spontaneous" lymphoid leukemia originates in the thymus of about 90% of AKR mice between 6.5 and 12 months of age. Therefore, we wish to identify the cells, and if possible, the organelles that concentrate ^{67}Ga in the AKR tissues as these animals develop leukemia. In particular, we wish to demonstrate the details of the changes in ^{67}Ga uptake in thymic cells as they undergo leukemic transformation. Such information should

1027975

give significant new insight into morphological and biochemical aspects of leukemogenesis in a model system.

Gallium-67 concentrates in both human and experimental animal tumors (5). Information concerning its localization in cells and organelles of a model system such as the AKR thymoma would be expected to contribute important information to studies involving ^{67}Ga localization in human tumors.

To identify the cells that concentrate ^{67}Ga, we have used both light and electron microscopic autoradiography. Quantitative data are necessary and an

TABLE 45

Comparison of ^{67}Ga concentration in tissues of 8- to 10-month-old leukemic and nonleukemic AKR/J mice

Mice (No.)	Thymus weight	% administered dose/g*								
		Thymus	Spleen	Liver	Kidneys	Lungs	Muscle	Femur	Blood	Lymph node
Nonleukemic (20)	0.071 g ± 0.009†	2.28 ± 0.27	2.70 ± 0.24	4.36 ± 0.48	8.87 ± 1.38	4.28 ± 0.92	0.45 ± 0.04	6.13 ± 0.38	1.71 ± 0.18	1.83 ± 0.20
Leukemic (17)	0.434 g ± 0.079†	16.94 ± 1.44	2.41 ± 0.43	2.91 ± 0.37	3.80 ± 0.52	3.78 ± 0.66	1.10 ± 0.33	4.89 ± 0.49	1.29 ± 0.22	8.55 ± 1.34

*Average values.
†Standard error.

evaluation of the autoradiographic technique using ^{67}Ga is a prerequisite for determining the validity of our data.

One problem is to determine the amount of ^{67}Ga activity lost during preparation of specimens, i.e., fixation in 2.5% glutaraldehyde, postfixation in OsO_4, dehydration through a graded series of alcohol, and infiltration of the tissue in a mixture of propylene oxide and epoxy resin. Table 46 shows a representative experiment in which an average of 27% of the initial ^{67}Ga activity was removed from the tissue samples before embedding in Epon 812.

For light microscopy thick (0.5 to 1 μm) and for electron microscopy thin (600 to 1,000 A) sections were cut from Epon-embedded tissues. Thick sections were mounted on glass slides, dipped in Kodak NTB-2 nuclear track emulsion, exposed for 3 to 10 days, developed, and stained with methylene blue. Thin sections were picked up on copper grids and coated with Ilford L-4 nuclear track emulsion, exposed for 10 to 15 days, developed and stained with uranyl acetate and lead citrate.

Grain counts were done on thick sections of thymus and peritoneal exudate cells to determine the amount of ^{67}Ga localization in nuclei versus cytoplasm. Peritoneal exudate cells were used because there are many macrophages in this population. Table 47 shows the data obtained from leukemic and nonleukemic animals. These results indicate that about 75% of the intracellular ^{67}Ga is localized in the cytoplasm. More data are required to determine whether there are differences between normal and leukemic tissues in the intracellular sites of localization.

TABLE 46

Gallium-67 activity lost from tissues during fixation and dehydration procedures determined in a well-scintillation counter.

Tissue	Total Counts/Tissue Aliquots: Beginning of fixation	End of procedure	Loss	Percent Activity Lost
Liver	12,644	10,134	2,510	20
	5,824	4,494	1,330	23
Thymus	14,628	8,378	6,250	43
	2,717	2,271	446	16
Mesenteric lymph node	5,332	3,656	1,676	31
Totals	41,145	28,933	12,212	
Averages				27

TABLE 47

Gallium-67 localization in nuclei versus cytoplasm of AKR thymus and peritoneal exudate cells determined by radioautography.

		Nuclei	Cytoplasm
Total grains*	8,001	1,943	6,068
Average		25	75

*Grain counts are corrected for background.

1027976

Grains were also counted on thick sections of AKR thymus to determine ^{67}Ga localization in cell types. In individual sections of both normal and leukemic thymus the individual macrophages had about 10 times as much gallium as the individual thymocytes. The evidence for this is shown in Table 48. In addition,

TABLE 48

Gallium-67 localization in cell types of AKR thymus determined by radioautography.

	Lymphoid cells*			Macrophages		
	Number Cells	Number Grains	Grains/ Cell	Number Cells	Number Grains	Grains/ Cell
Totals	1,546	1,337	0.9	138	1,530	11.1

*Includes thymocytes and typical lymphoma (leukemic) cells.

TABLE 49

An estimate of ^{67}Ga in cell organelles from grain counts of 114 electron micrographs.

Grains Localized Over:	Nuclei	Mitochondria	Cytoplasmic Dense bodies	Miscellaneous Cytoplasmic areas
Total counts (3,920)	315	93	2,389	1,123
Percent of total	8	2	61	29

there is clearly a greater number of these large gallium-labeled phagocytic cells in the leukemic than in the normal thymus. However, we do not know yet whether the absolute uptake in individual phagocytes is the same in the leukemic and the normal animals, and no accurate quantitative data are yet available on the uptake in normal versus leukemic thymocytes. Thus, we have not yet established on a cellular basis the explanation for all of the 8-fold increase in ^{67}Ga uptake in the leukemic thymus. Work is in progress to show whether or not the leukemic cells take up more gallium than the normal thymocytes.

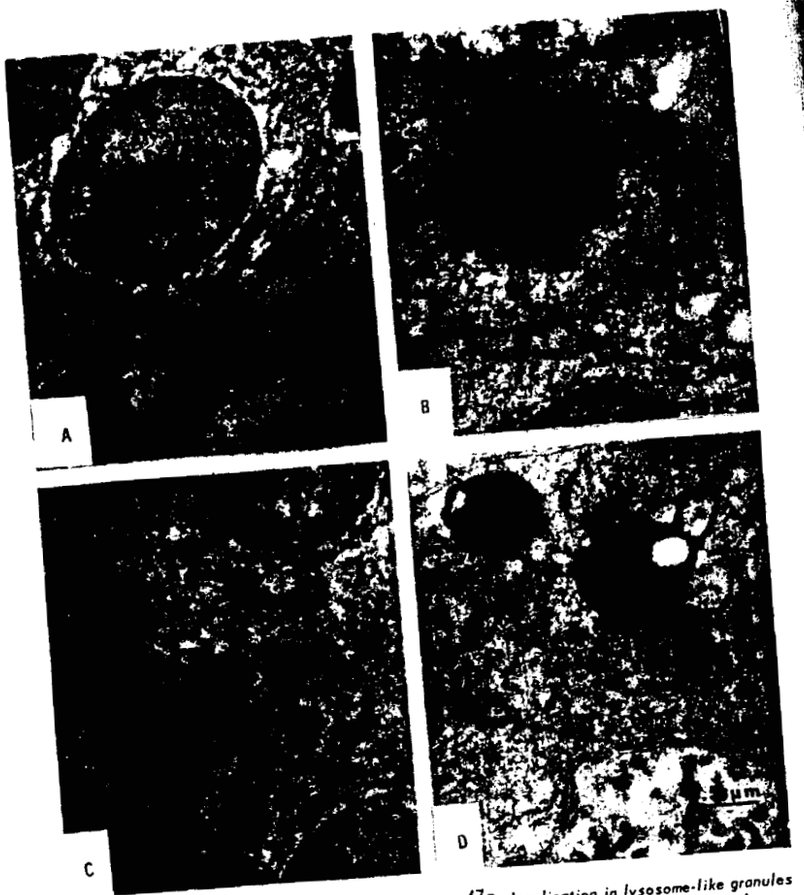


Fig. 79. Electron microscopic autoradiograms showing ^{67}Ga localization in lysosome-like granules of various AKR cell types. Fig. 79 A is from mesenteric lymph node; Fig. 79 B is from thymus; Fig. 79 C is a portion of a hepatic cell; and Fig. 79 D shows a part of a renal cell from the proximal convoluted tubules. All figures are 33,000 x.

On the subcellular level, we have shown that ^{67}Ga is associated with cytoplasmic electron-dense bodies morphologically identified as lysosomes (96) With electron microscopic autoradiography (Fig. 79) we find silver grains localized over these lysosome-like granules in several cell types, viz., macrophages, liver cells, and kidney cells of the proximal convoluted tubules. We attempted to quantitate these findings by counting grains on 8 x 10-in. photographic enlargements. About 60% of the silver grains were localized over these cytoplasmic dense bodies (Table 49). Dense bodies or lysosome-like granules comprised less than 5% of the total surface area of these photographs, demonstrating a high degree of ^{67}Ga localization in these dense granules. In a collaborative experiment, electron microscopic autoradiography demonstrated ^{67}Ga activity in a pellet of lysosome-like granules obtained from a rat tumor (RFT) tissue (see Fig. 46).

1027977

Ultrastructural Studies of Lymphoid Germinal Centers

In our continuing studies on the ultrastructure of lymphatic tissues, we reported new observations on the deposition of lanthanum in AKR splenic germinal centers (95). One unique ultrastructural feature of lymphoid germinal centers is the network of electron-dense material surrounding the characteristic large germinal-center cells (Fig. 80 A). Morphologic studies have not always

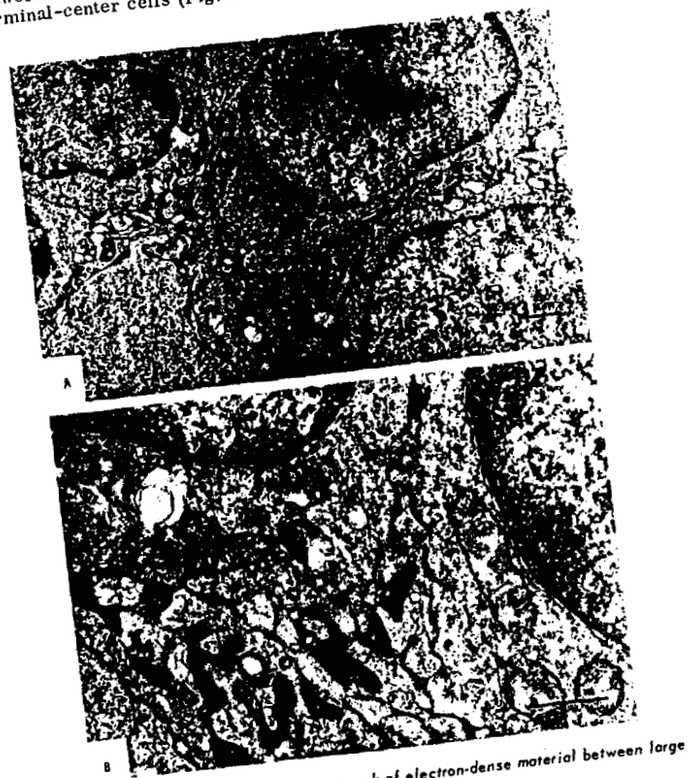


Fig. 80. (A) Electron micrograph showing network of electron-dense material between large germinal-center cells.
(B) Electron micrograph showing very dense deposits of lanthanum in the electron-dense material in splenic germinal center.

clearly distinguished between this apparent extracellular dense material and the narrow, elongated processes of dark reticular cells (Cancer Res 28, 421, 1968). We used lanthanum as a marker to investigate this problem.

Colloidal lanthanum nitrate when added to fixatives deposits notably in intercellular spaces in nerve and liver tissues (Proc Nat Acad Sci 53, 425, 1965). Lanthanum is electron-opaque, therefore can be visualized in the electron microscope as a dense line or area occupying intercellular spaces. We observed lanthanum deposition in splenic germinal centers in narrow intercellular spaces and in the characteristic electron-dense material between the large germinal center cells (Fig. 80 B). This finding confirms the extracellular nature of the electron-dense material in these centers and clearly distinguishes it from the narrow, elongated processes of dark reticular cells.

Induced Cytogenetic Abnormalities

Many exogenous agents – viral, chemical, and radiation – can cause chromosomal breaks. The cytogenetics group is working in this important area analyzing the blood leukocytes in patients who have been irradiated; also in a special study supported by NIH they are characterizing the changes in blood chromosomes of women taking oral contraceptives. Whether or not the increased abnormal chromosomes produced by these ubiquitous agents indicate any somatic or genetic damage in man is largely unknown. The aims are to shed light on how chromosome abnormalities are induced, to uncover the mechanisms of removal of abnormal forms ("recovery") and the possible clinical implications of increased numbers of abnormal metaphases in bone marrow or blood.

Chromosome Breakages in Women Taking Oral Contraceptives

We have completed the second year of study on chromosome breakages in women taking oral contraceptives (Gayle Littlefield, Mary Beth Klepper, and Eugene Joiner). Since the project was designed to cover a 3-year period, this interim report is merely an updating of data. We have completed analyses on several thousand metaphases obtained from lymphocyte cultures from control men, control women, and the study group of women taking oral contraceptives. The continuing accumulation of such a large number of observations has disclosed several trends in chromosomal breakage that could only have been seen in a long-range study.

As of November 30, 1970 we have initiated 649 lymphocyte cultures and are presently reporting data obtained from 390 of these cultures (124 cultures from 20 control women, 100 cultures from 10 control men, and 166 cultures from 29 women in the study group). We have now collected a sufficient number of observations to allow for the evaluation of variability within the three groups. To facilitate data tabulations, we have examined only the percentages of abnormal cells and abnormal chromosomes in each culture, without subdividing the breakage data on the basis of specific types of lesion. As an initial point of comparison we have pooled data from all cultures in each of the three groups and determined the mean, standard deviation, and standard error of the mean of the abnormal cells and abnormal chromosomes (Table 50).

The mean numbers of abnormal metaphases and chromosomes are somewhat higher than those we reported previously. In the study group, the average incidence of abnormal metaphases in 166 cultures was 9.24%. The value is 2.43% higher than

1027978

the average breakage frequency in 124 cultures from the control women, and 3.74% higher than the average breakage in 100 cultures from the control men. The standard deviation and standard error of the mean are also higher in the study group indicating that individual cultures from women who are taking oral contraceptives are somewhat more variable in the percentage of chromosome breakages per culture than either of the control groups.

TABLE 50

PERCENT ABNORMAL CELLS AND CHROMOSOMES IN CULTURES OBTAINED FROM CONTROL MEN, CONTROL WOMEN, AND STUDY GROUP*

Group	Number Cultures	Number Cells Analyzed	Mean	SD	SEM
Control Men	100	10,000	5.50†	3.82	0.38
			(6.43)‡	(4.97)	(0.50)
Control Women	124	12,173	6.81	5.49	0.49
			(7.62)	(6.38)	(0.57)
Study Group	166	14,313	9.24	6.97	0.54
			(10.69)	(7.90)	(0.61)

*Women taking oral contraceptives

†Abnormal cells

‡Abnormal chromosomes

To further define the variability in chromosome breakages in the three groups, we have subdivided the data on the basis of monthly time periods. We have determined the average number of abnormal cells per month for all cultures in each of the three groups for a total time span of 21 months (January 1969 to October 1970). The average number of abnormal metaphases, the number of cultures, number of metaphases, and individual culture ranges for each month from cultures from women in the study group ar shown in Fig. 81.

Several trends become apparent when the chromosome breakages in the three groups are tabulated on the basis of month of culture. First, in cultures from the control women and men, at least one culture had less than 4% abnormal cells in the majority of the monthly time periods. This would suggest that the corresponding high breakages in cultures from other persons during the same periods cannot be attributed to intrinsic culture factors such as technique, or specific media used. We assume that the variations in the number of abnormal cells in individual cultures during different months is related to extrinsic factors, perhaps the exposure of several subjects in the group to viruses, or various drugs. We would anticipate that the women in the study group would be exposed to similar extrinsic factors, and also

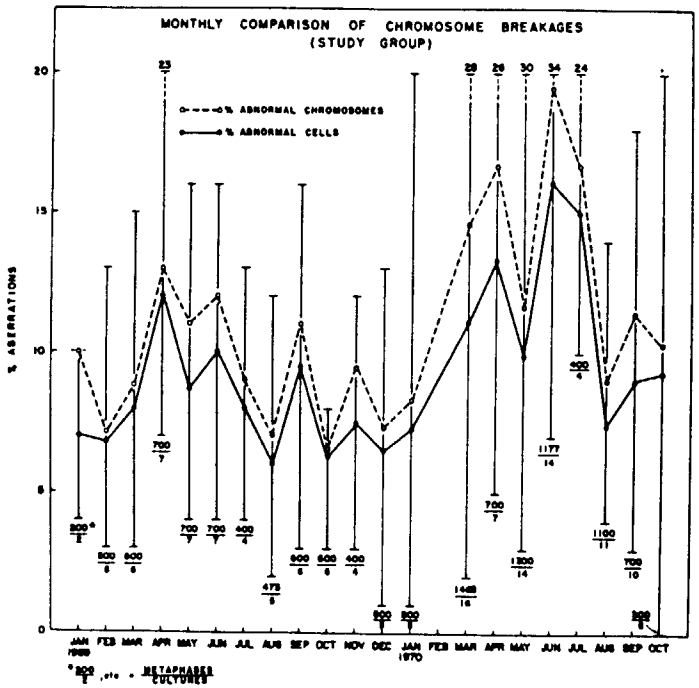


Fig. 81. Average breakages per month in cultures from women taking oral contraceptives. The breakage in these cultures is higher than that seen in either of the control groups, but the breakage is quite variable from month to month.

show fluctuations in chromosome breakage frequencies in different months. The increases in average breakage in the study group compared to the control groups probably represent the effect of the oral contraceptives. We have seen low breakages (less than 1% abnormal cells) in cultures from some women in the study group (lower limits of ranges, Fig. 81). These cultures with low breakages probably reflect variations in individual response to the medication.

A second observation which can be made from the monthly breakage tabulations, is that there appears to be a seasonal variation in the percent of abnormal cells in all three groups of subjects. In cultures from the control men, peak breakages occur during May, June, and July in both 1969 and 1970. The average breakages in cultures from the women are higher than those seen in cultures from the men, and month to month variation is more exaggerated. With slight variations in the two groups, peak breakages occur in March through August 1969 and in March through July 1970. We have reviewed all monthly health records; although sporatic illnesses have been reported in every month, more women recorded either viral or bacterial infections during March, April,

1027979

May, and June than during any other 4-month period. Comparable increases in various medications were recorded for these four months. Relatively few illnesses have been reported by the men, with no significant increases during the months of peak chromosome breakage. Attempts to correlate specific instances of high breakage with specific bouts of illness are quite difficult. Since the life span of some lymphocytes is quite long, it may be possible that cells which were damaged during the course of illness would persist in the circulation for a period of several weeks or months. We suspect that the fluctuations in the number of abnormal cells in different cultures may be related to illnesses, but obviously we have no methods available in a study of this nature for testing this hypothesis.

From our data it is obvious that the number of abnormal cells in the control women is lower than the number of abnormal cells in cultures from women taking oral contraceptives in each month with the exception of August 1969 and May 1970. To demonstrate the differences in the incidence of breakages in the two groups of women, we have subtracted the average breakage values per month in the control women from each comparable average value in the study group (Fig. 82). The differences in the breakage frequencies between the two groups of women in different months is quite variable. In 18 of the 20 months, the breakage in the study group is higher than in the control women. The difference between the two groups in these 18 months varies from 2% to 6.2%. In two other months the average breakages in the control women was slightly higher than the breakage seen in the study group. The variations in the differences in breakage between the two groups emphasizes the importance of long-range studies in projects of this nature. Obviously no one month's data can be considered representative of the effects of oral contraceptives on chromosomal breakage frequencies. We interpret this data as indicating that the medication does indeed cause increased breakage in this study group, but that the total effect of the medication may be overshadowed by the effects of other variables in specific instances.

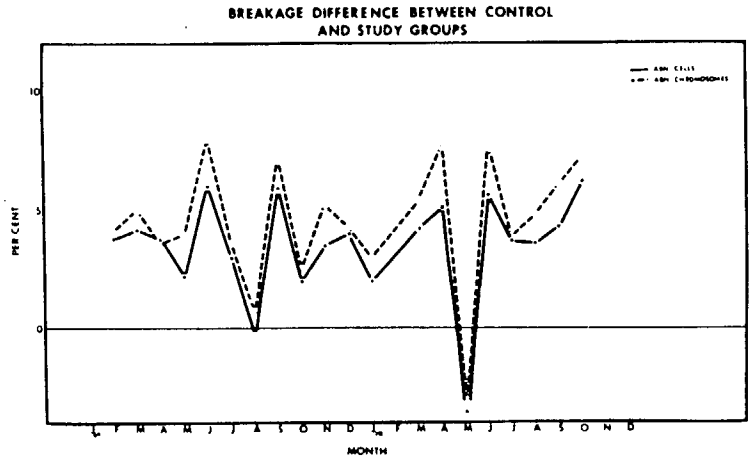


Fig. 82. Difference in breakages per month between cultures from control women and women taking oral contraceptives. We have subtracted the average breakages in the control cultures from the average breakages in the study groups for each month. Note that the differences in breakage between the two groups is quite variable.

Most of the aberrations that we have seen in all three groups of subjects have been "chromosome" breaks. We have identified three specific types of lesions that may be classified under this main heading; these are isochromatid breaks, chromosomes with deleted segments accompanied by the corresponding free fragment, and chromosomes with deleted segments in which the corresponding fragment is missing from the metaphase. To determine whether specific chromosomes were damaged more frequently than others, we have tabulated the distribution of all identifiable chromosome lesions into the following chromosome groups: A_1, A_2, A_3, B, C+X, D, E_{16}, E_{17} and 18, F, G+Y. If chromosome breakages were a random phenomenon, then the number of breaks in each chromosome should be proportional to the total length of that chromosome. We have employed data from the Chicago Conference (Standardization in Human Genetics, 1966) as an index of percent chromatin in the 10 chromosome groups, and compared these values with the percentage of breaks which we actually observed in each group of chromosomes (Fig. 83). We could identify 474 of the abnormal chromosomes in cultures from the control men, 847 breaks in cultures from the control women, and 1,386 breaks in cultures from women in the study group.

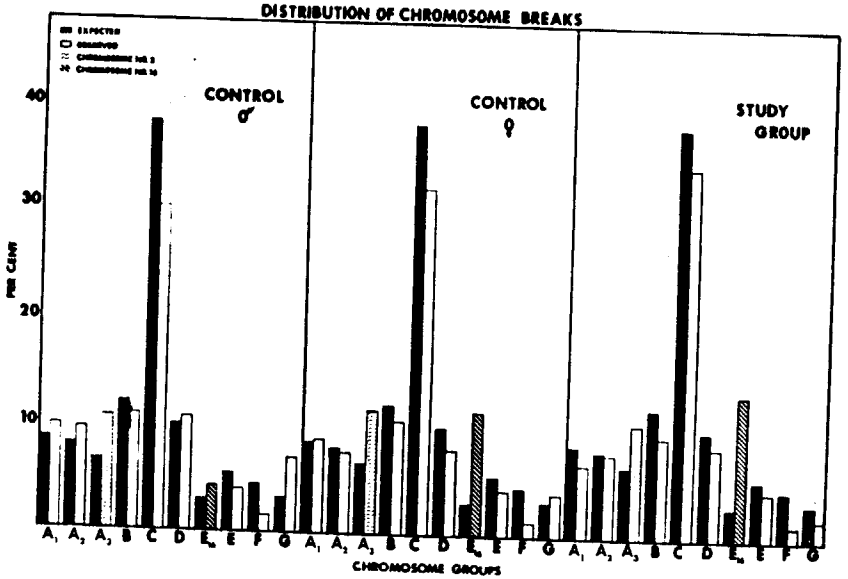


Fig. 83. Distribution of breaks in chromosome groups. We have compared the percent breaks which we observed in the various chromosome groups with the percent chromatin in each group. Note the large increases in breaks in chromosomes A_3 and E_{16}.

Comparison of the percentage breaks that occurred in the specific chromosome groups with the percentage chromatin in each chromosome group revealed a sizable increase in the observed number of breaks in chromosome A_3 with fewer breaks than would be expected in the C and F groups of chromosomes in cultures from the control men and women and in the study group. In addition there was a very large increase in

1027980

the observed breaks in the E_{16} chromosome in cultures obtained from the two groups of women. According to the Chicago Conference tabulations, the E_{16} contains only 3.1% of the total metaphase chromatin. In the control women and the women in the study group 11.4% and 13.4% of all identifiable breaks occurred in the E_{16} chromosome. In order to evaluate these discrepancies in breakage distributions statistically, we have converted the percent chromatin values to expected number of breaks in each chromosome group and compared the data by chi-square analyses. The chi-square tabulations support the visual observation of a nonrandom distribution of breaks in all three groups of cultures ($P<.001$). The breakage distribution in the cultures from the control men are the least deviant from random, whereas breakage in cultures from the women in the study group are the most deviant from random. In all three groups, large contributions to chi-square may be seen in the A_3 and F chromosomes. In cultures from both groups of women, the contribution to chi-square made by chromosome E_{16} is extremely large. Since the large contributions to chi-square made by the A_3 and E_{16} chromosomes were the result of an increase in the observed number of breaks, we have attempted to determine whether there were seasonal variations in breakage in these two chromosomes. The breakage in chromosome A_3 is greater than expected in all three groups of subjects during March 1970 through July 1970. It was during these months that the average breakage frequencies were highest in all three groups of cultures. Chi-square values for chromosome E_{16} in the cultures from control men are highest during November 1969 through May 1970. These months do not correspond to those times when breakage was highest in the control men. In cultures from the control women, E_{16} breakage was highest during those months when the average breakage per month was increased, whereas in the cultures from the study group, breakage in the E_{16} chromosome is greater than would be expected in all of the time periods.

The primary purpose of this study is to determine whether oral contraceptive medications cause increased breakages in lymphocyte chromosomes. Our data show a small but significant increase in chromosomal aberrations in women who are taking oral contraceptives. However, several factors should be considered in the interpretation of our findings. First, the chromosomal breakages that we have seen in control men and women are somewhat higher than those seen in many laboratories. The differences in control breakage frequencies among different laboratories have been attributed to variations in culture technique and in criteria for scoring lesions. One factor should be considered with regard to our technique. In an effort to reproduce the in vivo environment of the lymphocytes as nearly as possible, we have routinely supplemented each 7 cc culture with 3 cc autologous plasma, whereas many laboratories use heat-inactivated human AB+ or bovine serum as culture supplements. If we obtain samples from a person during a time when he is ill or taking medications, it may be that we are inoculating into our cultures in the 30% autologous plasma supplement various substances that could cause in vitro chromosome damage. Chromosome breaking properties of plasma have been shown in other experiments by Goh (Radiat Res 35, 171, 1969), Littlefield (Radiology 93, 879, 1969), and Scott (Cell Tissue Kinet 2, 295, 1969). In any event, our data show that abnormal cells may be found in cultures from "untreated" subjects, and that oral contraceptives cause small, but definite increases in chromosome breakages.

Since lymphocyte cultures generally tend to have a relatively high frequency of chromosome aberrations, an obvious question is "Can chromosomal breakages in lymphocytes be considered a reliable index of breakage in other somatic cells and in gametes?" In some types of studies on chromosome breakages, e.g., the exposure of cells to ionizing radiations, the lymphocyte may well be a very suitable cell for study. However, as the mediator of immune response, the small lymphocyte may have a unique cellular reaction when exposed to chemical or humoral agents. We cannot rule out the possibility that the variations in chromosome breakages that we

have seen in cultures from the same persons and the overall increases in average breakage in both the control and study groups at different times of the year, may be related to the lymphocyte's reactivity as an immune cell. It seems rather unreasonable to suppose that if 30% of the cells in a lymphocyte culture have abnormal chromosomes, then 30% of the gametes would also be damaged. Certainly in the female, this would be disastrous since all primordial oogonia are present in the ovary from birth and are not replenishable.

Although we cannot unequivocally state that oral contraceptives do not cause chromosome damage in gametes, our data suggest that one should be extremely cautious in the interpretation of data in this type of study.

Marker Chromosomes in Acute Leukemia

Chromosome studies on two patients with acute granulocytic leukemia revealed an aberrant stem-line in cells of bone-marrow direct preparations from each individual (Gayle Littlefield and Margo Steinman). In two initial marrow samples from one patient (A) (52-year-old man) who had been treated with 6-mercaptopurine and cytosine arabinoside, the abnormal cell clone comprised over 75% of the analyzable metaphases. In two marrow samples from the second patient (B) (23-year-old woman) who had not received any treatment for leukemia, the abnormal cell line comprised over 95% of analyzable metaphases. Chromosomes from phytohemagglutinin (PHA)-stimulated lymphocyte cultures were normal in both subjects; thus, the abnormal clones were apparently restricted to cells of myeloid or erythroid series, and probably represented the malignant marrow elements.

The abnormal cell clones were different in the two patients, but consistent from cell to cell in preparations from each individual. In patient A the cell line was hypodiploid, having 45 chromosomes with a consistent chromosomal rearrangement involving the G group chromosomes. In each marker cell, only three small acrocentric chromosomes were present (-2G's, or -1G, -Y) with an additional large acrocentric chromosome, which was often indistinguishable from the D group chromosomes. There are two reasonable explanations for the abnormal G chromosomes in this patient: (1) the stem-line may have been trisomic for a D chromosome and monosomic for two G chromosomes, or (2) the stem-line may have resulted from an unbalanced translocation between two G chromosomes with the loss of the centric fragment 45, XY, G-, G-, t(GqGq) + (Fig. 84 A). In patient B the abnormal stem-line was hyperdiploid, having 47 chromosomes. The G group was abnormal in this patient also, but in this instance an additional small acrocentric (+1G) was present. The stem-line lacked two C chromosomes (-2C) and had an extra E_{16} (+1E) and an extra metacentric marker chromosome. The marker could not be paired with any other chromosome in the metaphases (Fig. 84 B).

The identification of an abnormal marker cell line in the bone-marrow preparations was particularly significant in patient A. This patient was treated with 500 R total-body irradiation and 24 hr later received a bone-marrow transplant from his brother. Since both donor and recipient were male, it would have been impossible cytogenetically to follow the course of the transplanted marrow cells without the observation of a malignant-cell marker in the recipient. In the two pretransplant marrow preparations over 75% of the cells had the marker chromosomes. We have obtained marrow samples on five occasions since the marrow transplant, and have found only normal 46, XY cells in the last four samples. We interpret this data to indicate that the donor cells have become established in the host and are producing chromosomally normal myeloid and erythroid elements. The data obtained from the

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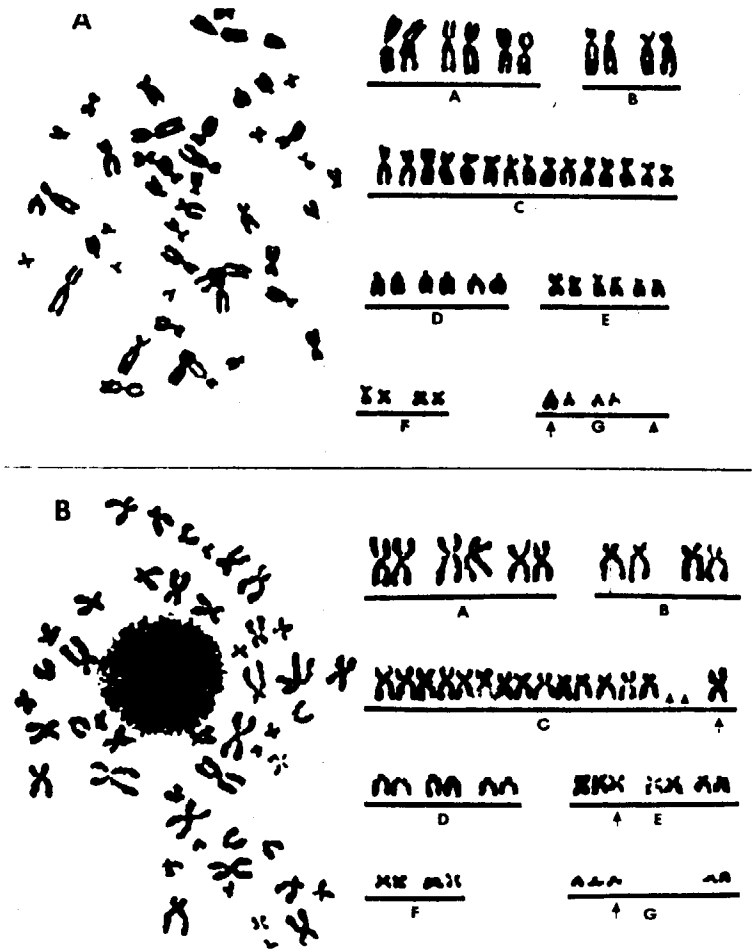


Fig. 84. (A) Representative metaphase from bone-marrow direct preparation from patient A. The marrow sample was obtained before total-body irradiation and marrow transplant. Note the chromosomal rearrangements in the G group chromosomes. Since metaphases from this patient were lacking two small acrocentrics and had an additional large acrocentric, the marker stemlines may have resulted from an unbalanced G/G translocation.

(B) Metaphase from patient B before treatment was begun for leukemia. The cell line was hyperdiploid, missing two C chromosomes, with an additional G, E, and marker chromosome.

direct bone-marrow and peripheral blood cultures from these two patients are shown in Table 51.

Since cytogenetic markers may be useful in distinguishing between donor and recipient cells, several laboratories perform chromosome analyses on patients who have received marrow transplants. If the donor and recipient are of opposite sexes, metaphases may be analyzed for the presence of the male Y chromosomes. If donor and recipient are of the same sex, then obviously the sex chromosome marker cannot be used to distinguish between donor and recipient metaphases. We feel that it is thus very important, particularly when an individual with a blood dyscrasia is to be the marrow recipient, to carefully study direct marrow preparations for the presence of malignant-cell markers.

TABLE 51

METAPHASES WITH MARKER CHROMOSOMES: TWO PATIENTS WITH ACUTE GRANULOCYTIC LEUKEMIA

Patient A	Aug 10	Aug 14	Aug 24	Aug 25	Aug 26	Aug 29	Sept 3	Sept 21	Oct 9	Nov 2
Bone Marrow	---	*3/21+	---	^{60}Co 500 R	11/39	4/4	9/0	22/0	100/0	99/1†
Peripheral Blood	259/0	196/0	200/0	Total-body Irradiation	150/0 Transplant (~18×10^9 bone marrow)					

Patient B	Oct 16	Oct 20
Bone Marrow	‡1/24§	0/25
Peripheral Blood	---	25/0

* Normal 46,XY
\- Marker 45,XY,C-,C-,t(CqCq)+
\- Normal 46,XX
† Marker 47,XX,G+,E+,C-,C-, Mar+

Cytogenetic Studies in a Patient Exposed to 500 Roentgens of Total-Body Irradiation

Although numerous papers have been published on the effects of partial-body irradiation on chromosome breakage frequencies in man, only a few studies have been done on patients who received acute total-body exposures. We have studied bone-marrow and peripheral blood preparations from a patient with acute leukemia before and after he was exposed to 500-R total-body irradiation (Gayle Littlefield, Margo Steinman, and Mary Beth Klepper). (See also page 174).

We studied duplicate cultures (supplemented with either autologous plasma

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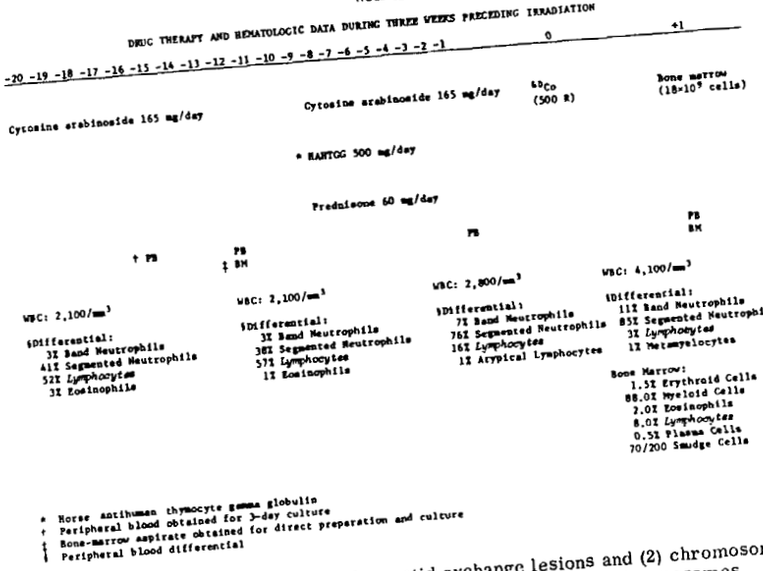
or fetal calf serum) of PHA-stimulated leukocytes on three occasions before the irradiation and on one occasion after the exposure to radiation.

Bone-marrow aspirates were obtained 10 days before and 20 hr after the irradiation. The patient was being treated with several drugs during the time that chromosome studies were initiated. Pertinent clinical and drug history in relation to cytogenetic sample dates are shown in Table 52.

Each abnormal chromosome that could be identified microscopically was classified according to specific lesions: (1) chromatid lesions including chromatid

TABLE 52

DRUG THERAPY AND HEMATOLOGIC DATA DURING THREE WEEKS PRECEDING IRRADIATION



* Horse antihuman thymocyte gamma globulin
† Peripheral blood obtained for 3-day culture
‡ Bone-marrow aspirate obtained for direct preparation and culture
§ Peripheral blood differential

breaks, isochromatid breaks, or chromatid exchange lesions and (2) chromosome lesions, including deletions, dicentric, translocated, and ring chromosomes. Pulverized chromosomes were scored separately. Any metaphase with a chromosome-type aberration was considered abnormal in data tabulations.

Since the patient had received numerous drugs that may have caused chromosome breakages, it was imperative to determine the number of damaged chromosomes in cells cultured before the irradiation exposure. Data obtained from the six pre-irradiation peripheral blood cultures are presented in Table 53. The number of abnormal metaphases seen in cultures containing fetal calf serum are reasonably consistent on the three culture days. However, the number of abnormal cells in cultures supplemented with autologous plasma was quite variable. It is possible that drugs or drug metabolites are introduced into in vitro culture systems when an autologous plasma supplement is used and this could possibly account for the variations in number of cells with aberrations in cultures from this patient. Since he was receiving daily three different drugs during part of this study, it is impossible

TABLE 53

CHROMOSOME BREAKAGES IN PREIRRADIATION CULTURES

Peripheral Blood Cultures	Cells Scored	CHROMOSOME LESIONS: Abnormal Cells	Deletions	Dicentrics	Translocations	Rings	CHROMATID LESIONS‡: Chromatid Exchange	Isochromatid Breaks	Chromatid Breaks	Pulverizations
Fetal Calf Serum										
-15 days	100	2 2%	2 2%	—	—	—	—	3 3%	15 15%	—
-11 days	96	6 6.3%	6 6.3%	—	—	—	—	2 2.1%	12 12.5%	—
-1 day	100	4 4%	3 3%	1* 1%	—	—	—	3 3%	6 6%	1 1%
Autologous Plasma										
-15 days	139	14 10%	14† 10%	1 0.72%	1 0.72%	—	1 0.72%	12 8.6%	23 16.5%	3.20 2.1%
-11 days	100	4 4%	4 75%	—	—	—	—	3 3%	4 4%	—
-1 day	100	—	—	—	—	—	—	1 1%	7 7%	

* Plus fragment
† Chromatid lesions and pulverized metaphases were not figured into tabulations of number of abnormal cells
‡ Seven deletions in G-group chromosomes

to determine what effects or countereffects may have been produced by them. We can state, from direct observations, that breakage frequencies were not drastically increased in the preirradiation cultures. The majority of the abnormal chromosomes were simple deletions; only one translocated and two dicentric chromosomes were identified in over 600 metaphases.

Pooled data from the control cultures are compared with data from the post-irradiation cultures in Table 54. Radiation-induced lesions in the peripheral blood cultures supplemented with fetal calf serum and in those supplemented with autologous plasma are quite similar. The variations in the number of specific lesions may have been the result of the relatively small number of cells scored. There is a considerable difference, however, between the number of chromosome exchange aberrations in the bone-marrow direct preparations and the peripheral blood cultures. This discrepancy might be explained on the basis of difference in stage of cell cycle, at the time of irradiation, between the marrow cells and peripheral blood cells. Since no blast cells were identifiable in differential blood smears from the patient on days PHA-stimulated cultures were set up, it might be assumed that only transformed lymphocytes were dividing in vitro. The great majority of these lymphocytes should have been in the G_1 (pre-DNA synthetic period) stage of interphase at the time of irradiation. Thus, a considerable number of two-hit aberrations should have been induced in these cells. The bone-marrow cells, on the other hand, were obtained from a heterogeneous population of immature and mature forms, and should have been in all stages of the cell cycle at the time of irradiation. Since our marrow samples were collected less than 20 hr after exposure to radiation, the majority of the cells that had time to reach metaphase in 20 hr were probably in the S-period at the time of exposure. If this is correct, fewer two-hit (G_1) aberrations would be expected in this sample of marrow cells than in blood leukocytes. Metaphases obtained from blood and bone-marrow direct preparations postirradiation are shown in Figs. 85 and 86.

1027983

TABLE 54

COMPARISON OF PREIRRADIATION AND POSTIRRADIATION CHROMOSOME BREAKAGES

Cultures	Cells Scored	CHROMOSOME ABERRATIONS: Abnormal Cells	Deletions	Dicentrics	Translocations	Rings	CHROMATID ABERRATIONS*: Chromatid Exchange	Isochromatid Breaks	Chromatid Breaks	Pulverizations
Pre-^{60}Co Bone marrow direct	23	—	—	—	—	—	—	1	—	—
Peripheral Blood Autologous Plasma†	339	18 5.3%	18 5.3%	1 0.3%	1 0.3%	— —	1 0.3%	16 4.7%	34 10%	3 9%
Peripheral Blood Fetal Calf Serum	296	12 4.1%	11 3.7%	1 0.34%	—	—	—	8 2.7%	33 11%	1 0.34%
Post-^{60}Co Bone marrow direct	50	32 64%	65 130%	12 24%	4 8%	—	2 4%	3 6%	13 26%	1 2%
Peripheral Blood Autologous Plasma	50	35 70%	43 86%	34 68%	14 28%	4 8%	—	10 20%	2 4%	1 2%
Peripheral Blood Fetal Calf Serum	100	84 84%	109 109%	76‡ 79%	17 17%	7 7%	1 1%	19 19%	5 5%	—

*Chromatid aberrations and pulverizations not included in tabulations of number of abnormal cells
†Pooled data from three pretreatment cultures
‡Plus three tricentrics

TABLE 55

FORMULA FOR YIELD OF RINGS AND DICENTRICS IN RELATION TO ABSORBED DOSE (X OR GAMMA RADIATION)

$$Y = a + bD^2$$

Y = Yield of rings and dicentrics

a = Control aberration frequencies

b = Coefficient of aberration production for specific cells

(for human lymphocytes -- 6.0×10^{-6})

(1) Y = 76/100 cells (peripheral blood autologous)

(2) Y = 86/100 cells (peripheral blood fetal calf serum)

$$D = \frac{Y}{b}$$

(1) $D = \sqrt{\frac{0.76}{6.0 \times 10^{-6}}} = 357$ rads

(2) $D = \sqrt{\frac{0.86}{6.0 \times 10^{-6}}} = 378$ rads

(Average for 1 and 2 = 368)

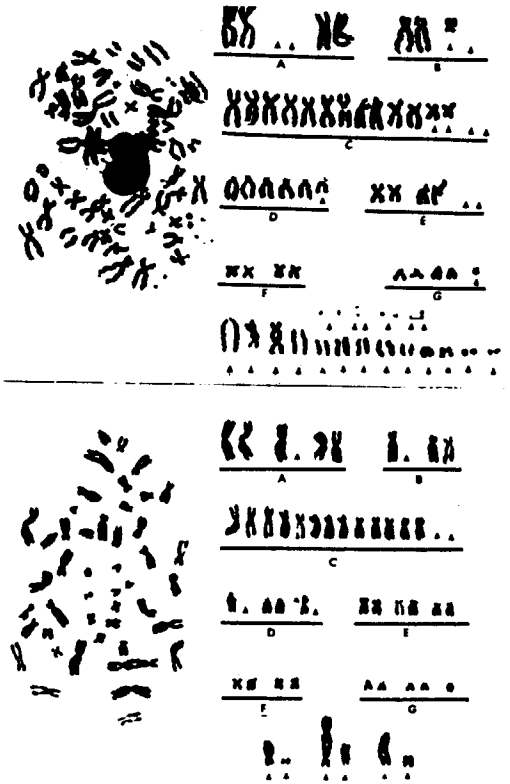


Fig. 85. Two lymphocyte metaphases from blood samples obtained within 24 hr postirradiation. Note two dicentric chromosomes, one translocated chromosome, and numerous fragments in upper metaphase. The lower metaphase has two dicentric chromosomes, one translocated chromosome and an additional fragment.

In the assessment of degree of injury in persons exposed during radiation accidents it has been suggested that chromosomal breakages might serve as a biological dosimeter since they increase in proportion to the total dose. The patient in this study was exposed to a known amount of radiation, and we have compared our data with previously reported in vitro breakage frequencies to determine whether the radiation-induced chromosomal aberrations could have been used to estimate exposure. We have made the calculations using the classical formula for ring and dicentric production, and the coefficient of aberration production obtained by Bender

1027984

for 3-day PHA-stimulated lymphocyte cultures (Radiat Res 29, 568, 1966). The tabulations are presented in Table 55.

The average absorbed dose in this patient as determined by instrumental monitors was 370 rads. The average value for absorbed dose that we could have predicted on the basis of ring and dicentric chromosomes in lymphocytes from this patient, is 368 rads. Thus in this instance, radiation-induced chromosome lesions appeared to offer a very accurate biological dosimeter.

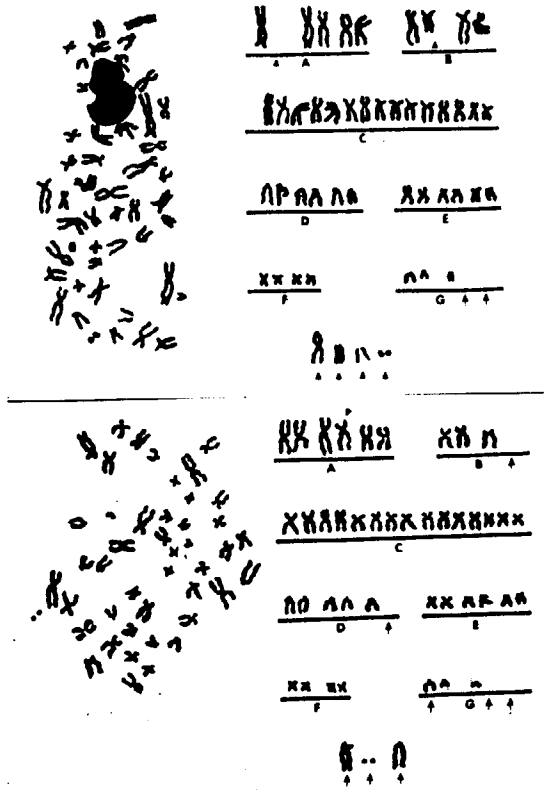


Fig. 86. Two metaphases from postirradiation bone-marrow direct preparation that have chromosome exchange aberrations. The upper metaphase has one dicentric chromosome with two additional fragments; the lower metaphase has one dicentric chromosome, and one translocated chromosome. Note that both metaphases have abnormal G group chromosomes, which suggest they were derived from the marker stem-line.

Subchromatid Structure in Human Leukocytes

Light microscopic studies of chromosomes from several species of plants have shown that each metaphase chromosome is composed of two chromatids joined at the primary constriction (centromere), and that each chromatid is made up of at least two smaller structural units, the subchromatids. Although it has been suggested that human chromosomes would have similar substructure, the relatively small size and intense staining properties of human metaphases make direct microscopic observations of subchromatids quite difficult. On occasion, individual chromosomes in preparations from human leukocytes appear coiled at light

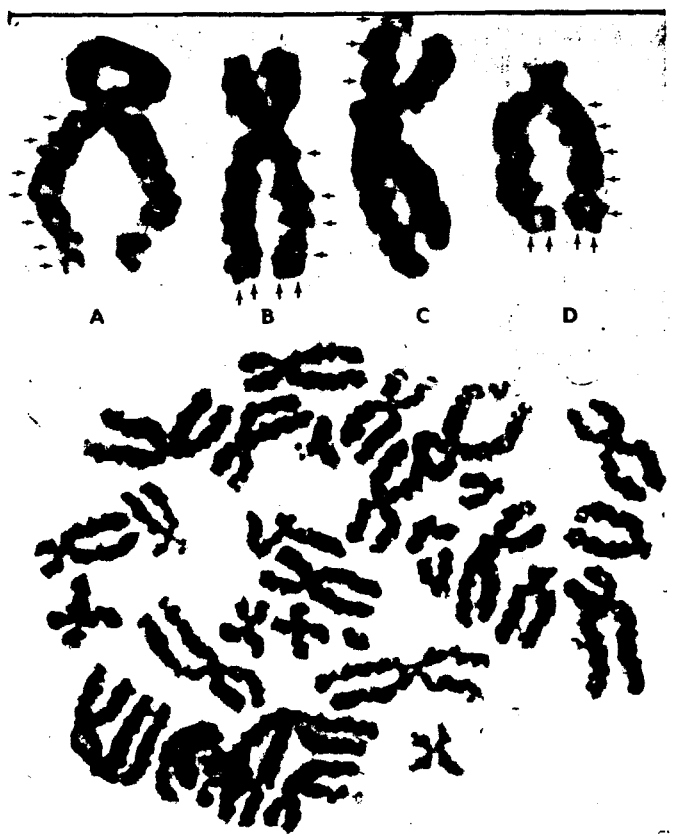


Fig. 87. Partial metaphase from bone-marrow culture showing subchromatid structure. An approximate magnification of metaphase is 2,900 x, and of chromosomes A, B, C, and D 5,200 x.

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microscopic magnifications. In these instances the chromosomes have darkly staining and lightly staining segments of chromatin that alternate along the length of the chromatid. Other investigators have determined that the number of darkly stained segments (gyres) is fairly consistent in analogous chromosomes from different cells. It has been suggested that the alternating dark and light bands in each coiled chromatid are produced by the specific substructural orientation of two subchromatids.

Recently we were able to obtain good quality photomicrographs that clearly show subchromatid structure in human leukocytes (Gayle Littlefield). The chromosomes were obtained from cultures of bone marrow obtained from the patient with acute granulocytic leukemia who had been exposed to 500-R total-body irradiation in preparation for marrow transplantation. Numerous metaphases from the bone-marrow preparation were striking in appearance. The chromosomes were loosely coiled in some prophase and many metaphase figures. Two subchromatids were clearly visible, particularly in the telomeric regions of the chromatids (Figs. 87 B, D). The subchromatids appeared to be loosely intertwined, separating slightly, then crossing at regular intervals along the length of the chromatid. In several chromosomes it was possible to count the number of loops that resulted from the crossing of the subchromatids (Figs. 87 A, B, C, and D). Detailed analyses of several metaphases are in progress. In the chromosomes that have been examined thus far, the number of subchromatid loops in the long arms and short arms of specific chromosomes approximate the number of gyres that have been seen in analogous chromosomes in other studies.

It is possible that the detailed substructure seen in this particular bone-marrow preparation was in some way made more visible as a result of the drug or radiation treatment of the patient. If this were the case, it might be possible to reproduce these conditions in vitro and thereby unmask the subchromatid structure in human cells.

Environmental Pollutants and Altered Metabolism

The work in the following section evolved out of earlier studies in whole-body counting and metabolism where special whole-body counting techniques were applied in studying the metabolic fate and turnover of radioactive compounds. A second stimulus for these studies came from the marmoset research project where a peculiar thyroidal lesion was noted in fetuses and adults. It was strongly suspected that exogenous goitrogens contributed to the lesion. Notes on marmoset aspects of the work are presented in the earlier section.

The Effect of DDT Poisoning in Pigeons on Thyroid Morphology and Function

Last year we reported work on a thyroid disorder occurring in marmosets and observations on this are continuing, but we find the study limited by the expense of the animal. In seeking a smaller species that could be studied in numbers and sacrificed for histologic studies, we were aware of interesting observations made by others in birds and we selected the homing pigeon (Columba livia) for the extension of our research (C. C. Lushbaugh, Gretchen Humason, W. D. Gibbs, and Harold D. Hodges). We needed direct evidence that our results, obtained by whole-body counting as a nondestructive method for determining thyroid

function in small animals, correlate with commoner means of measuring thyroid function that require killing the animals for histologic study and thyroid radioassay. The experiments by Jefferies and French (Science 166, 1278, 1969) demonstrated that chronic feeding of large amounts of P, P' DDT can cause symptoms suggesting hyperthyroidism in Bengalese finches (Lonchura striata) and can produce hepatomegaly and thyroid hyperplasia in homing pigeons. However, the status of thyroid function remained unanswered in that study since no metabolic or chemical studies on iodine were done. Jefferies and French held that the thyroid hyperplasia in the pigeons might reflect either a hyper- or hypofunctional state. They suggested that the lesion might be the result of a direct effect upon the thyroid or an indirect one caused by increased thyroxine metabolism by the liver as proposed by Fregly et al. (Canad J Physiol Pharmacol 46, 59, 1968) from physiologic studies of DDT effects in rats.

We repeated the experimental protocol of Jefferies and French as closely as possible except that we used technical grade DDT and force-fed the birds on Monday, Wednesday, and Friday of each week instead of every other day. After approximately 40 days Jefferies and French terminated their experiment to obtain DDT analyses and morphologic studies; instead, we continued the feedings for the next 14 days while determining the whole-body retention of 0.1 microcurie of ^{131}I using our whole-body counting technique. Estimates of the size of the unbound and bound fractions of the ^{131}I label and their respective turnover rates were made from lines fit to the whole-body retention data by an analog computer using the well known two-compartment exponential model. On day 56, after the retention data were obtained and the small ^{131}I tracer dose could no longer be followed, all birds were injected intramuscularly with 1.0 microcurie of ^{125}I; they were killed 18 to 40 hr later and necropsied for thyroid radioassay, autoradiograms, and microscopic study.

These results are summarized in Table 56. We found large changes in thyroid size, in thyroid iodine uptake, and in turnover rates for bound iodine that correlated with DDT dosage levels significantly ($P = < 0.05$). Although liver size also increased, change in liver weight was not significantly related to the size of DDT dose; some animals with medium levels of intoxication had larger livers than birds at the highest DDT intake. Brain weight, body weight, and renal iodine excretion rate also did not correlate with level of DDT intake.

Our findings confirm all of the observations of Jefferies and French and show that DDT has both direct and indirect effects upon thyroid morphology and function. In Fig. 88 whole-body-^{131}I retention curves are shown for four individual birds that demonstrate the typical retention patterns in the control and the three different dosage groups. In Fig. 88 A the normal curve and that of a bird fed at the highest (72 mg DDT/kg) level are contrasted. In the normal control about 60% of the tracer dose was found and it turned over with a half-time of 15 days. No evidence of any iodine binding (B_0) was found in some birds fed the highest dose of DDT; all of the tracer [marked T(U)] was excreted as rapidly as the unbound fraction (U_0) in the control bird. In Fig. 88 B the typical effects of 18 and 36 mg DDT/kg are contrasted. In comparing these retention curves with those in Fig. 88 A we find the bound iodine compartment size at the 18-mg/kg level (B-18) to be like that of the normal bird but its turnover rate was more rapid (t_{50}= 6 days). At 36 mg DDT/kg, the bound iodine compartment size (B-36) was reduced to ~ 25%. It was not completely eliminated as it was in the 72-mg/kg bird but the turnover rate was remarkably rapid (t_{50} = 4 days) and resembled that of the 18-mg/kg bird. In some birds the turnover rate in this compartment could not be estimated because the amount of iodine binding was too small to measure.

1027986

TABLE 56

Correlations of liver and thyroid weights with results of compartmental analysis of whole-body-^{131}I retention and control birds and birds fed three levels of DDT.

	Organ Weights		Unbound Iodine		Bound Iodine	
DDT* (mg/kg)	Liver (g)	Thyroid (mg)	U_0 (%)	U_{t50} (hr)	B_0 (%)	B_{t50} (day)
0	5.5	34	30	41	70	17
0	6.4	38	40	13	60	15
0	4.8	57	73	29	27	15
18	9.0	62	50	12	50	35
18	12.1	70	45	15	55	24
18	8.5	53	35	14	65	6
18	14.0	78	100	36	0	†
36	9.1	61	33	10	67	20
36	44.6	111	75	19	25	4
36	12.4	86	90	22	10	5
72	21.0	288	72	24	28	5
72	18.3	62	83	13	17	3
72	22.6	115	100	24	0	†
72	13.0	180	100	42	0	†

* The DDT doses were given orally 3 days each week for 6 weeks. U_0 = Unbound iodine compartment; B_0 = Bound (hormonal) iodine compartment.

† No bound iodine compartment found.

When these altered whole-body-^{131}I retention patterns were present, they were accompanied by increased thyroid weight, loss of colloid, hyperplasia of acinal epithelium, some increase in liver weight, hypertrophy of hepatic parenchymal cells, quantitative increase in hepatic fat content (not proportionally increased), and a decreased ^{125}I thyroid uptake. The extremes of our histologic and autoradiographic findings in these thyroid glands are shown in Fig. 89.

Some of the birds fed 18 mg DDT/kg had thyroid glands that were histologically

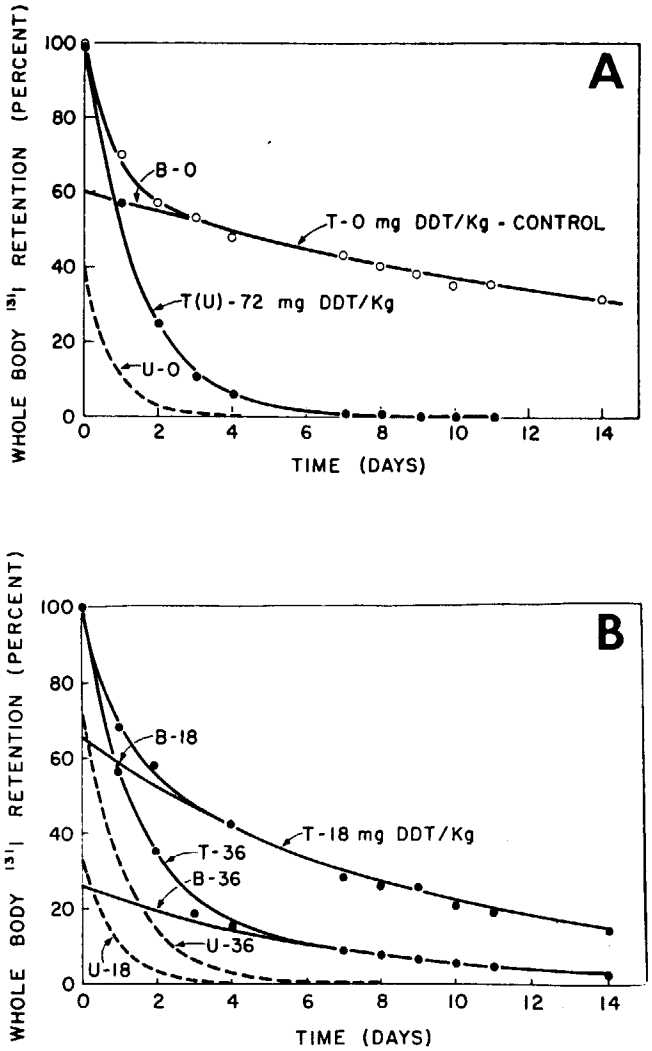


Fig. 88. Whole-body ^{131}I retention curves for four pigeons that typify the effects of DDT feeding upon thyroidal iodine-binding and bound-iodine turnover. The total-retention curves (T), bound-iodine curves (B), and unbound-iodine curves (U) are shown in (A) for a control pigeon (0 mg DDT/kg) and one fed 72 mg DDT/kg three times a week for 6 weeks; and in (B) for pigeons fed similarly at the 18- and 36-mg DDT/kg levels.

1027987

normal (Fig. 89 A) and showed colloid labeled normally with ^{125}I after 18 hr (Fig. 89 B). The birds fed at the 72-mg/kg level and those at other doses (see Table 56) with similar ^{131}I retention patterns [Fig. 88 A, T(U)-72] had, in the central portions of their enlarged glands, extreme thyroid hyperplasia (Fig. 89 C) and scantily ^{125}I-labeled thyroid epithelium (Fig. 89 D).

We conclude that chronic feeding to pigeons of DDT for 40-56 days induced thyroid hyperplasia associated with hypofunction of the gland. In the most severely

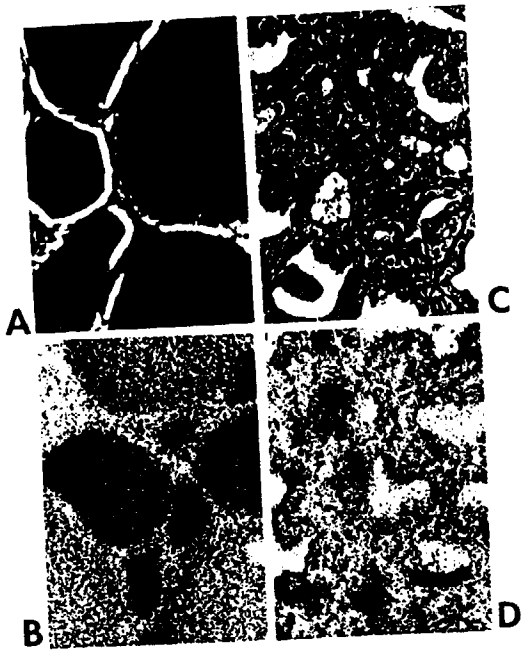


Fig. 89. Photomicrographs (reduced one half from 600 x) of sections of thyroid gland from a bird (A and B) with a normal whole-body iodine retention pattern and from a bird (C and D) without a demonstrable bound-iodine compartment after 72-mg DDT/kg feedings for 42 days. Photos A and C are of sections of glands fixed in mercuric chloride and formalin, embedded in paraffin and stained with PAS; B and D are autoradiograms of the other thyroid gland of the same birds fixed in cold formalin only, embedded in paraffin, and lightly stained with hematoxylin after 19 days exposure of the photographic dipping emulsion to ^{125}I radioactivity.

intoxicated birds, the thyroid becomes almost, if not completely, nonfunctional. The increased tunrover rate of the bound iodine fraction (the hormonal iodide pool) fits the suggestions of Fregly et al. that DDT or its metabolites DDE and DDD increase the rate of hepatic thyroxine destruction or excretion and thus affect the thyroid indirectly. The decrease, however, in thyroid iodine binding demonstrated by both our whole-body retention and autoradiographic studies must mean that DDT in high concentration has a direct goitrogenic effect, like thiouracil for example, and blocks thyroid iodine-binding directly. The rapid loss of whole-body iodine content also supports this conclusion.

These observations that DDT-fed pigeons have hyperplastic but hypofunctional thyroid glands do not explain the nervousness noted by Jefferies in his Bengalese finches. Our studies do not rule out the possibility of an early transient period of hyperthyroidism during DDT poisoning but we did not observe hyperkinetic signs in our birds; instead they became lethargic and weak as the experiment progressed. These symptoms and the pathologic changes in the thyroid glands of the pigeons resemble quite closely those seen in the animals that die unexpectedly in the ORAU marmoset colony.

This study shows well how whole-body retention measurements can be used to appraise thyroid function and to estimate the rate of hormonal iodide utilization. The total retention curve analyzed from the multicompartment viewpoint affords an accurate means of estimating the size of a fractional compartment hidden within the body without requiring destruction of the animal.

The Effect of DDT Feeding upon Thyroid Function of Quail

Our observations on the effects of DDT in pigeons indicated that their thyroid hyperplasia was hypofunctional, extending the work of Jefferies and French, but failed to offer an explanation for the appearance of extreme nervousness in other birds like the finch when fed DDT. It seemed to us that the explanation for their dichotomous effect might be found in studying thyroid function in a bird like the Japanese Quail (Coturnix) in which, for unknown reasons, hepatic microsomes are inhibited rather than stimulated by ingestion of this pesticide (Frank Mraz*, W. D. Gibbs, Gretchen Humason, Harold D. Hodges, and C. C. Lushbaugh). If the conclusions of Fregley and others are correct that stimulation of hepatic microsomes by DDT can lead to destruction of thyroxine and thus produce hypothyroidism, then in Coturnix feeding of DDT might produce no visible effect on the thyroid but a state of hyperthyroidism due to a shut down of hepatic - biliary thyroxine degradation and excretion.

This study became a collaborative one with the poultry research group of the UT-AEC agricultural project who raised the quail, prepared the diets and performed ^{131}I uptakes in one half of the 120 birds while the whole-body counting group in the Medical Division studied whole-body ^{131}I retention on the other 60. These birds also provided tissue specimens for determining organ weights, and for histologic study, autoradiograms and DDT analysis.

Table 57 lists the results obtained so far. The whole-body ^{131}I retentions were done every 2 weeks for 6 weeks of DDT feeding. The DDT was fed to three groups ad libitum at three different levels; 60, 120, and 240 ppm in dry poultry feed. These levels were calculated to be equivalent to the three dosages force fed in olive oil to the pigeons. The quail reacted quite differently. While the pigeons became lethargic

* UT-AEC Agricultural Research Laboratory.

1027988

TABLE 57

THE EFFECT OF FEEDING DDT TO JAPANESE QUAIL ON BODY AND SELECTED ORGAN WEIGHTS AND THEIR THYROID METABOLISM DETERMINED BY ^{131}I RETENTION STUDY

	Mean Weights				Iodine Metabolism*		
DDT	Body	Liver	Brain	Thyroid	B_0	B_0/thyroid	B_{t50}
(PPM)		(gms)			(%)	(%/gm)	(days)
			A. Two Weeks				
0	89	2.21	0.68	0.012	21	1.7	15
60	108	2.35	0.68	0.012	23	1.9	9
120	96	3.30	0.69	0.013	16	1.2	13
240	118	2.81	0.76	0.013	25	1.9	13
			B. Four Weeks				
0	106	2.80	0.69	0.012	19	1.6	2.2
60	110	2.74	0.75	0.019	23	1.2	30
120	95	3.01	0.71	0.010	7**		32
240	101	2.85	0.76	0.014	42	3.0	14
			C. Six Weeks				
0	107	3.64	0.71	0.011	19	1.7	30
60	91	2.94	0.76	0.015	8	0.5	17
120	99	2.87	0.77	0.011	38	3.5	19
240	76	2.50	0.76	0.011	50	4.5	*

*B_0 = Initial Iodine Bound
B_{t50} = Bound Iodine Turnover Rate

** Single Measurement

(one died), the quail became distinctly jumpy, developed tremors, and many in the two higher dosage levels died. The averaged organ weights at necropsy, Table 57 show that, unlike the pigeons, no increase in hepatic or thyroid weight occurred in the quail. Brain weight increased in excess of normal growth but the importance of this change is questionable since many birds injured their heads jumping against the padded roofs of their cages. The size of the bound iodine compartment changed and is graphed in Fig. 90 as the average percent of ^{131}I bound per milligram of thyroid. On the lowest intake of DDT iodine binding progressively decreased while at the highest level (and perhaps at the intermediate level) it increased significantly (about 2.5 times normal). We confirmed this whole-body retention result by determining the ^{131}I content of extirpated thyroid glands on the four consecutive postinjection days in quail that had been fed DDT at these levels for 6 weeks. Thyroidal iodine uptake had definitely increased.

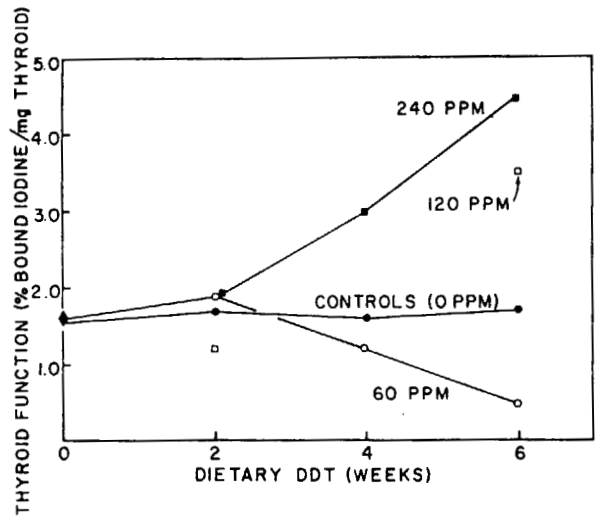


Fig. 90. The effect of feeding DDT to Coturnix quail upon the average percent of ^{131}I label bound per milligram of thyroid. At 240 ppm of DDT, the quail were definitely hyperthyroid by this measurement.

Histologically the thyroid glands of all quail were similar with normal appearing cuboidal epithelium and uniform follicles filled with colloid. The liver morphology was unchanged and so uniform it prompted us to study the enlarged livers of the pigeons more closely. (These observations are described later.)

These studies indicate that the quail became hyperthyroid on the larger doses of DDT. "Clinically" this change was associated in time with increased nervousness and the onset of tremors. At the highest dose of DDT fed for 6 weeks the quail lost weight significantly in the last 2 weeks.

While these results, as we had predicted, were the opposite of those obtained in pigeons they are logically unexplainable only on the basis of an extrathyroidal (hepatic) effect. They are more compatible with the conclusion that DDT is a goitrogen acting directly on thyroid function. Apparently, however, this direct thyroidal effect can be modified by a DDT effect upon hepatic metabolism of thyroxine.

Gold Thioglucose and the Satiety Center

One role of ORAU is to provide access for the academic community to special Oak Ridge resources; the following report is a study that grew out of a collaboration with a university faculty member who required an activation analysis resource in his experiment.

1027989

This year our collaborative study of the satiety center investigated the early development of the gold thioglucose-produced lesion and the focal gold deposits in the satiety center scar (Roger J. Cloutier, Evelyn E. Watson, and Albert F. Debons*).

The ventromedial portion of the brain's hypothalamus contains the satiety center which regulates food intake to caloric expenditure so that the weight of mature animals remains fairly constant. Animals whose satiety center has been destroyed physically or chemically become hyperphagic and in a few weeks become obese.

We were able to show that the necrotic lesion produced in the satiety center by the intraperitoneal injection of gold thioglucose (800 mg/kg) in mice begins at a

* State University of New York, Downstate Medical Center, Brooklyn, N.Y.



Fig. 91. Autoradiogram of hypothalamus 5 hours after gold thioglucose injection, showing the selective deposition of gold at the periphery of the oligodendrocytes.

specific cell, the oligodendrocyte (13). The first departure from the normal hypothalamic structure observed by light microscope examination of sections occurs about six hours postinjection. The sharp boundary between some oligodendrocytes and the surrounding neuropil is lost. The neuropil surrounding the affected oligodendrocytes progressively breaks down during the next 10 hours. Where the area of neuropil dissolution includes a capillary, the capillary becomes irregularly constricted and dilated. A fully developed lesion contains only fragmented filaments from the neuropil, and capillaries in various stages of disintegration.

When ^{198}Au-thioglucose was injected into normal mice which were killed at 5 hours postinjection, gold distributed diffusely throughout the hypothalamus with a selective distribution at the periphery of the cytoplasm of some of the oligodendrocytes (Fig. 91). The ^{198}Au-thioglucose, specific activity of 1.7 mCi/mg, was prepared in an Oak Ridge National Laboratory nuclear reactor.

When the same procedure was done in diabetic mice, autoradiography showed a diffuse distribution of the gold, but failed to reveal a specific relation of gold

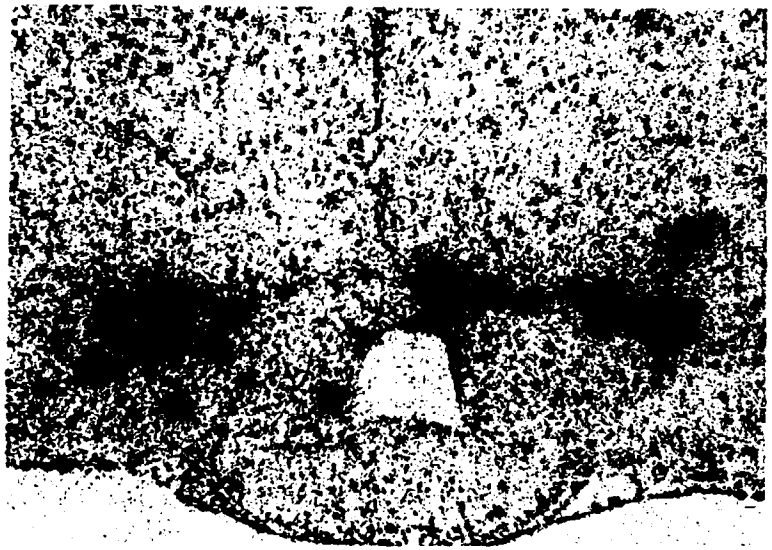


Fig. 92. Autoradiogram of hypothalamus after the gold thioglucose-caused lesion becomes a scar showing the focal deposits of gold. The gold thiomalate + bipiperidyl mustard autoradiograms are quite similar.

1027990

deposits with oligodendrocytes. In earlier work (Amer J Physiol 214, 652, 1968) gold thioglucose failed to produce a lesion in diabetic mice.

Although gold thioglucose produces a lesion in the hypothalamus of normal mice, gold thiomalate when injected at a dose of 800 mg/kg fails to produce one, presumably because it lacks the glucose moiety necessary to react with glucoreceptor cells in the satiety center. Brains from gold thiomalate-treated animals show a diffuse distribution of gold similar to that observed just before a lesion develops in gold thioglucose-treated animals; however, there is no specific deposition of gold at the periphery of the oligodendrocytes.

In the normal mice treated with gold thioglucose, the lesion later collapses into a scar and produces focal deposits of gold (Fig. 92). Since the gold thiomalate-treated animals never develop a lesion, they never exhibit the focal gold deposits. This year we found that focal gold deposits could be produced in gold thiomalate animals if we first injected bipiperidyl mustard. Although the mechanism is unknown, bipiperidyl mustard alone produces a sequence of histologic changes leading to a lesion quite similar to the gold thioglucose lesion.

The focal gold deposits produced by gold thioglucose and those produced by gold thiomalate in combination with bipiperidyl mustard are quite similar (13). This result indicates that most of the gold deposited in the scar of gold thioglucose-treated animals is derived from the diffuse distribution of gold rather than from the gold that may have affected specific gold thioglucose receptor cells.

1027991

PUBLICATIONS

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JOURNAL ARTICLES

Published

1. Balish, E., Pearson, T. A., and Chaskes, S. Irradiated humans: microbial flora, immunoglobulins, complement (C'3), transferrin, agglutinins, and bacteriocidins. Radiat. Res. 43, 729-756, 1970.

2. Barclay, T. R., Morris, A. C., Jr., Hayes, R. L., Carlton, J. E., and McDow, A. E., Jr. On-line computer interfacing of a densitometer film reader for measuring the size of radioactive colloid particles. Anal. Chem. 42, 685-686, 1970.

3. Blank, M. L. and Snyder, F. Long chain fatty alcohols in normal and neoplastic tissues. Lipids 5, 337-341, 1970.

4. Blank, M. L. and Snyder, F. Specificities of alkaline and acid phosphatases in the dephosphorylation of phospholipids. Biochemistry 9, 5034-5036, 1970.

5. Blank, M. L., Wykle, R. L., Piantadosi, C., and Snyder, F. The biosynthesis of plasmalogens from labeled O-alkylglycerols in Ehrlich ascites cells. Biochim. Biophys. Acta, 210, 442-447, 1970.

6. Carlson, D. E. and Gengozian, N. Radiation exposure-rate dependence of the immune response in mice. Radiology, 97, 433-438, 1970.

7. Cloutier, R. J. Symposium on medical radionuclides: Radiation dose and effect. Nucl. Safety 11, 475-481, 1970.

8. Comas, F. V. The radiosensitivity of rat bone-marrow cells. Int. J. Radiat. Biol. 17, 549-557, 1970.

9. Comas, F. V., Edwards, C. L., and Vodopick, H. Splenic irradiation in chronic granulocytic leukemia: Changes in leukocyte values. Radiat. Res. 42, 413-423, 1970.

10. Cosgrove, G. E., Humason, G., and Lushbaugh, C. C. *Trichospirura leptostoma*, a nematode of the pancreatic ducts of marmosets (*Saguinus spp.*) J. Amer. Vet. Med. Ass., 157, 696-698, 1970.

11. Cosgrove, G. E., Nelson, B. M., and Self, J. T. The pathology of pentastomid infection in primates. J. Lab. Animal Care 20, 354-360, 1970.

12. Debons, A. F., Krimsky, I., From, A., and Cloutier, R. J. Gold thioglucose induction of obesity: significance of focal gold deposits in hypothalamus. Amer. J. Physiol. 219, 1403-1408, 1970.

13. Debons, A. F., Krimsky, I., From, A., and Cloutier, R. J. Site of action of gold thioglucose in the hypothalamic satiety center. Amer. J. Physiol. 219, 1397-1402, 1970.

14. Edwards, C. L. and Hayes, R. L. Scanning malignant neoplasms with gallium-67. JAMA 212, 1182-1190, 1970.

15. Gengozian, N. and Merritt, C. B. Effect of unilateral ovariectomy on twinning frequency in the marmoset. J. Reprod. Fertil. 23, 509-512, 1970.

16. Gibbs, W. D., Hodges, H. D., Bailey, M. and Lushbaugh, C. C. Accurate whole-body quantitation of ^{131}I retention by counting a scatter window. J. Nucl. Med. 11, 487-490, 1970.

17. Goh, K. O. Inner chromosomal structure of human leukemic metaphase: A light microscopic observation. Cytologia 35, 465-470, 1970.

18. Hayes, R. L., Nelson, B., Swartzendruber, D. C., Carlton, J. E., and Byrd, B. L. Gallium-67 localization in rat and mouse tumors. Science 167, 289-290, 1970.

19. Pearson, T. A. and Balish, E. Unusual Sphaerophorus species from the large intestine of man. Appl. Microbiol. 19, 458-462, 1970.

20. Piantadosi, C., Ishaq, K. S., and Snyder, F. Synthesis of O-alkyldihydroxyacetone and derivatives. J. Pharm. Sci. 59, 1201-1202, 1970.

21. Piantadosi, C. and Snyder, F. Plasmalogens and related derivatives: Their chemistry and metabolism. J. Pharmacol. Sci. 59, 283-297, 1970.

22. Sansone, G., Swartzendruber, D. C., and Snyder, F. Inclusions in mitochondria of preputial glands from mice. A combined biochemical and morphologic study. Biochim. Biophys. Acta 201, 401-409, 1970.

23. Snyder, F., Blank, M. L., and Malone, B. Requirement of cytidine derivatives in the biosynthesis of O-alkyl phospholipids. J. Biol. Chem. 245, 4016-4018, 1970.

24. Snyder, F., Blank, M. L., Malone, B., and Wykle, R. L. Identification of O-alkyldihydroxyacetone-phosphate, O-alkyldihydroxyacetone, and diacyl glyceryl ethers after enzymic synthesis. J. Biol. Chem. 245, 1800-1805, 1970.

1027992

25. Snyder, F. and Malone, B. Enzymic interconversion of fatty alcohols and fatty acids. Biochem. Biophys. Res. Commun. 41, 1382-1387, 1970.

26. Snyder, F., Malone, B., and Blank, M. L. Enzymic synthesis of O-alkyl bonds in glycerolipids. J. Biol. Chem. 245, 1790-1799, 1970.

27. Snyder, F., Malone, B., and Cumming, R. B. Synthesis of glyceryl ethers by microsomal enzymes derived from fibroblasts (L-M Cells) grown in suspension cultures. Can. J. Biochem. 48, 212-215, 1970.

28. Snyder, F., Piantadosi, C., and Malone, B. The participation of 1- and 2-isomers of O-alkylglycerols and acyl acceptors in a cell-free system. Biochim. Biophys. Acta 202, 244-249, 1970.

29. Snyder, F., Rainey, W. T., Jr., Blank, M. L., and Christie, W. H. The source of oxygen in the ether bond of glycerolipids: ^{18}O studies. J. Biol. Chem. 245, 5853-5856, 1970.

30. Soodsma, J. F., Piantadosi, C., and Snyder, F. The biocleavage of alkyl glyceryl ethers in Morris hepatomas and other transplantable neoplasms. Cancer Res. 30, 309-311, 1970.

31. Swartzendruber, D. C., Byrd, B. L., Hayes, R. L., Nelson, B., and Tyndall, R. L. Preferential localization of gallium-67 citrate in tissues of leukemic mice. J. Nat. Cancer. Inst. 44, 695-700, 1970.

32. Winchell, H. S., Sanchez, P. D., Watanabe, C. K., Hollander, L., Anger, H. O., McRae, J., Hayes, R. L., and Edwards, C. L. Visualization of tumors in humans using ^{67}Ga-citrate and the Anger whole-body scanner, scintillation camera and tomographic scanner. J. Nucl. Med. 11, 459-466, 1970.

33. Wood, R. Plasma membranes: Structural analyses of neutral lipids and phospholipids of rat liver. Arch. Biochem. Biophys. 141, 174-182, 1970.

34. Wood, R. Tumor lipids: Identification of sterol ester species in Ehrlich ascites cells. Cancer Res. 30, 151-154, 1970.

35. Wood, R., Anderson, N. G., and Swartzendruber, D. C. Tumor lipids: Characterization of the lipids isolated from membranous material. Arch. Biochem. Biophys. 141, 190-197, 1970.

36. Wood, R. and Harlow, R. D. Tumor lipids: Carbon number distribution of triglycerides and glyceryl ether diesters. Lipids 5, 776-781, 1970.

37. Wood, R. and Harlow, R. D. Tumor lipids: Structural analyses of the phospholipids. Arch. Biochem. Biophys. 141, 183-189, 1970.

38. Wood, R. and Healy, K. Liberation of aldehydes from alk-1-enyl-glyceryl ethers by acid hydrolysis. Lipids 5, 661-663, 1970.

39. Wood, R. and Healy, K. Tumor lipids: biosynthesis of plasmalogens. Biochem. Biophys. Res. Commun. 38, 205-211, 1970.

40. Wood, R. and Healy, K. Tumor lipids: biosynthesis of plasmalogens. J. Biol. Chem. 245, 2640-2648, 1970.

41. Wood, R., Walton, M., Healy, K., and Cumming, R. B. Plasmalogen biosynthesis in Ehrlich ascites cells grown in tissue culture. J. Biol. Chem. 245, 4276-4285, 1970.

42. Wykle, R. L., Blank, M. L., and Snyder, F. The biosynthesis of plasmalogens in a cell-free system. FEBS Letters 12, 57-60, 1970.

43. Wykle, R. L. and Snyder, F. Biosynthesis of an O-alkyl analogue of phosphatidic acid and O-alkylglycerols via O-alkyl ketone intermediates by microsomal enzymes of Ehrlich ascites tumor. J. Biol. Chem. 245, 3047-2058, 1970.

In Press

44. Carlson, D. E. and Gengozian, N. The effect of acute radiation exposure rates on formation of hemagglutinating antibody in mice. J. Immunol.

45. Carlton, J. E. and Hayes, R. L. Rapid separation of generator-produced gallium-68 from EDTA eluate. Int. J. Appl. Radiat. Isotop.

46. Gengozian, N., Congdon, C. C., Allen, E. A., and Toya, R. E. Immune status of allogeneic radiation chimeras. Transplantation Proceedings.

47. Gengozian, N and Hübner, K.F. An "In Situ" visualization of a graft-versus-host reaction. J. Immunol.

48. Morris, A. C., Jr., Barclay, T. R., Tanida, R., and Nemcek, J.V. A miniaturized probe for detecting radioactivity at thyroid surgery. Phys. Med. Biol.

49. Piantadosi, C., Ishaq, K. S., Wykle, R. L., and Snyder, F. Synthesis and Characterization of 1-O-alkyldihydroxyacetone phosphates and derivatives. Biochemistry.

50. Snyder, F., Hibbs, M., and Malone, B. Enzymic synthesis of O-alkyl glycerolipids in brain and liver of rats during fetal and postnatal development. Biochim. Biophys. Acta.

51. Swartzendruber, D. C., Nelson, B., and Hayes, R. L. Gallium-67 localization in lysosomal-like granules of leukemic and non-leukemic murine tissue. J. Nat. Cancer Inst.

1027993

CHAPTERS IN BOOKS AND SYMPOSIUM PROCEEDINGS

Published

52. Arimizu, N. and Morris, A. C., Jr. Quantitative measurements of radioactivity deposited in internal organs using computer analysis of scintigraphy. In: Proceedings of the 9th Japan Conference on Radioisotopes, Tokyo, Japan, May 12-15, 1969, (Publ. 1970) pp. 378-381.

53. Bernard, S. R. and Hayes, R. L. Dose to various segments of the gastrointestinal tract. In: Medical Radionuclides: Radiation Dose and Effects. R. J. Cloutier, C. L. Edwards, and W. S. Snyder, eds., AEC Symposium Series No. 20, CONF-691212, 1970, pp. 295-314.

54. Cloutier, R. J. and Watson, E. E. Radiation dose from radioisotopes in the blood. In: Medical Radionuclides: Radiation Dose and Effects. R. J. Cloutier, C. L. Edwards, and W. S. Snyder, eds., AEC Symposium Series No. 20, CONF-691212, 1970, pp. 325-346.

55. Dickson, H., Roberts, E., Jr., Butler, H. M., Cloutier, R. J., Smithwick G., Watson, E. E., Haywood, F. F., and Berger, J. D. Radiation safety in the operation of a low-energy accelerator. In: Proceedings of the Second Oak Ridge Conference on the Use of Small Accelerators for Teaching and Research, Oak Ridge Associated Universities, Special Training Division, March 1970, J. L. Duggan, ed., USAEC Report CONF-700322, 1970, pp 441-458.

56. Nelson, B. Postmortem studies of radionuclides in man. In: Medical Radionuclides: Radiation Dose and Effects. R. J. Cloutier, C. L. Edwards, and W. S. Snyder, eds., AEC Symposium Series No. 20, CONF-691212, 1970, pp. 103-113.

57. Nelson, B. and Kniseley, R. M. Marrow fibrosis in myeloproliferative disorders. In: Myeloproliferative Disorders of Animals and Man; Proceedings of the Eighth Annual Hanford Biology Symposium, Richland, Washington, May 1968. AEC Symposium Series No. 19, CONF-680529, 1970, pp. 533-555.

58. Snyder, F. Glycerolipids in the neoplastic cell: methodology, metabolism, and composition. In: Methods in Cancer Research, Vol. 6, edited by H. Busch, New York, Academic Press, 1971, pp. 399-436.

59. Snyder, F. Liquid scintillation radioassay of thin-layer chromatograms. In: Proceedings of the International Symposium on the Current Status of Liquid Scintillation Counting, E. D. Bransome, Jr., ed., New York, Grune and Stratton, 1970, pp. 248-256.

60. Snyder, F. Thin-layer radiochromatography and related procedures, In: Progress in Thin-Layer Chromatography and Related Methods, Vol. 1, A Niederwieser and G. Pataki, eds., Ann Arbor-Humphrey Science Publishers, 1970, pp. 52-73.

61. Vodopick, H., Rupp, E. M., and Gengozian, N. An in vitro histocompatibility test in a search for tumour antigen. In: In Vitro Procedures with Radioisotopes in Medicine. Vienna, International Atomic Energy Agency, 1970, pp. 199-208.

In Press

62. Andrews, G. A., Kniseley, R. M., Vodopick, H., Bergner, P.-E., and Lushbaugh, C. C. Hematologic responses to total-body irradiation. Radioisotope in Pharmakokinetik und Klinischer Biochemie, edited by G. Hoffman and H. A. Ladner. Schattauer Verlag.

63. Gengozian, N. Male and female cell populations in the chimeric marmoset. Proceedings of the 2nd Conference on Experimental Medicine and Surgery in Primates, New York, 1969. Edited by E. I. Goldsmith and J. Moor-Jankowski. S. Karger.

64. Gengozian, N. and Porter, R. P. Transplantation immunology in the marmoset. Proceedings of the 2nd Conference on Experimental Medicine and Surgery in Primates, New York, 1969. Edited by E. I. Goldsmith and J. Moor-Jankowski. S. Karger.

65. Lushbaugh, C. C. and Ricks, R. C. Some cytokinetic and histopathologic considerations of irradiated male and female gonadal tissues. Sixth Annual San Francisco Cancer Symposium, October 1970. Proceedings.

66. Snyder, F. The chemistry, physical properties, and chromatography of lipids containing ether bonds. Progress in Thin-Layer Chromatography and Related Methods, Vol. 2, edited by A. Niederwieser and G. Pataki, Ann Arbor Science Publishers.

67. Snyder, F. and Moehl, A. Mass measurements in a liquid scintillation spectrometer: Quantitation of sulfhydryl moieties by color quenching. Proceedings of the International Conference on Organic Scintillators and Liquid Scintillation Counting. Academic Press.

GOVERNMENT REPORTS

Published

68. Andrews, G. A., Comas, F. V. Edwards, C. L., Kniseley, R. M., Lushbaugh, C. C., and Vodopick, H. Hematologic and therapeutic effects of total-body irradiation (50 R-100 R) in patients with malignant lymphoma, chronic lymphocytic and granulocytic leukemias, and polycythemia vera. USAEC Report ORAU-112, Oak Ridge Associated Universities, 1970.

69. Medical Radionuclides: Radiation Dose and Effects. Proceedings of a symposium held at Oak Ridge Associated Universities, December 8-11, 1969. R. J. Cloutier, C. L. Edwards, and W. S. Snyder, eds. USAEC Symposium Series No. 20, CONF-691212, June 1970.

1027994

70. Snyder, F. Lipids and cancer, a bibliography: 1947-1970. USAEC Report ORAU-111, Oak Ridge Associated Universities, 1970.

ABSTRACTS

Published

71. Arimizu, N., Kakehi, H., and Saegusa, K. New accessories of the scintillation camera for producing the simultaneous section scintigrams of multiple layers. J. Nucl. Med. 11, 618, 1970.

72. Beck, W. L., Cloutier, R. J., Comas, F. V., and Lushbaugh, C. C. Human depth-dose estimates in multidirectional gamma-ray fields. Phys. Med. Biol. 15, 144, 1970.

73. Beck, W. L., Stokes, T. R., Cloutier, R. J., and Lushbaugh, C. C. Dosimetry for clinical total-body irradiation. AAPM Quart. Bull. 4, 66, 1970. Abstract T2-2.

74. Blank, M. L., Wykle, R. L., Piantadosi, C., and Snyder, F. Biosynthesis of O-alkyl and O-alk-1 enyl ether-linked lipids in neoplastic cells. Prog. Amer. Assoc. Cancer Res. 11, 9, 1970. Abstract 32.

75. Chaskes, S. and Balish, E. Immunoglobulins (IgG, IgA, and IgM), complement (C'3), and transferrin in irradiated humans. Bacteriol. Proc. 70, 77, 1970. Abstract M19.

76. Cloutier, R. J., Smith S. A., Snyder, W. S., and Warner, G. G. Dose to the fetus from gamma emitters present in the bladder. Health Phys. 17, 362, 1969. Abstract P/16.

77. Corrill, L. S., Stokes, T. R., and Lushbaugh, C. C. Accumulation rates of GI and marrow damage in pair-irradiated mice during continuous and fractionated daily exposures. Radiat. Res. 43, 271, 1970. Abstract Gd-7.

78. Edwards, C. L. and Hayes, R. L. Tumor scanning with ^{67}Ga citrate. Year Book of Cancer, 350-352, 1970.

79. Edwards, C. L., Hayes, R. L., Nelson, B., and Tehranian, N. Clinical investigation of ^{67}Ga for tumor scanning. J. Nucl. Med. 11, 316-17, 1970.

80. Edwards, C. L., Nelson, B., and Hayes, R. L. Localization of gallium in human tumors. Clin. Res. 18, 89, 1970.

81. Goswitz, F. A. and Vodopick, H. Pitfalls to avoid in the interpretation of $^{*}CoB_{12}$ tests. S. Med. J. 63, 1369, 1970.

82. Goswitz, F. A. and Vodopick, H. Radiocolloid scans in chronic granulocytic leukemia. J. Nucl. Med. 11, 630-631, 1970.

83. Goswitz, F. A., Vodopick, H., and Clevenger, M. The day one index and organ erythropoiesis. S. Med. J. 63, 1368-1369, 1970.

84. Gottlieb, C. F. and Gengozian, N. X-ray and neutron depression of the mouse immune system. Radiat. Res. 43, 217, 1970. Abstract Cd-10.

85. Hagen, P. O. and Blank, M. L. Minor lipid components of Clostridium butyricum. J. Amer. Oil Chem. Soc. 47, 83A, 1970. Abstract 55.

86. Hayes, R. L., Byrd, B. L., Carlton, J. E., and Rafter, J. J. Factors affecting the localization of ^{67}Ga in animal tumors. J. Nucl. Med. 11, 324, 1970.

87. Lee, T-c. The metabolism of lipids in smooth- and rough-surfaced membranes of rat liver microsomes. Fed. Proc. 29, 674, 1970. Abstract 2411.

88. Lee, T-c., Swartzendruber, D. C., Morris, H. P., and Snyder, F. Zonal centrifugation profiles of smooth- and rough-surfaced microsomes from normal and neoplastic tissues. Tenth International Cancer Congress, Houston, Texas, May 22-29, 1970, pp. 278-279. Abstract 449.

89. Lushbaugh, C. C., Corrill, L. S., Stokes, T. R., Humason, G., and Lushbaugh, D. Mouse splenic stem-cell response to protracted radiation exposure. Radiat. Res. 43, 212-213, 1970. Abstract Cc-9.

90. Morris, A. C., Jr., Barclay, T. R., and Lushbaugh, C. C. Monitoring physiological responses during total-body irradiation therapy. Phys. Med. Biol. 15, 192, 1970.

91. Pearson, T. A. and Balish, E. Serum agglutinins and bacteriocidins from irradiated patients. Bacteriol. Proc., 70, 111, 1970. Abstract M248.

92. Ricks, R. C. and Edwards, C. L. The effect of dose rate and exposure fractionation on plasma iron kinetics in rats and man. Radiat. Res. 43, 240-241, 1970. Abstract Ed-1.

93. Ricks, R. C., Lushbaugh, C. C., and Frome, E. Changes in the power spectrum of the impedance pneumograph trace during radiation-induced gastro-intestinal distress in man. Fed. Proc. 29, 451, 1970. Abstract 1159.

94. Soodsma, J. F. In vitro studies on the oxidative cleavage of alkyl glyceryl ethers by rat liver. Fed. Proc. 29, 931, 1970. Abstract 3909.

95. Swartzendruber, D. C. Electron microscopic studies of lymphoid germinal centers. In: XIII International Congress of Hematology, Munich, August 1970. Abstract Volume. J. F. Lehmanns Verlag, Munich, 1970. p. 398

96. Swartzendruber, D. C., Hayes, R. L., and Nelson, B. Subcellular localization of gallium-67 by high resolution radioautography. J. Cell. Biol. 47, 207a-208a, 1970. Abstract 549.

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97. Winchell, H. S. Sanchez, P. D., Watanabe, C. K., Hollander, L., Anger, H. O., McRae, J., Hayes, R. L., and Edwards, C. L. Visualization of tumors in humans using ^{67}Ga-citrate and the Anger whole-body scanner, scintillation camera and tomographic scanner. Clin. Res. 18, 145, 1970.

98. Winchell, H. S., Sanchez, P. D., Watanabe, C. K., Hollander, L., Anger, H. O., McRae, J., Hayes, R. L., and Edwards, C. L. Visualization of tumors in humans using ^{67}Ga-citrate and the Anger whole-body scanner, scintillation camera and tomographic scanner. J. Nucl. Med. 11, 378, 1970.

99. Wood, R. and Healy, K. Tumor lipids: Biosynthesis of plasmalogens. J. Amer. Oil Chem. Soc. 47, 79A, 1970. Abstract 12.

100. Wood, R., Walton, M., Healy, K. and Cumming, R. B. Plasmalogen biosynthesis in Ehrlich ascites cells grown in tissue culture. J. Amer. Oil Chem. Soc. 47, 79A, 1970. Abstract 13.

101. Wykle, R. L., Blank, M. L., Piantadosi, C., and Snyder, F. Identification of intermediates in the biosynthesis of O-alkyl-linked lipids. Fed. Proc. 29, 674, 1970. Abstract 2412.

MEDICAL DIVISION CONFERENCES-1970

January 6: Some comments regarding management of patients with lymphocytic disorders. Frank Goswitz, Division staff.

January 13: Rare earths as bone-scanning agents. R. L. Hayes, Division staff.

January 27: Some metabolic and pathologic aspects of hyperplastic marmoset disease. C. C. Lushbaugh, Division staff.

February 3: Experimental acute pancreatitis in dogs. Per-Otto Hagen, Division staff.

February 10: Hormonal regulation of gene expression. Francis Kenney, ORNL Biology Division.

February 17: Development of fast clinical analyzers. N. G. Anderson, MAN Program, ORNL.

March 3: Molecular approaches to learning and memory: a progress report. William L. Byrne, University of Tennessee Medical Units, Memphis.

March 10: Characterization of the active site of triose-p isomerase. Fred Hartman, ORNL Biology Division.

March 17: Bone-marrow transplantation in acute leukemia; clinical studies. A. H. Owens, Johns Hopkins University School of Medicine, Baltimore, Md.

March 24: In vivo modification of the effects of mutagenic and carcinogenic compounds. Robert Cumming, ORNL Biology Division.

April 14: Autoradiography. William D. Gude, ORNL Biology Division.

April 21: Toxic and lethal properties of Listeria monocytogenes hemolysin. G. Charles Kingdon, Division staff.

April 23: Lipogenesis in mammary gland. Samuel Abraham, Children's Hospital Medical Center of Northern California, Oakland.

April 28: Mechanism of repair of ultraviolet irradiated DNA in cells possible implications of UV-induced skin cancer. Gary L. Whitson, Department of Zoology, University of Tennessee, Knoxville.

May 5: Biocleavage of alkyl glyceryl ethers. James F. Soodsma, Division staff.

May 7: Biochemistry of the visual process. R. E. Anderson, Baylor University School of Medicine, Houston, Texas.

May 12: Fine structure of genes in action. O. L. Miller, Jr., ORNL Biology Division.

June 2: Hemodialysis against l-asparaginase: its biochemical effects. Jere M. Bauer, Department of Internal Medicine, University of Michigan, Ann Arbor.

June 9: Distribution of gallium-67 in human tissues. Bill Nelson, Division staff.

June 16: Lymph-node scanning with ^{67}Ga. Steven Ominsky, Division staff.

June 23: Report on the Miami meeting on quantitative organ visualization. W. D. Gibbs and Alvis E. McDow, Jr., Division staff.

June 30: Panel discussion: responsibility of the scientist to inform the public, and responsibility of the news media to the scientist and to the public. R. D. Smyser, Editor, The Oak Ridger, and E. D. Aebischer, Division staff.

July 14: Subcellular particle separations by zonal centrifugation techniques. David Brown, Division staff.

July 21: Total-body irradiation and iron kinetics. Robert Ricks, Division staff.

July 28: Spontaneous cyclic granulocytosis and thrombocytosis in patients with chronic granulocytic leukemia. Helen Vodopick, Division staff.

August 11: The origin of the moon. P. R. Bell, ORNL.

August 25: Computer-generated populations of cells. Arthur L. Kretchmar, U. T. Memorial Research Center.

September 1: General principles of protein-binding tests. Irving Glover, Special Training Division, ORAU.

1027996

September 8: Radiation protection agents. David G. Doherty, ORNL Biology Division.

September 15: Protein synthesis and cellular organelles. William E. Barnett, ORNL Biology Division.

September 23: Radioactive contamination and decontaminiation of edible oils and oil seeds. Professor Dr. A. Seher, Director, Institute of General and Analytical Chemistry, Münster, Germany.

September 29: Radioisotope production at the Oak Ridge National Laboratory. L. C. Brown and J. K. Poggenburg, ORNL.

October 6: Graft-versus-host reactivity and its early detection. K. F. Hübner, Division staff.

October 13: Clinical report on the bone-marrow transplantation. C. L. Edwards, Division staff.

October 20: In vitro propagation of blood elements from marrow and peripheral blood from the marmoset. Raymond P. Porter, Division staff.

November 4: Hyperthyroidism due to hyperfunction and metastasis of thyroid carcinoma. Efraim Otero-Ruiz, Bogota, Colombia, South America.

November 17: Hematologic disorders of interest to the clinical program. Frank Goswitz, Division staff.

December 8: Experimental respiratory carcinogenesis. Paul Nettesheim, ORNL.

December 15: Cytogenetic studies on patients with leukemia treated with bone-marrow transplantation. Gayle Littlefield, Division staff.

December 22: Ecology program at ORNL. J. P. Witherspoon, Jr., ORNL.

LIPID SEMINARS-1970

June 18: Nuclear membranes and intranuclear particles. Carl Albrecht, Molecular Anatomy Program, ORNL.

July 16: Studies on plasmalogen synthesis in the developing rat brain. Oliver E. Bell, Jr., Division summer research participant.

August 13: Biocleavage of glyceryl ethers. James Soodsma, Division staff.

September 24: Structural analysis and turnover studies of phospholipids in smooth- and rough-surfaced membranes of rat liver microsomes. Ten-Ching Lee, Division staff.

October 29: Assay of ether synthesis in mice showing neurological mutations. Roger Lumb, Division staff.

November 10: The absorption and malabsorption of fat. Part I of an illustrated lecture series from the National Medical Audiovisual Center of the National Institute of Health, Atlanta, Georgia.

December 3: Part II of a slide and tape lecture series on the absorption and malabsorption of fat which included lecture 5, Properties of Pancreatic Lipase, and lecture 6, Assessment of Fat Digestion.

December 17: Part III of a slide and tape lecture series on the absorption and malabsorption of fat which included lecture 7, Differential Diagnosis of Steatorrhea; lecture 8, Steatorrhea in the Blind Loop Syndrome; and lecture 9, The Usefulness of Medium Chain Triglycerides.

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1027997

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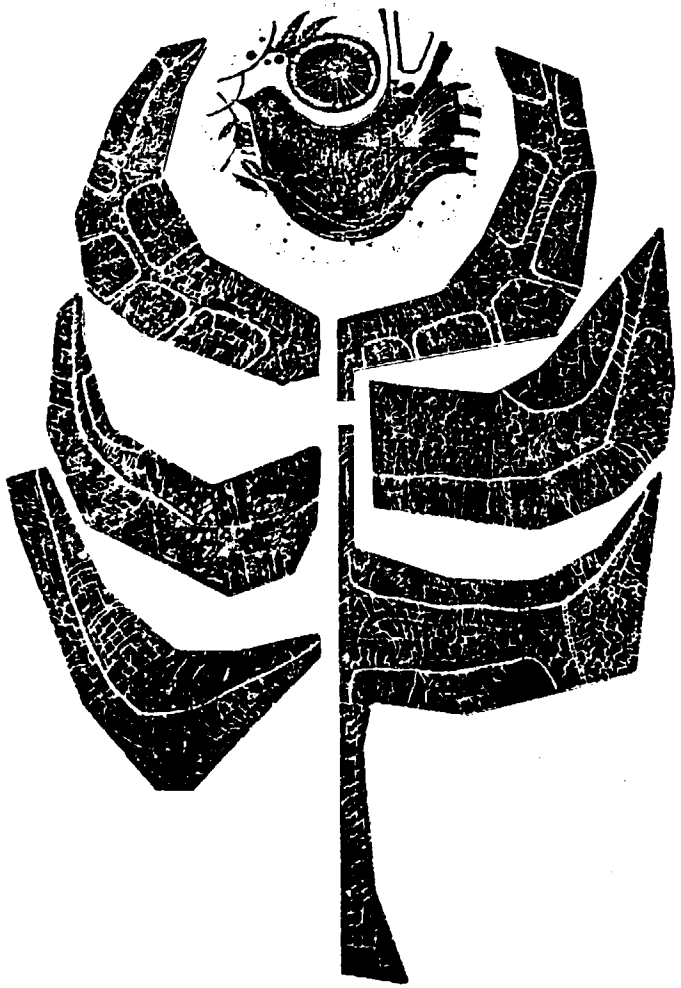
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RESEARCH REPORT

for the year ending December 31, 1971



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Oak Ridge Associated Universities

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1028004



COVER—The figure is taken from the ceramic mural, "Hymn to Life," by East Tennessee artist Charles Counts, which is on the facade of the Medical Division's research hospital building. The work was commissioned by the division from funds contributed by friends and patients, and is reproduced on the cover from plates prepared for a special ORAU 25th anniversary report on the Medical Division to the faculties of its member colleges and universities.

NOTICE

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ORAU-116

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Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

Printed in the United States of America. Price $3.00.
Available from: National Technical Information Service, Springfield, Virginia 22151.

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CONTENTS

ORGANIZATION OF RESEARCH iv

INTRODUCTION 1

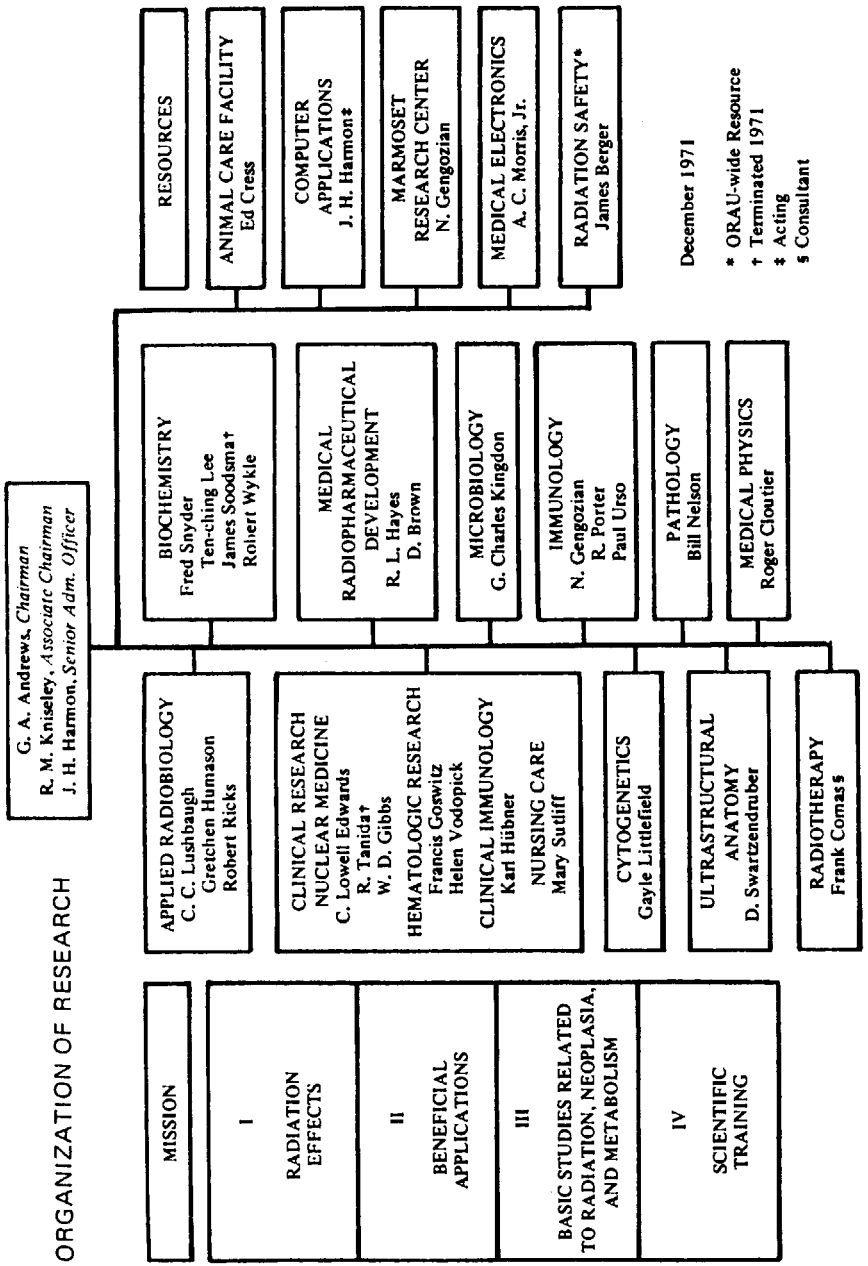
RADIATION EFFECTS AND RELATED TOPICS 5
- Clinical Total-Body Irradiation 5
- Summary of Accomplishments 5
- Clinical Observations 7
- Infections in Irradiated Patients 21
- Dosimetry Studies and Monitoring 39
- Applied Radiobiology 48
- Radiation Immunology and Marrow Transplantation 64

RESEARCH IN NUCLEAR MEDICINE 80
- Summary of Accomplishments 80
- Radiopharmaceutical Development 81
- Clinical Investigations in Nuclear Medicine 101
- Computer Methods 106

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM 117
- Summary of Accomplishments 117
- Biological Chemistry 118
- Marmoset Research Project 136
- Ultrastructural Studies of Tumor 152
- Induced Cytogenetic Abnormalities 159

MISCELLANEOUS 183
- Publications 183
- Medical Division Conferences—1971 189
- Lipid Seminars—1971 190
- Fellowships and Term Appointments 191
- Research Participants—1971 191
- Student Trainees—1971 191
- ORAU Medical Division Advisory Committee 192
- Medical Committee, ORAU Council 193
- Medical Division Employees 194
- Consultants and Collaborators 196

NOTE: Numbered references in the text may be located in the list of publications beginning on page 183.



1028006

INTRODUCTION

The Medical Division of Oak Ridge Associated Universities conducts a biomedical research program, including a small hospital for clinical investigation. The work is supported mainly by the United States Atomic Energy Commission, with some additional funds from other sources, both federal and private. The main research objectives lie in three closely related areas: (1) mechanisms of radiation injury, especially in man; (2) beneficial applications of radiation for diagnosis and therapy of disease, especially cancer; and (3) basic biological and physical problems arising from ongoing research and mainly related to cancer. In addition, the staff devotes a significant effort to training activities. In this report brief summaries beginning on pages 5, 80, and 117 highlight the accomplishments of the year.

During 1971 there were a number of changes in our senior scientific staff. In the middle of the year Dr. Ryosaku Tanida left the Division to take a new position at the Veterans Administration Hospital in Fresno, California. Dr. Tanida, during his period of 8 years on the staff here, contributed devoted clinical care to many of our research patients. A change that occurred in 1970, but not commented on in the introduction of last year's report, was the addition of Roger Cloutier to the senior staff of the Division as Head of the section on Medical Physics. He had previously been radiation safety officer for Oak Ridge Associated Universities; in his new position he is directing a program on Internal Dosimetry of Radiopharmaceuticals, supported by the Atomic Energy Commission and the Food and Drug Administration. Dr. David Brown, who has been on a temporary basis, became a regular senior staff member this year. Dr. Ten-ching Lee and Dr. Robert Wykle, both of whom were trainees at the Medical Division, continued working in biochemical research as temporary members of the regular staff. Dr. Karl Hübner, who for the previous 3 years had been doing laboratory research in immunology, has shifted a major portion of his effort to clinical research, while continuing with some laboratory research in immunology. Dr. Paul Urso, an immunologist who had formerly worked at Oak Ridge National Laboratory and most recently had been at Seton Hall Medical School, joined our staff to work with Dr. Gengozian in immunology.

As in 1970, the Division's training activities were again somewhat limited due to funding restrictions. Dr. Dieter Emrich, Chief of Radioisotope Laboratory, University of Göttingen, Göttingen, Germany, visited the Division for six months, participated actively in the clinical program, and conducted studies involving the binding of ^{67}Ga by serum proteins. Dr. Yukio Tateno, from Chiba University in Japan, completed his nuclear instrumentation studies under a Damon Runyon Fellowship. Dr. Kazuo Kasama visited here as a guest scientist from Japan and carried out experiments on the identification and metabolism of hydroxy-substituted alkyl glyceryl ethers isolated from rabbits. Dr. Roger Lumb worked with Dr. Snyder while on a Damon Runyon Fellowship. Dr. Murray R. Grigor came to us from New Zealand and also worked in lipid research, his initial

support from the Medical Research Council of New Zealand and subsequently from a Damon Runyon Fellowship. Graduate students from the University of Tennessee include John Kateley, Michael Yurconic, Elizabeth Allen, and Andrew So, all in immunology, and from the University of North Carolina, Kun Chae, in biochemistry. Mr. Thomas F. Hogan, Student Fellow in Nuclear Medicine, was assigned here for 10 weeks from the Medical School of Virginia Commonwealth University.

The Board of Directors of Oak Ridge Associated Universities have generously voted a portion of corporate funds to support of certain aspects of the Medical Division. For some years a significant part of the marmoset research project has been supported in this way. During the current year the Corporation, in addition, began the support of a postdoctoral fellow in the Medical Division. The first one appointed, Dr. Donald Barnhart, is working in the marmoset project; in the future the position will be rotated among various units of the Division. Corporate funds have also been provided to support four medical students per year in a 10-week assignment at the Division; this is the continuation of a training activity that was formerly sponsored by a group of pharmaceutical companies. One further new form of support provided from corporate funds, not yet in use, is an allowance to provide for extra housing and incidental costs of a scientist spending a sabbatical period in Oak Ridge and whose main salary is paid by his own institution.

On November 15–19, 1971, the Medical Division held its 13th major symposium. This one was titled, "Clinical Uses of Radionuclides; Critical Comparison with other Techniques," and was cosponsored by the American College of Physicians as one of their 1971–72 postgraduate courses. Program directors were Francis Goswitz and Gould Andrews of the Medical Division and Manuel Viamonte, Professor of Radiology at the University of Miami. Speakers included 34 prominent scientists from other institutions and three members of the Medical Division's staff. The Proceedings will be published by the Technical Information Center of the Atomic Energy Commission as part of the AEC Symposium Series.

A major development during the year was the growth and enthusiasm of the cooperative project on radiopharmaceutical development. A large group of clinical nuclear medicine centers, mostly in medical schools who are members of ORAU, are testing ^{67}Ga as a diagnostic agent for lung tumors and lymphomas. Additional financial support has been forthcoming and many problems of joint research and data collection have been dealt with. The details of the project are reported on pages 101-102.

During the year N. Gengozian was appointed editor of two journals, *Transplantation* and *Journal of Medical Primatology*. Fred Snyder became an editor of *Cancer Research* and *Archives of Biochemistry and Biophysics*. C. C. Lushbaugh was appointed to a 2-year term on the Space Medicine Radiobiological Advisory Panel. R. M. Kniseley has been named as Founding Member of the newest medical specialty board, The American Board of Nuclear Medicine.

1028007

Dr. Ralph Kniseley, Associate Chairman of the Medical Division, in addition to carrying a full load of duties within the Division, has been instrumental in two projects somewhat separate from the Division but important to the scientific future of Oak Ridge. Along with other Oak Ridge scientists, he has helped to set up a group of regional demographers who will cooperate with each other and take advantage of the computer facilities and census data available at Oak Ridge National Laboratory to further research in demography. A related and larger project, which is particularly attributable to the leading role played by Kniseley, is the Population Research Center in Oak Ridge, a new research unit that will build its efforts around existing programs of Oak Ridge National Laboratory, Oak Ridge Associated Universities, and the University of Tennessee. The Center will be supported initially by corporate funds of ORAU. Dr. Melvin Ketchel has recently been appointed its Director, and will assume his duties in a few months. The Medical Division has some research activities that will contribute to the new Center, and the Medical Division's staff takes pride in Dr. Kniseley's role in helping to create it.

In spite of limited travel funds, two of our staff made extensive overseas trips. Dr. Gayle Littlefield attended the 4th International Congress on Human Genetics and presented a paper entitled, "Chromosomal Breakages in Women Taking Oral Contraceptives: A Two-Year Study." In October, Dr. Raymond Hayes visited Japan as a guest of the Japan Society for Cancer Therapy. He spoke on "Application of Radioisotopes for Cancer Diagnosis," and made visits to several laboratories in Japan where he gave talks and exchanged information.

Our primary base of support continues to be from the United States Atomic Energy Commission, Division of Biology and Medicine, John Totter, Director. We have continued to receive outside support for some important research areas: The National Aeronautics and Space Administration project, "Radiosensitivity in Man," under Dr. C. C. Lushbaugh's direction continued for its 8th year. Our contract with the Army Medical Research and Development Command entitled "A Study of Infections in Irradiated Patients" was renewed for another year. This project is supervised by Dr. G. C. Kingdon. In marmoset research, Dr. Nazareth Gengozian continues his immunologic studies under the combined support of a National Institutes of Health grant and ORAU. The title of this study is "Immunology of Hematopoietic Chimerism in Tamarins." The contract with the NIH National Center for Population Research, "Chromosomal Breaks in Women Taking Oral Contraceptives," was renewed and expanded to include a study of chromosome damage in other somatic cells. The chromosome study is under the direction of Dr. L. Gayle Littlefield. Dr. Fred Snyder's grant from the American Cancer Society on "The Occurrence, Metabolism, and Function of Glyceryl Ethers in Neoplasms" has been renewed, as has been an NIH grant to the University of North Carolina in which he participates with Dr. Claude Piantadosi on "Basic Studies on Alkoxy Alkenyloxy Glycerol Ethers and Derivatives." Dr. Snyder's grant entitled "Biosynthetic Mechanisms for Ether Bonds in Lipids" was renewed for its 2nd year. Likewise, the NIH grant "Concentration of Gallium by Malignant Neoplasms" was renewed and given

supplemental funds for its 2nd year under the direction of Dr. Raymond L. Hayes.

As in 1970, we have continued to encourage cooperation among our staff members and the Oak Ridge National Laboratory, as well as our member institutions. The formation of the Cooperative Group to Study Localization of Radiopharmaceuticals which was mentioned in our 1970 Research Report has led to several new research projects. The National Cancer Institute awarded it a contract "Studies of Gallium-67 in Lymphomas and Lung Cancer" which is supervised by Dr. Ralph M. Kniseley. The American Cancer Society also awarded a grant to the Cooperative Group for a project entitled "Gallium-67 as a Tumor-Localizing Radioindicator" which is directed by Dr. Ralph M. Kniseley and Dr. C. Lowell Edwards. The Food and Drug Administration awarded a contract "Center for Information on Internal Dosimetry of Radiopharmaceuticals" under the direction of Mr. Roger Cloutier.

We are somewhat encouraged by the number of new grants which were approved and funded during 1971. We have a number of quality proposals which are currently pending and we have hopes that these projects will be approved. We plan to continue efforts directed at establishing closer relationships with our member universities as evidenced by a new cooperative study with the University of North Carolina which is scheduled to begin early in 1972.

During the year the staff spent a considerable amount of time on planning, particularly with a view to determining how the Medical Division could best contribute to the plans for an increased federally supported effort to control cancer. An initial prospectus was worked out representing a joint effort with Oak Ridge National Laboratory. We later learned that the staff of the National Cancer Institute believed that a preferable arrangement would be a fusion of Oak Ridge and Knoxville University of Tennessee groups having appropriate resources. Subsequently, a joint proposal for a planning grant was submitted by the combined institutions. Advantages and disadvantages of increased interaction with the University of Tennessee and the U. T. Memorial Research Center have been extensively discussed, and the planning efforts themselves resulted in increased visits and exchanges of ideas and services. The Medical Division's already extensive ties with Oak Ridge National Laboratory have meanwhile continued to grow.

Gould A. Andrews
Chairman

The Medical Division staff was saddened by the death on August 11, 1971 of James O. White who was killed in an auto accident while he was driving to work. Mr. White had been employed here since the early days of the Division. Starting as an unskilled employee he had become, through his own vigorous efforts, a very competent histology technician. His fine spirit and warm personality made him many friends among his co-workers.

1028008

RADIATION EFFECTS AND RELATED TOPICS

CLINICAL TOTAL-BODY IRRADIATION

The major part of the Division's research is directed toward acute effects of total-body irradiation (TBI) in man for two distinct reasons. First, TBI offers an effective way to control and palliate a number of myeloproliferative and lymphoproliferative disorders; we are searching for improved protocols of fractionation and protraction to yield the maximum benefits to the patient. Second, it is most important to develop effective treatments for the occasional victim of a radiation accident. We have managed to study a number of acute accident victims and have consulted on problems arising in accidents throughout the U. S. and elsewhere in the world, collecting as much of the world-wide information on human irradiation sensitivity as possible.

In closely related work we have also a major program in radiation immunology and bone-marrow transplantation. With new transplantation knowledge, and radiobiological findings on dose-rate effects, we have initiated an experimental protocol using high-dose rates and high doses of TBI as part of the therapy to suppress the immune response of selected leukemic patients so that they may accept marrow transplants. Closely related laboratory work continues on the important graft-vs-host (GvH) reaction, and the underlying mechanisms that operate in selective depression of the immune system and which are affected by dose rate and quality of radiation.

SUMMARY OF ACCOMPLISHMENTS

The TBI program this year moved further into its protocols which were designed to find optimum combinations of fractionation and protraction. We accumulated 26 additional patient-treatments for myeloproliferative and lympholiferative disorders. These included exposure to 1.5 R/min fractionated at 10 R/day and at 30 R/day; as well as exposures at 1.5 R/hr either protracted (30 R/day) or fractionated (10 R/day). The results numerically are too small for broad conclusions but suggest that 10 R/day may not provide a satisfactorily prolonged hematologic response. We have made attempts to predict dose-response relationships by mathematical formulae comparing values based on retrospective analyses of other clinical radiation data. While the equation predicts average response, a wide range of individual responses still prevents one from accurately predicting the drop in blood values of a given patient.

An additional serious radiation accident occurred in Oak Ridge during 1971. We treated and studied the victim in the Medical Division, monitoring his physiologic responses, his clinical and hematologic course, cytogenetic aberrations, and microfloral patterns during his confinement to the sterile facility. Fortunately he recovered without serious complications or the need of radical therapy.

We have continued physiologic monitoring after TBI, looking for decrements in performance induced by this kind of stress. One of the tests is based on measuring pulmonary impedance and variance of cardiac rate (R-R interval) during periods of controlled exercise on an ergometer. In the radiation accident victim, periods of increased fatigability were associated with elevated variance in pulmonary impedance but not with increased resting cardiac rate.

Microfloral studies of irradiated patients housed in open hospital environments are being compared with patients housed in the controlled sterile unit. After irradiation the flora changes significantly in the open environment. Frequently patients acquire additional exogenous organisms in their upper respiratory and intestinal tracts. In comparing microfloral data it is suspected that both endogenous and exogenous organisms can alter the flora, and in the absence of a normal "complete" flora, it is increasingly difficult to control individual components. The relative importance of the endogenous-versus-exogenous organisms in causing infection is still to be established. We are also looking at humoral and cellular factors in host resistance after irradiation. Serum immunoglobulins decline after TBI and return slowly toward normal over many weeks. Phagocytic and killing capacities of granulocytes are diminished early after radiation, but this impaired capacity persists in leukocytes produced after the irradiation.

The Center for Information on Internal Dosimetry of Radiopharmaceuticals developed during the year a modified internal-dose equation that corrects for previous overestimations of dose to a target organ. They have also calculated doses to the fetus from radioactivity in the mother's urine. This latter effort is a collaborative project with ORNL Health Physics Division using computer programs for a series of photon energies and for each month of pregnancy.

Further external dosimetry efforts have been productive. Comparisons have been made of two personnel monitors—film badge and thermoluminescent dosimeters (TLD)—used on patients during their treatment in the low-exposure-rate TBI facility. The film badges are significantly less reliable than the TLD for monitoring high radiation exposures.

Time-motion studies and phantom irradiations were used to reconstruct and estimate the exposure in the radiation accident. This was analyzed in relation to the patient's clinical blood response. The estimated dose under-predicted the level and severity of the course; possibly the high-exposure rate has a more important effect than is generally appreciated. We have also started an investigation in the possible use of whole-body counting as a way of individualizing dose estimates in TBI. This is a way for correcting for individual differences in sizes of patients, based on the concept that the absorption by the body of an internal isotope would be identical to the body's absorption of radiations from an external source of the same nuclide aimed at the target bone marrow.

Radiation immunology in marrow transplant work this year was highlighted by another successful graft, the patient surviving at the time of this writing. Detailed review of the successful graft mentioned in last year's report has been completed. In brief, that patient, after 122 days, died with a complicated terminal course of pulmonary infection, preexisting cardiovascular disease, and possibly drug-related renal failure. He had no recurrence of leukemia, no rejection of the graft, and no demonstrable GvH reaction. In the laboratory, animal work has continued on GvH problems with development of an immunofluorescent technique for assessing this phenomenon, and on further progress and understanding the radiation dose-rate effect and the influence of the quality of radiation on immunosuppression. Maximal suppression, regardless of dose rate, has been found to occur at 24 hr. Of additional interest is the finding that even with low dose rates (1.6 to 6 R/hr), a differential effect on immunosuppression is measurable in animal systems.

1028009

CLINICAL OBSERVATIONS

Blood Responses with Protracted and Fractionated Exposures of Total-Body Irradiation

In the past year 26 treatment courses (Table 1) of total-body irradiation (TBI) were given for several blood diseases (C. C. Lushbaugh, C. L. Edwards, Helen Vodopick, F. A. Goswitz, Ann Sipe, and J. Yuhas*). Twenty-two had myeloproliferative disease [13 chronic granulocytic leukemia (CGL), 6 polycythemia rubra vera (PRV), and 3 idiopathic thrombocythemia (IT)] and 3 had lymphoproliferative diseases [chronic lymphocytic leukemia (CLL) or lymphosarcoma (LS)]. Twelve of these treatments were given in the medium-exposure-rate total-body irradiator (METBI) (1.5 R/min) facility and 14 were given in the low-exposure-rate total-body irradiator (LETBI) (1.5 R/hr) facility (Table 2). Since 1967 all TBI exposures have been fractionated or protracted over 3.5 days to more than 20 days. Nineteen treatments were at 30 R/day and 7 treatments (5 patients) were given as daily exposures of 10 R. (See below.)

10 R/Day Treatments

1. 49-yr-old female	CGL	200 R	LETBI
2. 37-yr-old female	CGL	200 R	LETBI
3. 49-yr-old female	CGL	150 R	LETBI
4. 71-yr-old male	LS	150 R	LETBI
5. 61-yr-old male	CLL	100 R	LETBI
6. 67-yr-old male	LS	100 R	METBI
7. 67-yr-old male	LS	50 R	METBI

Those treated in LETBI either slept in the facility at night (6.7 hr) while receiving their treatment or stayed in the facility during the daytime hours and returned home at night.

*Biology Division, Oak Ridge National Laboratory.

TABLE 1
DISTRIBUTION OF TOTAL-BODY IRRADIATION THERAPY CASES OBTAINED DURING 1971 ACCORDING TO DIAGNOSIS, TYPE OF EXPOSURE AND EXPOSURE DOSE

Diagnosis	Exposure (R)	METBI (1.5 R/min.) 30 R/day	METBI (1.5 R/min.) 10 R/day	LETBI (1.5 R/hour) Protracted* (~30 R/d)	LETBI (1.5 R/hour) 10 R/day	Totals
CLL	100	–	–	–	1	1
LS	50	–	1	–	–	
	100	–	1	–	–	3
	140	–	–	–	1	
CGL	150	8	–	1	1	
	200	–	–	–	2	13
	250	–	–	1	–	
PRV	100	–	–	2	–	
	150	2	–	4	–	8
IT	150	–	–	1	–	1
	Totals	10	2	9	5	26

*Continuous exposure for 20 hrs/day.

TABLE 2

LOW-EXPOSURE RATE (1.5 R/hr) TOTAL-BODY IRRADIATION OF PATIENTS

No.	Diagnosis	Sex	Age	Total residence Hr	Total residence Days	Total R	Exposure hr	Fractional days (%)	Prodromal symptoms
37	IT	M	65	115	4.8	150	100.0	86.0	0
38	CLL	M	61	67	10.0	100	67.0	29.0*	0
39	PRV	M	60	110	4.6	150	101.4	93.0	0
40	LS	M	70	95.2	14.0	140	95.2	29.0*	0
41	CGL	F	49	103.5	15.0	150	103.5	29.0*	Nausea
42	CGL	F	37	127	5.3	150	104.1	78.0	0
43	PRV	F	57	106	4.4	150	96.8	92.0	0
44	PRV	M	73	120	5.0	150	97.4	80.0	0
45	CGL	F	49	130	20.0	200	130.0	27.0*	Nausea
46	CGL	F	38	130	20.0	200	130.0	27.0*	0
47	PRV	F	66	73	3.0	100	66.2	92.0	0
48	PRV	F	58	75	3.1	100	68.0	90.0	0
49	PRV	M	64	108	4.5	150	98.0	90.0	0
50	CGL	M	67	112	4.7	150	100.0	90.0	0

*Fractionated daily exposures of 10 R.

1028010

This change in the protocol relying more on protracted or fractionated exposures was prompted by our earlier finding that the desired hematological responses could be achieved with much less gastrointestinal distress when the total dose of radiation is given over a longer interval. Our retrospective analysis shows that individual differences and the specific disease probably affect the hematologic response to a greater degree than the amount of the initial radiation damage, thus accounting for a wide range of responses seen after any given exposure to TBI. With smaller daily exposures (fractions of total dose) and therefore with more numerous exposures over longer treatment periods (fractionation period) one should be able to use the patient's own responses as the criteria for giving more or fewer exposures. Hence this would give the clinician better control of the blood changes and help him avoid hazardous suppression of normal cell lines. The bar graph of Fig. 1 depicts the chronology of our TBI protocols.

Fractionation or protraction at 30 R/day seems to have substantially reduced the gastrointestinal symptoms and these can probably be decreased still more by using 10 R/day exposures. As yet no patient has been exposed to a greater number of roentgens than we had previously used in the single-exposure treatments (50 to 250 R). Before exceeding these limits, we need to demonstrate the predictability of response in order to be certain that there will not be a further significant depression of the blood values after discontinuing the radiation. Although in each case there was hematologic and clinical improvement, in the three treatments of CGL the changes were short-lived, with an

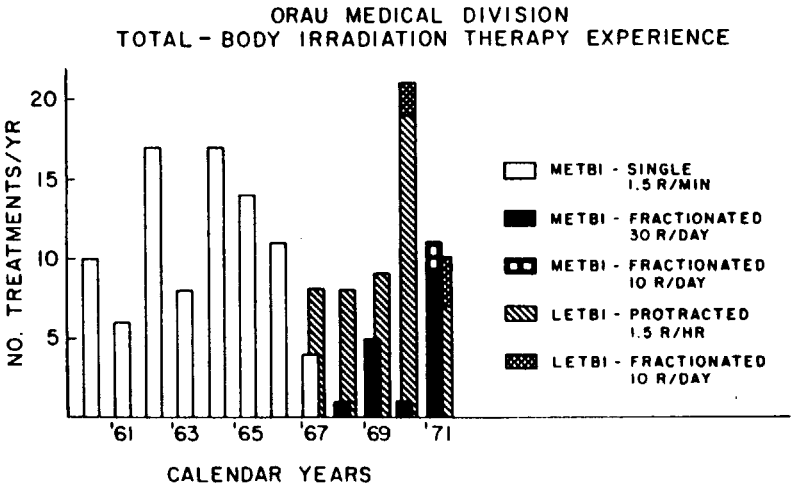


Fig. 1. Histogram of TBI treatments given 1960-1971.

apparent sparing of the platelet count. In fact in one patient the platelet count, which had been moderately suppressed (70% of pretreatment levels) at the end of the treatment, rapidly increased, exceeding the pretreatment levels 2 to 3 weeks after the radiation was ended. Although the same platelet-sparing effect was not seen in the patient with CLL or LS, further experience with this treatment schedule is necessary to determine if this observation holds for patients with lymphoproliferative diseases. If these smaller daily fractions do somehow spare the platelets, this would mean they hold two distinct advantages over the single acute doses to compensate for the greater investment of time.

The apparent short-lived effect seen in the patients with CGL plus the uneconomic aspects of prolonged therapy suggest that 10 R daily exposures will not be advantageous for patients with CGL. Because of the brevity of the response seen, we have used the 10 R/day schedules in only three studies of patients with CGL. In preference we have given single exposures of 100 to 150 R at 30 R/day as initial therapy; we then give single exposures of 30 R weekly to monthly as necessary to gradually lower and maintain the blood count at optimal levels.

The uncertainty in the radioresponsiveness (% decrease in blood count/R) for a given patient makes it unsafe to prescribe a single exposure large enough to return the blood counts to normal. Although there is some variation in the radiation dose given patients exposed to the same number of R, the error in predicting a patient's response is not due to variation of marrow dose. Within an exposure group (250 R for example) marrow dose extremes are ±5% (~6 rads) while the responses in % of initial white blood cell (WBC) count at nadir vary in excess of ±25% around the logarithmic mean. The histopathologic study of marrow changes after irradiation, described in later paragraphs, suggests that measurement of the number of primitive marrow (progenitor?) cells may be required for predicting individual radiosensitivity. While this number could be obtained for an individual bone marrow biopsy, its expansion to total marrow organ would require simultaneous estimate of the total-body distribution and volume of the marrow, numbers that would be difficult to obtain with present technology.

Without the means of determining individual radiosensitivity directly, it remains necessary to estimate what it was in retrospect and from these averaged responses to construct predictive equations that generalize the dose-response relation. So far our best effort (Proc. Nat. Symp. Natural and Manmade Radiation in Space, E. A. Warman, ed. NASA TM X-2400, 1972, p. 233) used the data of others and resulted in a multifactorial equation in which the slope constants (power function) of time-protraction on dose appeared to vary directly with the different sensitivities of "normal," CGL, CLL, and LS marrows.

This year the substantial numbers of patients who have been irradiated in both LETBI and METBI with daily fractions of their total exposure afford us a new, large sample for dose-response analysis. To keep the analysis simple in the first trial, we chose to ignore the conclusion that the degree of depression of the

1028011

WBC at nadir is a complicated combination of disease status and exposure and considered it to be the function of marrow dose alone. In addition, we ignored the length of time in which the exposure was made since the time required was related to the size of the total dose. Marrow dose was the computed logarithmic average of the corrected, average marrow-organ-dose of the patients given 100 R, 150 R, and 200 to 250 R in LETBI and 100 R and 150 R in METBI. These exposure selections grouped the patients into four average marrow-dose groups of 56, 85, 98, and 138 rads. In these groups, there were respectively 15, 20, 14, and 12 persons. The logarithmic averages of the percent depression of the WBC at nadir (point of maximum depression in the peripheral blood) of the patients within these dose groups are shown in Table 3, along with the ranges of responses, and the responses predicted by using a semilogarithmic relation of response to dose and the power function relation of the multifactorial equation. These equations are shown in the footnote of Table 3. The value (.693/44) in the semilogarithmic equation is lambda, here the WBC-depressing efficiency per rad, when 44 rad cause a 50% depression in WBC. Lambda is found to be 1.58% per rad.

Because these data were used to derive the semilogarithmic equation, it "predicts" as shown in Fig. 2 the actual level of average response quite well except after 250 R exposure where an overprediction is obtained. The other equation, derived from old data of others, predicts the levels of response well for the higher exposures when the slope factor for CGL is used. It overpredicts the degree of depression at 100 R. The slope factor for "normal marrow" greatly underpredicts the level of depression at 250 R as would be expected if "normal"

TABLE 3

COMPARISON OF TWO PREDICTIONS OF RESPONSE WITH THAT OBSERVED IN BLOOD LEUKOCYTE COUNTS OF PATIENTS AFTER TBI

	No. pt.	Exposure (R)	Average marrow dose (rad)	Blood leukocyte response (%)				
				Log average response	Range of responses	Exponential prediction*	Multifactorial prediction	
							Normal	CGL†
LETBI	15	100	56	41	22-95	42	35	29
LETBI	20	150	85	27	3-72	26	29	22
METBI	14	150	98	19	1-40	21		
LETBI	12	200-250	138	14	2-30	11	24	16

*$\%\ \text{WBC (nadir)} = e^{\frac{.693}{44}(\text{marrow dose})}$

†$\text{WBC (nadir)} = \frac{k \times 100 \times (\text{time})^{b_2}}{\text{Exposure (R)}}$

$b_2 = 0.63$ for "normal"

$b_2 = 0.39$ for CGL

marrow has greater than diseased-marrow reparability. The better fit of the average 250-R response with the prediction of the power equation may likewise be explained by a decreased radiation efficiency (increased reparability or a decreasing radiosensitivity of the surviving marrow cells after long protracted exposures). These data and those for platelet depression are being analyzed further, using subgroupings for the various diseases being treated.

These analyses have radiobiologic and radiotherapeutic implications that encourage our selection of progressively lower daily exposures and prolongation of total treatment period. In therapy we are finding this important for better

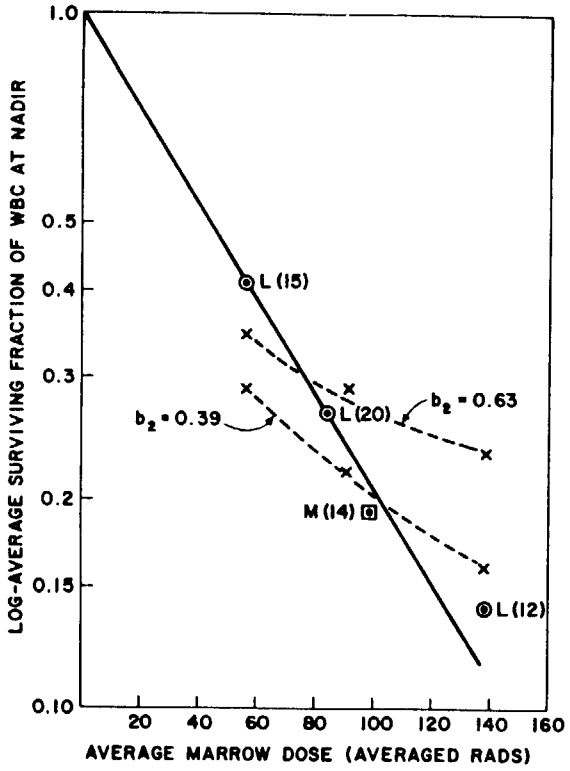


Fig. 2. Human peripheral leukocyte survival curves after TBI in LETBI ◉ and METBI ◉ and those (dashed lines) practiced by Yuhas et al. (1972) from a retrospective analysis of clinical data of others. The lower dashed line is computed using the repair factor (b_2) of 0.39 for CGL and the upper using that of 0.63 for "normal" man. The number of ORAU patients are in parenthesis. See text for further explanation.

1028012

day-to-day clinical control of the response to obtain only desired effects. For human radiobiologic purposes they should enable us to extrapolate safely to those small daily exposures and the periods of exposure duration that will not produce immediately dangerous hematologic effects in normal man.

Radiation Accident

Because of its interest in the effects of radiation exposure, the Medical Division maintains special facilities for the care of persons exposed accidentally to radiation. This past year such an accident occurred in the Oak Ridge area. The patient, a research laboratory technologist, had been irradiating seeds in an experiment using a high energy ^{60}Co source (7,700 Ci). When the patient entered the room where the radiation was performed, he was unaware that the ^{60}Co source was out of its shield. (The details of the mechanical difficulties encountered are not covered here.) Using his right hand he changed the seeds directly in front of the source. The dose rate at this distance from the source was 350 R/min. The entire procedure, according to later estimates, required 40 sec. Soon after it was recognized that the man had received a radiation dose, he was transferred to the ORAU Medical Division for further care (Helen Vodopick and Gould A. Andrews). The exact dose of radiation was not immediately known since the monitoring badge was sent to the supplier for reading. When eventually reported, the badge, which had been worn at waist level, showed an exposure of 260 R; however, because of the arrangement of the ^{60}Co source and the relation of its shield to the patient, the dose was very nonuniform. Further data on the dose will be found on p. 42.

After his admission, and about 2.25 hr after exposure, he began to vomit; the suddenness of some episodes even interrupted his conversation on several occasions. No diarrhea was experienced. His admission blood count 2 hr after his exposure was normal (Fig. 3) but 2.5 hr later the WBC count had doubled (15,800/mm^3), and the absolute lymphocyte count had dropped from 2840/mm^3 to 948/mm^3. The leukocyte count returned to the admission level in 12 hr. Except for an isolated rise to 1900/mm^3 at 16.5 hr after exposure, the absolute lymphocyte count remained close to 1000/mm^3 for the next 4 weeks. Bone marrow aspirations could not be done until 29 hr after exposure. Cellularity and the myeloid-to-erythroid ratio were within normal range. Cytogenetic analysis of the marrow preparations showed numerous divisions with the majority exhibiting changes consistent with radiation damage.

After 48 hr he was asymptomatic. On the 7th hospital day he was allowed to go home to avoid the unnecessary risk of exposure to nosocomial bacteria. He returned daily for blood counts. When the anticipated fall of his platelets occurred on day 25, he was readmitted to the sterile laminar-air-flow unit where his exposure to foreign microflora would be reduced (see p. 21). No prophylactic, antimicrobial agents were given. The platelet count fell to its nadir (37,000/mm^3) on day 29 and the WBC count was lowest (2000/mm^3) on day 36. No bleeding or temperature elevation occurred during this time. On day 36 an infection in his mouth, diagnosed as Vincent's angina, was treated

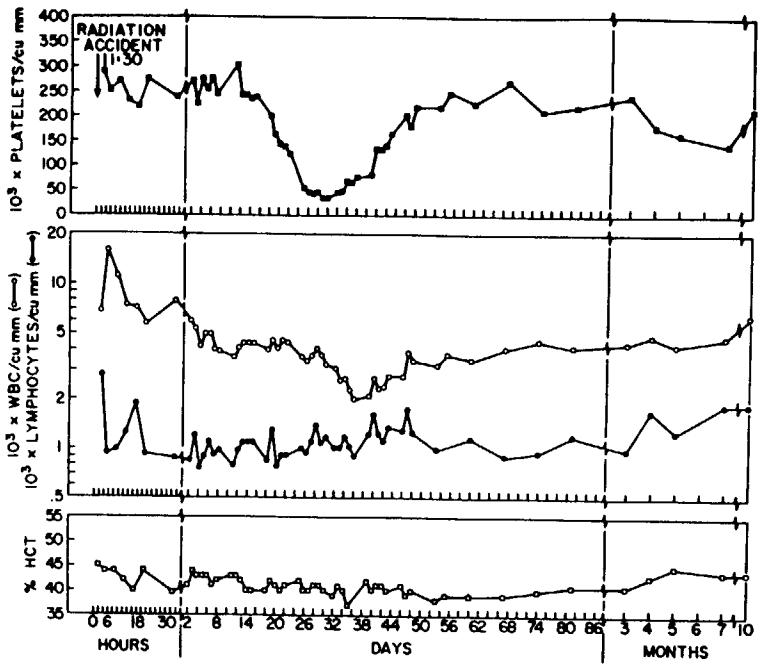


Fig. 3. The hematologic changes were observed in a patient who was accidentally irradiated with 260 R.

with hydrogen-peroxide mouthwashes and oral penicillin for 5 days. After recovery of all his cellular blood elements occurred, he was sent home on day 48. He continues to be seen in the outpatient department.

His right hand, which presumably received more irradiation than the rest of his body, has shown no visible signs of radiation damage. For 4 months after the accident he did, however, have subjective pain in this hand described as a dull aching sensation especially after using it for prolonged periods. This pain has gradually subsided and he has no further discomfort. He has returned to full time work as a research technician but is not allowed to work in any area of radiation.

His eyes when examined by an ophthalmologist on two occasions have been normal. Semen analysis 6 months after exposure revealed a low concentration of sperm with only slight morphological abnormality. Since previous sperm counts were not obtained, there can be no comparison.

Of interest but unexplained was the observed rise in the creatine phosphokinase (CPK) level on days 8 and 9 after its normal range on admission. It is possible that this was related to the monitored bicycle exercise he performed.

1028013

The CPK value, however, fell to normal range even with further exercise (see p. 55.)

Elevated serum iron was found on day 9. (On admission the serum iron was 180 μg/100 ml and on day 9 was 310 μg/100 ml.) This rise probably represents depression of erythroid production and the marrow's inability to effectively utilize the iron released from destroyed RBC.

Management of this patient was expectant and little actual therapy was required. We believe that the sterile environment may have decreased the risk of morbidity from infection during the patient's maximum bone marrow depression.

Human Marrow Changes after Treatment with Low-Dose-Rate Total-Body Irradiation

Marrow changes in man after continuous irradiation at the low-dose rate of 1.5 R/hr have not been reported. Since 1967 we have studied the marrow changes in 30 patients with malignant hematologic disorders (F. A. Goswitz, C. C. Lushbaugh, and Helen Vodopick). We compared the marrow at approximately 18 days after the start of treatment with marrow taken before therapy. We evaluated the cellularity, fat granulocytic-erythroid (G:E) ratio, maturation of the granulocytic and erythroid elements, number of lymphocytes, megakaryocytes and platelets, and concentration of iron in histiocytes.

These patients, treated in our LETBI facility, were in a protocol that investigated the therapeutic usefulness of TBI. The radiation field is uniform to within ±10%. The treatments, given at average-exposure rate of 1.5 R/hr, lasted from 3 to 8 days to provide a total protracted exposure of 100, 150, or 250 R.

Thirty-three treatments have been given to 21 patients with myeloproliferative disorders: CGL, PRV, and IT, and 11 treatments to 10 patients with lymphoproliferative disorders: CLL, lymphosarcoma-cell leukemia (LSL), and LS. Of these 44 treatments, 15 were given with a total exposure of 100 R, 19 with 150 R, and 10 treatments with 250 R. One CGL patient treated with 150 R had no marrow aspirated at the posttreatment time designated by the study, and therefore, his data were excluded.

To the 20 patients with myeloproliferative disorders, 32 treatments were given: 15 CGL, 12 PRV, and 5 IT. In contrast we studied fewer patients with lymphoproliferative disorders administering 11 treatments: 4 CLL, 3 LSL, and 4 LS. This makes a total of 43 marrows evaluated after continuous low-exposure rate. Five patients (1 CLL, 1 IT, and 3 PRV) were treated twice in the LETBI facility. Four patients (2 CGL, 1 PRV, and 1 IT) were treated 3 times.

The calculated radiation-absorbed dose to marrow is 60 rads per 100 R, 90 rads per 150 R, and 150 rads per 250 R. Generally we aspirated marrow from the iliac spine, occasionally using the sternum when necessary. If the sternum was the site for the preirradiation sample, it was also used for the postirradiation specimen. If satisfactory aspirates were not obtained, we biopsied the marrow with a Westerman-Jensen needle.

The marrow sections helped us to interpret the ratio of cellularity to fat. If the cellularity was 75% of the total area of tissue, the marrow was interpreted as showing a slight increase (↑). If 85% of the tissue were occupied by cells, a moderate change had occurred (↑↑); if 95% or more of the area were cellular, we classified it as pronounced cellularity (↑↑↑). On the other hand, a pronounced decrease (↓↓↓) indicates only 5% cellularity or less, a moderate decrease (↓↓) about 15%, and a slight decrease (↓) in cellularity is approximately 25%.

TABLE 4

MARROW DATA IN PATIENTS WITH CGL AND PRV BEFORE AND AFTER 250 R*

	Cellular		Fat		G:E Ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
Pt.	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)
Chronic granulocytic leukemia																
1	↑↑↑	↑↑↑	A	↓↓↓	32.0	17.0	N	N	L↑↑	L↑↑	↓↓↓	↓↓↓	↑↑↑/↑↑↑	↑/↑	A	A
2	↑↑↑	↑↑	↓↓↓	↓↓	30.0	13.7	L↑	N	L↑↑	L↑↑	↓↓↓	↓↓↓	↑↑↑/↑↑	↑↑/↑↑	A	A
3	↑↑↑	↑	A	N	42.0	8.8	N	N	L↑↑	L↑↑	↓↓	↓↓↓	↑↑/↑	↑↑/↑	↓↓↓	↓
Polycythemia rubra vera																
1	↑↑	N	↓↓	N	2.0	1.6	N	N	N	N	N	N	↑/N	N/N	A	A
2	↑↑↑	↑↑	↓↓↓	↓↓	1.5	0.8	N	N	N	N	N	N	N/N	N/N	A	A
3	↑↑↑	↑↑	↓↓↓	↓↓	5.0	5.2	N	N	N	L↑↑	↓↓↓	N	↑↑↑/↑↑	↑↑/N	A	↑

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift

Table 4 reports the marrow changes in 3 patients with CGL and 3 patients with PRV, each treated with 250 R. The most consistent change is the increase of fat. The other striking change is the increase of fat. The other striking change is the moderate or pronounced reduction in G:E ratio for the 3 patients with CGL, whereas no change in this ratio occurred in the 3 patients with PRV. In 4 patients with lymphoproliferative disease treated with 250 R, the most significant changes also were the increase in fat and the decrease in cellularity (Table 5). Reduced numbers of megakaryocytes and platelets were particularly evident in these diseases treated with 250 R. A moderate reduction in the immature cells of the granulocytic and erythroid series or shift to the left and a moderate increase in stored iron were noted in 2 of the 4 patients. Five of the 10 patients treated with 250 R had an absence of iron after treatment. Seven treatments of 150 R were studied in 5 patients with CGL (Table 6). We found a slight decrease in cellularity in 6 of the 7 marrows but a greater increase in fat after all treatments in these patients. The second major change was a pronounced decrease in the G:E ratio of 5 marrows, less pronounced than in those treated with 250 R which accounted for the reduced ratio after all treatments in the CGL group. Less immaturity in the granulocytic series was observed after treatment also.

1028014

TABLE 5

MARROW DATA IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASES TREATED WITH 250 R*

	Cellular		Fat		G:E Ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
Pt.	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)
Lymphosarcoma																
1		↓		↑		2.8		N		N		N		↓↓/↓↓↓		A
2	↑↑	↓↓	↑↑	↓	1.5	0.4	N	L↑↑	N	N	N	N	N/N	N/N	↑	↑↑↑
Lymphosarcoma-cell leukemia																
3	↑↑	N	↓↓	N	2.6	1.6	N	N	N	N	↑↑	↑↑↑	↓↓↓/↓↓↓	A/↓↓↓	↑	↑↑↑
4	↑↑	N	↓↓	N	2.0	4.5	N	N	L↑	L↑↑	↑↑	↑↑	↓/↓	↓↓↓/↓↓↓	↑↑	↑↑

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift

TABLE 6

MARROW DATA IN CGL PATIENTS TREATED CONTINUOUSLY WITH 150 R*

	Cellular		Fat		G:E Ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
Pt.	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)
1	↑↑↑	↑↑↑	A	↓↓↓	18.7	1.4	N	N	L↑	N	↓↓	N	↑↑/↑↑	↓/N	A	A
1†	↑↑↑	↑↑	↓↓	↓↓	99.9	6.7	N	N	L↑↑	L↑↑	A	↓↓	↑/↑	↓/N	A	A
2	↑↑↑	↑↑	A	↓↓	13.0	1.8	N	N	L↑↑↑	L↑	N	N	↑/N	N/N	A	A
3	↑↑↑	↑↑	A	↓↓	30.0	0.3	N	L↑↑	L↑↑	L↑↑	N	N	↓↓/N	↓/↓	↓	↓
4	↑↑↑	↑↑	↓↓↓	↓↓	9.0	3.4	N	N	L↑	N	↓↓↓	↓↓↓	↓/N	N/N	A	A
4†	↑↑↑	↑↑	A	↓↓	11.1	9.5	N	N	L↑↑	L↑	↓	↓	↑↑/N	N/N	A	A
5	↑↑↑	↑↑	↓↓↓	↓↓	98.5	96.5	N	N	L↑	L↑	↓↓	↓↓	↑↑/↑↑	↑↑/N	A	A

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift
†Second treatment

Table 7 shows the marrow data after 11 treatments to 7 patients (4 with PRV and 3 with IT) treated with 150 R. In contrast to the CGL group, the G:E ratio didn't change significantly nor did the maturation of granulocytes. As expected, the cellularity decreased including megakaryocytes, platelets, and lymphocytes; and fat increased in most patients after treatment. Histiocyte iron decreased slightly or moderately in patients with IT and also in most of those with PRV. However, one patient with PRV showed a moderate increase (absent before irradiation to moderate decrease after treatment) in stored iron.

In 5 patients with CGL and 3 patients with PRV treated once each with 100 R, the marrow changed slightly or not at all (Table 8). Only 2 had reduced G:E ratio; little if any change occurred in the megakaryocytes or platelets. We also found no maturation change.

Table 9 demonstrates the findings in patients with lymphoproliferative disorders treated with 100 R. We had a total of 7 treatments in 6 patients. The cellularity decreased more than in the myeloproliferative group. Little change was seen in erythroid or granulocytic maturation.

1028015

TABLE 7

MARROW DATA IN PATIENTS WITH PRV AND IT TREATED WITH 150 R IN LETBI*

Pt.	Cellular (b)	Cellular (a)	Fat (b)	Fat (a)	G:E Ratio (b)	G:E Ratio (a)	Erythroid maturation (b)	Erythroid maturation (a)	Granulocytic maturation (b)	Granulocytic maturation (a)	Lymphocytes (b)	Lymphocytes (a)	Megakaryocytes/ platelets (b)	Megakaryocytes/ platelets (a)	Iron (b)	Iron (a)
Polycythemia rubra vera																
1	↑↑	↑	↓↓	N	3.0	5.8	N	N	N	N	↓↓↓	↓	↑↑/↑↑	N/N	A	A
2	↑↑↑	↑↑	↓↓↓	↓↓	1.4	3.7	N	N	N	L↑	↓↓↓	↓↓	↑↑↑/↑↑↑	↑↑/↑↑	A	A
2†	↑↑↑	↑	↓↓	↓	3.0	4.7	N	N	N	N	N	↓↓	↑↑/↑↑	↑/N	A	↓↓
3	↑↑	↓↓	↓↓	↓	4.1	2.8	N	N	N	N	↓↓	↓	↑↑/↑↑	↑/↑	↓↓↓	↓↓↓
4	↑↑↑	↑	↓↓↓	↓↓	2.2	3.4	N	N	N	N	↓↓	↓	↑↑/↑	N/N	A	A
Idiopathic thrombocythemia																
5	↑	N	↑	N	2.8	0.7	N	N	N	N	N	N	↑↑/↑↑	N/N	↑↑↑	↑
5†	↑↑	N	↑	N	2.4	1.5	N	N	L↑	N	N	N	↑↑↑/↑↑↑	↑↑/↑↑	↓	A
5‡	N	N	N	N	2.8	1.0	N	N	N	N	↓↓	N	↑↑↑/↑↑↑	↓/↑	A	A
6	N	↓↓	N	↑↑	2.0	3.7	N	N	N	N	N	↓	↑↑↑/↑↑↑	N/↑↑	↑	N
6†	N	↓↓	N	↑↑	3.2	3.1	N	N	N	N	N	N	↑↑/↑↑↑	N/↑	N	N
7	↑↑	↑↑	↓↓	↓	2.0	1.5	N	N	N	L↑	N	N	↑↑/↑	↑↑/↑	A	↓↓↓

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift
†Second treatment
‡Third treatment

TABLE 8

MARROW DATA IN PATIENTS WITH CGL AND PRV TREATED WITH 100 R*

Pt.	Cellular (b)	Cellular (a)	Fat (b)	Fat (a)	G:E Ratio (b)	G:E Ratio (a)	Erythroid maturation (b)	Erythroid maturation (a)	Granulocytic maturation (b)	Granulocytic maturation (a)	Lymphocytes (b)	Lymphocytes (a)	Megakaryocytes/ platelets (b)	Megakaryocytes/ platelets (a)	Iron (b)	Iron (a)
Chronic granulocytic leukemia																
1	↑↑↑	↑↑↑	↓↓↓	↓↓↓	54.2	60.0	N	N	L↑	L↑	↓↓↓	↓↓↓	↑↑/N	↑↑/N	N	↓↓↓
2	↑↑↑	↑↑↑	A	A	50.4	50.0	N	N	L↑↑	L↑↑	↓↓	↓↓	↑↑/↑	↑↑/↑	A	A
3	↑↑↑	↑↑↑	↓↓↓	↓↓↓	10.0	5.0	N	N	L↑	L↑	↓↓	↓↓↓	↑↑/N	↑/N	A	A
4	↓↓↓†	↓↓↓†		↑↑↑												
5	↑↑↑	↑↑↑	↓↓		28.0		N		L↑↑		N		↑↑/N		↓↓	
Polycythemia rubra vera																
1	↑↑↑	↑↑↑	↓↓↓	↓↓↓	1.5	2.0	N	N	N	N	N	N	↑↑↑/N	↑/N	A	A
2	↑↑	↑	↓↓	↓	6.9	2.9	N	L↑	N	N	↓↓	↓	↑/↑	↑/↑	A	A
3	↑↑↑	↑	A	↓↓↓	1.6	1.7	N	N	N	N	N	N	↑↑↑/↑↑↑	N/↑↑↑	A	A

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift
†Westerman–Jensen needle biopsy

TABLE 9

MARROW DATA IN LYMPHOPROLIFERATIVE DISEASES TREATED WITH 100 R*

Pt.	Cellular		Fat		G:E Ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)	(b)	(a)
Lymphosarcoma																
1	N	↓↓	N	↑↑	1.1	1.8	L↑	N	N	N	↑	↑↑	↓/N	↓↓/N	N	A
2	↑	↓↓	↓↓	↓↓	1.9	2.1	N	N	N	N	↑↑	↑↑	↓↓/↓	↓↓/↓	↑↑	↓
Lymphosarcoma-cell leukemia																
1	↑↑↑	↑↑	↑	↓↓	3.2	0.5	N	N	N	N	↑↑↑	N	N/N	N/N	A	A
Chronic lymphocytic leukemia																
1	↑↑↑	↑	↓↓↓	↓	3.8	1.6	N	N	N	N	↑↑↑	↑↑	N/N	↓/↓↓	A	A
2	↑↑↑	↑↑	↓↓↓	↓↓	3.1	0.3	N	L↑	R↑↑	N	↑↑↑	↑↑↑	↓↓/↓↓	↓↓/↓↓	↓↓↓	A
3	↑↑↑	↑↑	↓↓↓	↓↓	0.4	0.2	N	N	N	N	↑↑↑	↑↑↑	↓/N	↓↓↓/↓↓	A	↓↓↓
3†	↑↑↑	↑↑↑	A	A	2.1	1.4	N	N	N	N	↑↑↑	↑↑↑	↓↓↓/↓↓↓	↓↓↓/↓↓↓	A	A

*Symbols and abbreviations
↑ = increase; ↓ = decrease
(b) = before; (a) = after
A = absent; N = normal; L = left shift; R = right shift
†Second treatment

For many patients the marrow changes were less than we anticipated, even in those who had the greatest reduction in blood-cell counts at this interval. The myeloproliferative disorders could be controlled with TBI alone, particularly IT where control was established for 12 to 18 months. Remission for the CGL patients ranged from 4 to 8 months; patients with PRV had a slightly longer remission. Certainly in CGL the length of remission with TBI compares favorably to these remissions induced with busulfan. Although the majority of our patients with CGL developed myelofibrosis, we believe that this secondary myelofibrosis is not due solely to the irradiation. Certainly the IT patients treated similarly did not develop it. In the lymphoproliferative disorders, we have not yet treated any patients with 150 R in a continuous dose; the marrow changes, however, are more pronounced in those given 250 R and 100 R than those in the myeloproliferative group. Our clinical staff has commenced recently the cautious treatment of these lymphoproliferative disorders with fractionated daily doses to obtain more precise control.

In summary, 44 treatments with TBI at the low-dose-rate exposure of 1.5 R/hr were given in continuous exposures of 100, 150, or 250 R to 31 patients with a proliferative hematologic disorder. The marrow, approximately 18 days after the start of TBI, was compared with that obtained before irradiation. This study points out the effect of low-dose-rate irradiation on the parent cells. The data also demonstrate our not unexpected limited success in obtaining complete remissions for several patients, particularly in CGL; we do not find normocellular marrow at the time of the maximum effect of irradiation—about 3 weeks after treatment.

INFECTIONS IN IRRADIATED PATIENTS

Infection is the most serious complication of sublethal total-body irradiation (TBI) in experimental animals. It is also well known that infections occurring in human beings after accidental or therapeutic exposure is an important cause of death. A great deal of information is available on the increased susceptibility of laboratory animals to experimental or spontaneous infection after irradiation; however, the underlying causes of infections in irradiated human beings have not been adequately studied. It is also important to develop more adequate procedures to prevent or control infections after radiation exposure. The objectives of the microbiology project are aimed at these questions.

The Bacterial Flora of Patients in Open and Ultra-Clean Environments

The relative importance of endogenous and exogenous microorganisms in postirradiation infections has not been completely defined. Foreign microorganisms (either genus, species, or strain) introduced before or during

1028016

leukopenia may suddenly shift the microflora balance to a microorganism with which the host has had no recent immunological experience.

A study of the microflora of irradiated patients housed in an open environment was completed during 1971 (G. Charles Kingdon and Jean McIntyre). The study included 44 patients who were exposed to between 100 R and 350 R TBI in either the medium-exposure total-body irradiator (METBI) (1.5 R/min) or low-exposure total-body irradiator (LETBI) (1.5 R/hr) facility. The patients also received additional supportive therapy and medication (including antibiotics) as deemed necessary by the clinical staff. Nares, mouth, throat, feces, urine, skin, and blood were cultured twice weekly during hospitalization, which averaged less than 4 weeks, and then weekly until 52 days after irradiation.

The aerobic and anerobic bacterial flora changed significantly after irradiation. Some components increased in number, while others decreased. The patients frequently acquired additional, presumably exogenous, organisms in the upper respiratory tract, intestinal tract, and urine. They appeared to become established in the upper respiratory tract and intestinal tract more easily after treatment. The number and varieties of transient microorganisms from the upper respiratory tract and intestinal tract also increased.

More subtle changes occurred in the physiology of certain normal aerobic components. This is illustrated by the strain variability of *Escherichia coli* found in the stools of a group of 20 patients. One or more biochemical types of *E. coli* were present in the feces before irradiation. After treatment new biochemical types were found, either as transient microorganisms which were present for varying intervals or as persistent components of the microflora. The newly established strain occasionally replaced the original component as the predominant representative of the genus and was in turn replaced by yet another biochemical type. The feces of several patients followed for long periods (up to 160 days) contained up to seven distinct biochemical types of *E. coli* during the study.

This biochemical variability of *E. coli* in the feces supports the interpretation that the patient is unable to maintain a stable microflora, and at the same time raises a question of whether the reported infections by endogenous microorganisms in fact may have been caused by exogenous strains of normal components. Since these data were from patients housed in an open environment, it was impossible in many instances to distinguish between changes due to exogenous microorganisms and those caused by alteration of endogenous components.

Studies to determine the relative importance of endogenous and exogenous flora were continued on patients housed in the Medical Division's laminar air flow (LAF) facility.

Microbiological studies have been carried out on the use of ultraclean environments during occupancy by patients. The microbial contamination level of surfaces, furnishings, and fixtures in the patient and preparation rooms was determined by contact plate at least once weekly. Air contamination was

1028017

determined by liquid impinging samples 5 days a week. The microflora of the patient's nose, throat and mouth, skin, and feces were cultured two or three times each week; blood and urine were also cultured.

The microflora and LAF data presented here were obtained from a male patient confined for 20 days. Diphtheroids and *Staphylococcus albus* predominated on the patient's skin before (Table 10) entry to the ultraclean environment: *Klebsiella-Enterobacter* gp and nonhemolytic streptococci were found on day 2; *Pseudomonas aeruginosa* was first cultured on day 4 and *E. coli* on day 15, and showering more often with antibacterial soap did not eliminate them. The nonhemolytic streptococci appeared only in the external auditory canals of both ears. *Staphylococcus albus* was found on all skin sites before he entered LAF, and was eliminated only from the prepuce. In contrast the diphtheroids were cleared from the skin microflora.

TABLE 10

MICROORGANISMS CULTURED FROM THE SKIN OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (Days)	*S. albus*	Diphtheroids	*P. aeruginosa*	*E. coli*	*Klebsiella-Enterobacter* gp	Nonhemolytic streptococci
−3	x	x	–	–	–	–
+2	x	x	–	–	x	x
+4	x	–	x	–	x	x
+8	x	–	x	–	x	x
+11	x	–	x	–	x	x
+15	–	–	x	x	x	x
+18	x	–	x	x	x	x
+22	x	–	x	x	x	x
+23	x	–	x	x	x	x
+24	x	–	x	–	x	x

*Returned to open environment on day 20.

The progressive spread of *P. aeruginosa* to various sites presents an interesting picture (Table 11). From mouth washings and from a small skin lesion, present on the patient's chest before isolating him, *P. aeruginosa* was cultured on day 2. On day 4 the organism appeared in the microflora of the groin. Later it was found in rectal swabs, and on skin cultures from the prepuce and umbilicus. This pathogen was not in urine until he returned to the open environment.

Prophylactic, nonabsorbable antibiotics were given to reduce the microbial load in the intestinal tract. Nystatin, paromomycin, Vancomycin, oxacillin, and polymyxin B were started when the patient entered the LAF room and

TABLE 11

ISOLATION OF *PSEUDOMONAS AERUGINOSA* FROM VARIOUS BODY SITES OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (days)	Chest lesion	Urine	Mouth washings	Rectal swabs	Groin	Prepuce	Umbilicus
−3	–	–	–	–	–	–	–
+2	x	–	x	–	–	–	–
+4	–	–	–	–	x	–	–
+8	x	–	–	–	x	–	–
+11	x	–	–	x	x	–	–
+15	x	–	–	x	x	x	x
+18	–	–	–	x	x	x	x
+22	–	x	–	x	–	x	x
+23	–	x	–	x	–	x	x
+24	–	–	–	x	–	x	x

*Returned to open environment on day 20.

discontinued on day 18. *Staphylococcus albus* and diphtheroids predominated in the nares before antibiotic treatment; both organisms were temporarily eliminated by day 4 but *S. albus* had reappeared by day 11 (Table 12). The microflora of his throat and mouth was normal before admission to LAF (Tables 12 and 13). Antibiotic treatment eliminated all aerobic components except for *P. aeruginosa* and a bacillus found in mouth washings on day 2. The anerobic components persisted although their numbers dropped significantly. Fecal specimens were not obtained; however, the microbial profile on rectal swabs was normal (Table 14). Antibiotic treatment eliminated all components from the rectum; *P. aeruginosa* was first found on day 11 and persisted on rectal swabs in spite of increasing the polymyxin B.

His urine before isolation contained small numbers of *S. albus* and diphtheroids (Table 15). No growth was found in any urine cultures while he was in LAF; however, *E. coli, P. aeruginosa,* and *Klebsiella-Enterobacter* gp appeared after he returned to the open environment.

The most consistent contaminants in the environment were a gram-negative rod, saprophytic fungi, and *S. albus* (Table 16). The gram-negative rod and saprophytic fungi were recovered almost exclusively from the bathtub-shower while *S. albus* was recovered from table tops which the patient had touched. *Pseudomonas aeruginosa,* despite its presence on the skin, was not found in his environment.

In comparing microflora data from patients in open and ultraclean environments, we suspect that both endogenous and exogenous microorganisms can alter the microflora, consequently increasing the risk of infection. The data also indicate that in the absence of a complete, normal flora it becomes

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TABLE 12

MICROORGANISMS CULTURED FROM THE THROAT AND NARES OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (days)	S. salivarius	S. mitis	Alpha hemolytic streptococcus	Beta hemolytic streptococcus (not gp A)	S. aureus	S. albus	Neisseria sp	Diphtheroids	Veillonella sp	Diphtheroids	Diphtheroids (pleomorphic)	Lactobacillus sp	Eubacterium sp	B. melaninogenicus
−3	†	†	†		†	‡	†		†	‡‡		†	†	–
+2						‡				‡	–		–	–
+4						–								+
+8						–					–			–
+11						‡						+	+	+
+15						‡						+	+	+
+18						‡								–
+22					†	‡				‡	‡			–
+23	†	†			†	‡			†	‡	‡	+	+	+
+24	†	†	†		†	‡	†		†	‡	‡	+	+	+

*Returned to open environment on day 20.
†Isolated from throat swab.
‡Isolated from nares.

TABLE 13

MICROORGANISMS CULTURED FROM MOUTH WASHINGS OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (days)	S. salivarius	S. mitis	Alpha hemolytic streptococcus	S. aureus	S. albus	Micrococcus sp	Neisseria sp	Diphtheroids	Diphtheroids (pleomorphic)	Bacteroides sp	B. melaninogenicus	Fusobacterium sp	Lactobacillus sp	P. aeruginosa	Bacillus sp	Veillonella sp
1	5.85†	5.70	5.48	2.0	2.30	5.95	6.0	5.0	–	–	5.60	5.20	3.60	–	–	–
+2	–	–	–	–	–	–	–	–	–	–	3.26	2.70	–	4.0	5.95	–
+5	–	–	–	–	–	–	–	–	–	–	4.60	–	–	–	–	–
+8	–	–	–	–	–	–	–	–	–	–	–	2.70	3.0	–	–	–
+11	–	–	–	–	–	–	–	–	–	–	4.0	–	–	–	–	–
+15	–	–	–	–	–	–	–	–	–	2.30	3.85	–	–	–	–	–
+18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
+22	–	–	–	3.0	–	–	–	5.43	–	–	5.30	4.30	4.30	–	–	–
+23	3.70	5.08	4.26	2.70	4.26	–	–	6.04	–	–	4.95	4.30	4.85	–	–	3.0
+24	5.04	6.0	5.30	–	2.48	–	4.0	5.70	–	–	4.18	4.32	4.48	–	–	

*Returned to open environment on day 20.
†Log$_{10}$ per ml.

TABLE 14

MICROORGANISMS CULTURED FROM RECTAL SWABS OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (days)	*S. salivarius*	*S. mitis*	*S. albus*	*E. coli* (nonhemolytic)	*E. coli* (beta hemolytic, lactose negative)	*Bacteroides* sp	*B. melaninogenicus*	*P. aeruginosa*	*Klebsiella-Enterobacter* gp
−3	x	x	x	x	x	x	–	–	–
+2	–	–	–	–	–	–	–	–	–
+4	–	–	–	–	–	–	–	–	–
+8	–	–	–	–	–	–	–	–	–
+11	–	–	–	–	–	–	x	–	–
+15	–	–	–	–	–	–	x	–	–
+18	–	–	–	–	–	–	x	–	–
+22	–	–	–	x	–	–	–	–	–
+23	–	–	–	x	–	–	x	–	x
+24	–	–	–	–	–	–	–	x	–

*Returned to open environment on day 20.

TABLE 15

MICROORGANISMS CULTURED FROM THE URINE OF A PATIENT HOUSED IN AN ULTRACLEAN ENVIRONMENT*

Time (days)	*S. albus*	Diphtheroids	*E. coli*	*P. aeruginosa*	*Klebsiella-Enterobacter* gp
−3	2.00†	3.70	–	–	–
+2	–	–	–	–	–
+4	–	–	–	–	–
+8	–	–	–	–	–
+11	–	–	–	–	–
+15	–	–	–	–	–
+18	–	–	–	–	–
+22	–	–	3.48	5.00	3.08
+24	–	–	3.00	5.00	3.70
+33	–	–	–	–	–

*Returned to open environment on day 20.
†Log_{10} per ml.

TABLE 16

MICROORGANISMS CULTURED FROM LAF ROOM DURING PATIENT OCCUPANCY

	Day of occupancy			
	0	5	11	18
Bacillus sp	0	0	3	0
Diphtheroids	0	0	1	0
S. albus	0	2	2	1
P. aeruginosa	0	1	0	0
Klebsiella-Enterobacter gp	0	1	0	6
Fungi (sap.)	0	0	5	14
Unidentified gram-negative rod	0	0	100	2
Positive samples (+/T)	0/17	4/17	4/17	5/17
Average colonies/inch²	0	.074	1.63	0.34

increasingly hard to control the individual components. More patients will have to be studied in the ultraclean environment before the relative importance of endogenous and exogenous microorganisms *in causing infections* can be evaluated.

Serum Immunoglobulins in Irradiated Patients

Previous work here has shown that bactericidal and agglutinating antibody titers decline in the serum of some patients after whole-body irradiation. The

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present study (G. Charles Kingdon and Stuart Chaskes), completed during 1971, sought to determine whether altered serum immunoglobulin levels after irradiation might cause the reduced natural antibody activity found previously and the consequent decrease in resistance to infection. The experimental group included 42 patients treated with 100 R to 350 R irradiation in either the METBI or LETBI facility. Serum was obtained twice weekly for the week before and 4 weeks after treatment, then weekly until day 52. Immunoglobulin (IgG, IgA, IgM) and complement (C'3) concentrations were measured by the radialimmunodiffusion technique using heavy chain specific antisera. We set the baseline level for each patient by averaging the three preirradiation concentrations; individual values were within ±20% of the baseline. Postirradiation concentrations that varied from the baseline by more than ±20% were considered to differ significantly from preirradiation values.

The study showed that human serum immunoglobulins can be altered after TBI (Fig. 4). A reduction in IgA of 20% or more occurred in 66% of the

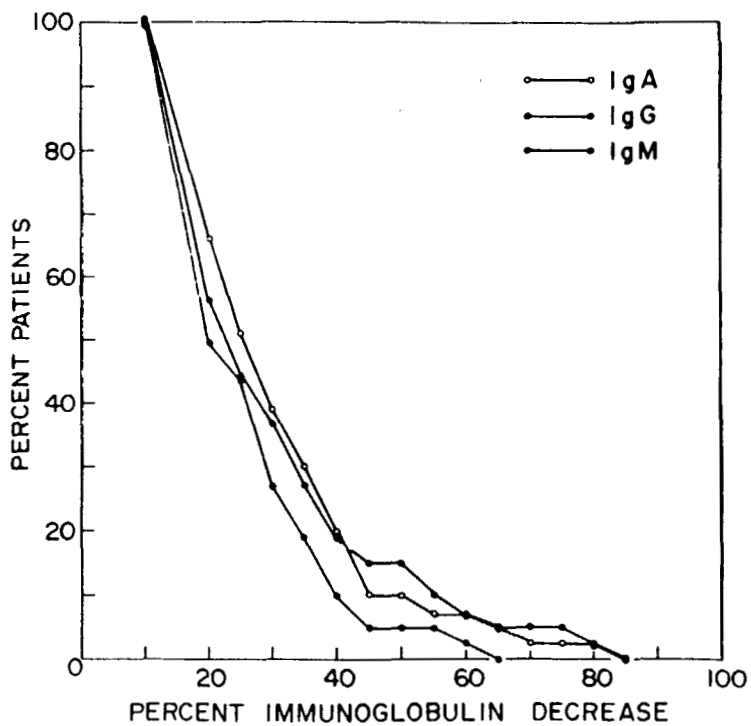


Fig. 4. Cumulative distribution of patients showing depression of serum immunoglobulin levels after TBI.

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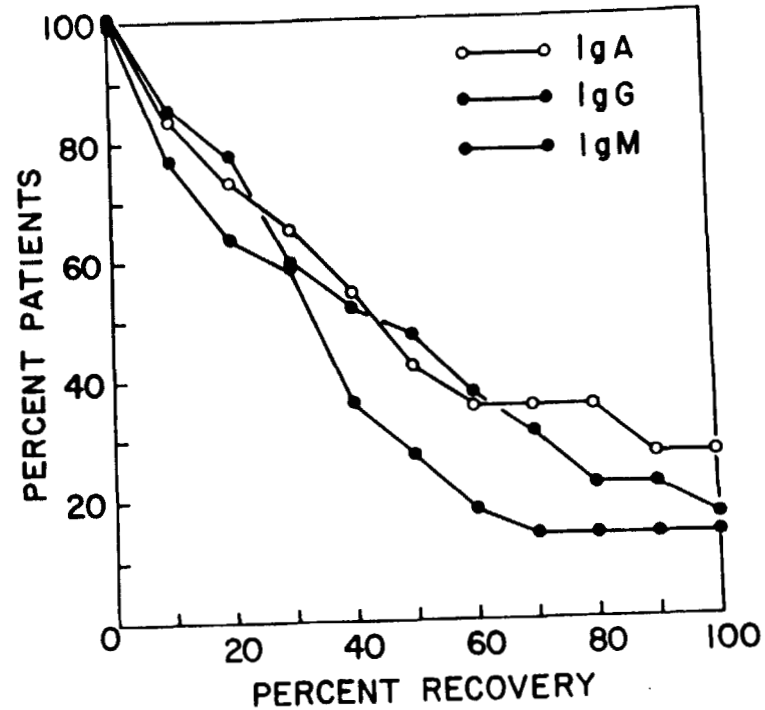


Fig. 5. Cumulative distribution of patients showing various degrees of recovery of serum immunoglobulin levels 7 weeks after TBI.

patients, while 56% showed an IgM decrease and 49% an IgG decrease of that magnitude. IgM and IgA concentrations dropped 40% or more in 20% of the patients while only 10% showed an IgG decrease in excess of 40%. A few patients (7.5%) had a greater than 65% depression in IgA and IgM levels but IgG decreases did not exceed 60%. All three immunoglobulin concentrations decreased 20% of the time while the levels in 12% of the patients remained unchanged. A drop in IgA concentrations alone occurred in 20% of the subjects while IgM or IgG alone decreased in 10% and 2.5% of the patients respectively. Reduced levels of C'3 were also occasionally found; however, the decrease was usually small, rarely falling below low normal values.

The fall in IgA began on an average of 7.4 days after TBI, followed by IgM at 8.4 days and IgG on 9.3 days. The average time for the nadir was 14.6 days for IgA, 17.6 days for IgM, and 20.7 days for IgG.

Return toward preirradiation levels was substantial but incomplete for most patients by 7 weeks after TBI (Fig. 5). Fifty percent recovery or more occurred

with a 48% frequency for IgG, 43% for IgA, and 28% for IgM. Presumably more patients would have returned to normal if studied longer than 52 days.

The relationship between lowered immunoglobulin and complement levels and postirradiation infection is still only partially understood. The incidence of minor infections was increased in irradiated patients with pathogens frequently being isolated from the urine and throat. There was no apparent correlation between the reduced serum immunoglobulin levels and the incidence of minor infection. Secretory IgA levels in saliva or mucus secretions were not measured; possibly secretory IgA may be affected by irradiation in a way similar to serum IgA, an explanation for the trend toward increased infection rates of mucosal surfaces in the absence of lowered serum immunoglobulin levels.

During the study four patients with lymphoproliferative disorders died within 40 days of TBI. Three had septicemia, the probable cause of death, and had seriously reduced levels of two or more immunoglobulins as well as a severe leukopenia. Death of the fourth was attributed to his primary disease.

We found instances both of increases and decreases in serum bactericidal activity after irradiation; frequently no changes were observed. Where changes in serum inhibitory activity occurred we could not correlate them with decreased or increased levels of immunoglobulins or C'3 levels.

We conclude that reduced serum immunoglobulin and complement levels alone don't explain fully the increased susceptibility to infection of some irradiated patients. Fatal infections were not found in any patient who had low immunoglobulin levels without a severe leukopenia, or in patients possessing normal immunoglobulin levels with or without a coexisting granulocytopenia. Three out of four patients who displayed both a serious leukopenia (below 1500/mm^3) and severely reduced immunoglobulin levels died from infection. Clearly increased susceptibility to infection is due to damage of a number of natural defense mechanisms, with reduced serum immunoglobulin and complement levels being only one facet.

Phagocytosis and Killing by Polymorphonuclear Leukocytes from Irradiated Patients

The rapid coarse and high mortality rate of postirradiation infections clearly indicate a reduced capacity of the patient to suppress growth of the invading pathogen, and to interrupt or slow the sequelae of infection that lead to cellular destruction and death. It is well known that leukopenia follows radiation exposure; however, the effects of radiation on other parameters of the natural defense mechanisms have not been adequately studied in man.

Studies were begun into the effects of radiation on phagocytic activity and killing potential of blood leukocytes (G. Charles Kingdon, Shirley Colyer, and William Tankersley). Heparinized blood was drawn once or twice weekly and the experiment started within 30 min. We added washed, overnight cultures of *P. aeruginosa* or *Staphylococcus aureus* and incubated the mixture with rolling for 3 hr at 37°C. All glassware was silicone coated to minimize loss of leukocytes

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by attachment to glass. We determined the number of bacteria required to give an initial bacterium-granulocyte ratio of 1 : 1 with the patient's first preirradiation blood granulocyte count. The number of bacteria was held constant during the study while the bacterium-granulocyte ratio increased by inverse proportion to the granulocyte decrease after irradiation. We removed an aliquot of the bacteria-blood suspension immediately after adding bacteria, and enumerated viable bacteria by pour plates. Aliquots were removed at intervals during incubation, the formed blood elements sedimented by low speed centrifugation and washed twice with pH 7.0 saline. The centrifugation pellet containing leukocytes was lysed and the bacteria enumerated by pour plates. The supernatant fluids were combined and viable bacteria counted. Blood smears were made at intervals, stained with Giemsa stain and the phagocytic number and phagocytic index determined by direct microscopic count.

Patients being treated for chronic granulocytic leukemia (CGL) or polycythemia vera (PV) were exposed to 150 R in either the LETBI or METBI facility. Detailed information on one of these patients with PV (150 R, LETBI) is presented here. Phagocytic and killing activity of his granulocytes on represented days is presented in Figs. 6 and 7. Five days before irradiation (−5 days) the granulocytes removed 97% of the *P. aeruginosa* (Fig. 6) and 97.5% of the *S. aureus* (Fig. 7) during the first 30 min of incubation. The rate of removal decreased greatly between 30 min and 60 min; this was more pronounced with *S. aureus* than with *P. aeruginosa*. After 60 min the removal rate recovered and at 180 min only 0.15% of the *S. aureus* and 0.015% of the *P. aeruginosa* remained in the supernatant. During the first 30 min approximately 85% of the *S. aureus* and 96.5% of the *P. aeruginosa* was killed by the granulocytes. Viability of *P. aeruginosa* continued to decrease and at 180 min less than 0.03% of the bacteria were still viable. *Staphylococcus aureus* was killed much more slowly. Viability decreased to 8% at 60 min and then slowly increased to 10% by 180 min, not unexpectedly since it is well known that blood granulocytes kill *S. aureus* only with difficulty.

Significant changes in phagocytosis and killing were seen 7 days after irradiation. Phagocytosis of *S. aureus* was increased; 99.6% of the bacteria was removed during the first 30 min compared with 97.5% removed by preirradiation granulocytes, in spite of an increase in the bacterium-leukocyte ratio from 1 : 1 to 1 : 1.6. Bacteria in the supernatant increased between 30 min and 60 min and then decreased to a value not greatly different from preirradiation values at 180 min. Increased removal of *P. aeruginosa* during the first 30 min was not observed. Phagocytosis stopped between 30 and 60 min and then resumed. The number of viable microorganisms in the supernatant fluid was not greatly different than preirradiation values at 180 min. Bacterial killing was temporarily reduced on day 7. Approximately 25% of the *S. aureus* and 8% of the *P. aeruginosa* was viable at 30 min in comparison to 15% and 3.5% for preirradiation values respectively. Little loss of bacterial viability was seen between 30 and 60 min for either microorganism. Killing increased after 60 min

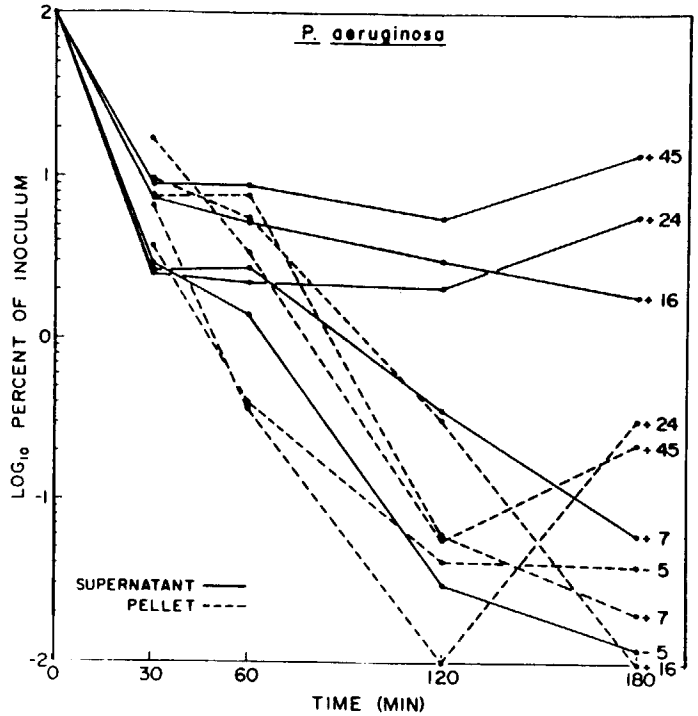


Fig. 6. Phagocytosis and killing of P. aeruginosa by blood leukocytes of a patient with PV after 150 R TBI.

and by the end of 180 min fewer viable bacteria were present than for the preirradiation samples.

Blood samples obtained subsequent to 7 days postirradiation showed a progressive exaggeration of the changes seen at 7 days. Removal of *P. aeruginosa* from the supernatant fluid appeared to cease after 30 min. On days 24 and 45 bacteria in the supernatant fluid increased between 120 and 180 min. Although the viability of bacteria in the leukocytes usually continued to decrease, it is difficult to assess the killing activity since ingestion of viable bacteria had stopped. Increased numbers of viable bacteria were found between 120 and 180 min on days 24 and 45.

Little difference was seen in the rate that *S. aureus* was phagocytosed during the first 30 min. All postirradiation samples showed increased bacteria in the supernatant between 30 and 60 min. On days 24 and 45 the bacteria also increased between 60 and 120 min. Significantly less killing of *S. aureus* occurred at 30 min, with a peak of 80% viability on day 45 in comparison to

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15% viability before irradiation and 25% on day 7. Viable bacteria in the leukocyte fraction declined by 180 min in all samples. We are, however, uncertain whether this was due to bacterial killing or reflects a release of the bacteria from the leukocytes back into the supernatant fluid.

Significant differences in the percent of granulocytes participating in phagocytosis occurred after irradiation. Approximately 40% of the granulocytes in preirradiation samples contained one or more bacteria by 30 min. During the next 120 min this percentage increased to 50%. No decrease was observed at 180 min with *S. aureus;* however, the percent of granulocytes containing *P. aeruginosa* fell from 50% at 120 min to 30% at 180 min, probably from digestion of the bacteria. The percent of granulocytes containing bacteria increased progressively after irradiation. The phagocytic number for *S. aureus* at 30 min on day 24 was twice the value obtained before irradiation.

We also saw increases in the phagocytic index (average number of bacteria in each phagocyte) after irradiation; preirradiation samples were approximately 4 and 2 for *S. aureus* and *P. aeruginosa* respectively. After irradiation the

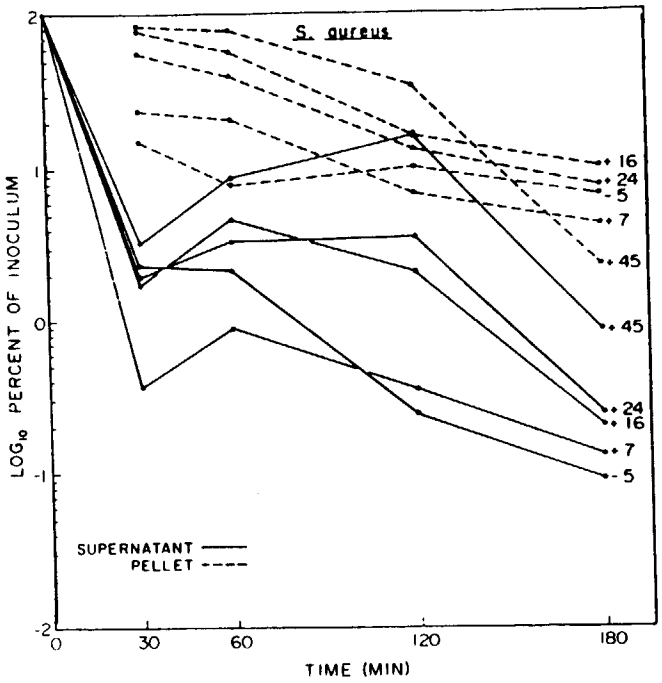


Fig. 7. Phagocytosis and killing of S. aureus by blood leukocytes of a patient with PV after 150 R TBI.

phagocytic index increased for both microorganisms to a peak of 13 for *S. aureus* at 120 min and 6 for *P. aeruginosa* at 180 min on day 45.

Interpreting phagocytic activity based on either the phagocytic number or phagocytic index alone is usually impossible, since an increase in the phagocytic number may be balanced by a corresponding decrease in the index. This can be avoided if the numerical product of the phagocytic number and phagocytic index (N-I product) is used. Ideally, where essentially all the bacteria are phagocytosed, the N-I product should approximate the bacterium-granulocyte ratio but should not greatly exceed it. The N-I product values for this patient are presented in Figs. 8 and 9. Five days before irradiation the N-I product for *P. aeruginosa* was slightly greater than 1 through 120 min and then decreased to less than 1 at 180 min (Fig. 8). This value agrees well with the bacterium-granulocyte ratio of 1 : 1. After irradiation the N-I product increased in proportion to the increased bacterium-granulocyte ratios. The increased N-I product after 30 min on days 16, 24, and 45 is inconsistent with the decreased

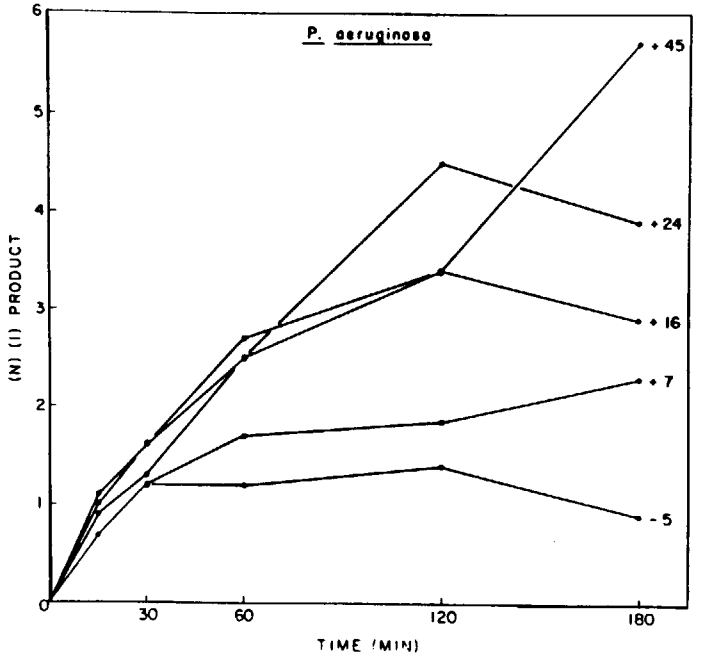


Fig. 8. N-I product (phagocytic number x phagocytic index) for P. aeruginosa of a patient with PV after 150 R TBI.

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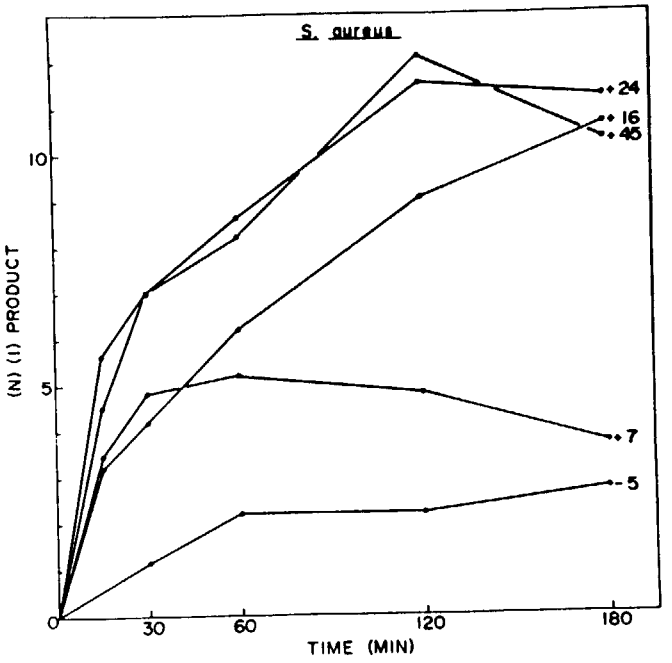


Fig. 9. N-I product (phagocytic number x phagocytic index) for S. aureus of a patient with PV after 150 R TBI.

removal of *P. aeruginosa* from the supernatant fluid and sometimes is also greater than predicted from the bacterium-granulocyte ratios.

The N-I product for *S. aureus* was approximately 2 in samples 5 days before irradiation (Fig. 9). We can explain apparent discrepancies between an N-I product of 2 and a bacterium-granulocyte ratio of 1 : 1 by the fact that *S. aureus*, present as paired cocci and counted as two units under the microscope, appeared as a single viable unit when enumerated by the pour plate method. Here the N-I product should not greatly exceed twice the viable bacterium-granulocyte ratio. After irradiation the N-I product increased, consistent with an increasing bacterium-granulocyte ratio up through 60 min; however, the N-I product had greatly exceeded twice the ratio by 120 min on days 16, 24, and 45. The increasing N-I product after 60 min is inconsistent with the decreased removal of *S. aureus* from the supernatant fluid during these intervals.

These data are preliminary and more patients will need to be studied before a final evaluation of the effects of radiation on phagocytosis and killing can be made. However, these data suggest that the phagocytes may have sustained both

an early and a late injury. Early postirradiation blood showed a decrease in the ability of the phagocytes to kill bacteria. This reduced killing was most pronounced with *S. aureus* but was also observed with *P. aeruginosa*. Later postirradiation blood had a reduced removal rate of bacteria, release of bacteria into the supernatant fluid, and an inconsistent increase in the N-I product values in the absence of apparent bacterial phagocytosis. This inconsistency might be explained by lysis of the granulocytes and redistribution of bacteria (both viable and killed) back through a smaller leukocyte pool.

Protection of Natural Defense and Immune Mechanisms During Irradiation

Because infections after irradiation frequently have a rapid course and a high mortality rate, it is important to assist, protect, or enhance the host's natural defense systems during exposure. A number of chemicals used experimentally can modify the effects of irradiation in several parameters including acute lethality, splenic atrophy, intestinal mucosal cell damage, and leukopenia; their efficacy in modifying infection rates after irradiation has not been examined.

Studies were begun in late 1971 to determine the effect of radioprotective chemicals on infections occurring after TBI (G. Charles Kingdon and William Tankersley). Twenty-gram female Swiss Webster pathogen-free mice were used in all experiments. Routine microbiological surveys were performed before irradiation and all cages showing contamination by *Pseudomonas* sp. were discarded. The mice were exposed 12 at a time to a ^{60}Co source at a dose rate of 50 R/min. The experimental $LD_{50/30}$ using 60 animals at each dose was 753 R. For protection studies the mice were given a total dose of 800 R since 60 to 80% of the animals died before 20 days, with few deaths occurring after 3 weeks.

Mercaptoethylamine (MEA) or β-aminoethylisothiuronium (AET) at a dose of 200 mg/kg body weight was injected intraperitoneally 5 to 10 min before irradiation. Radiation exposure was completed within 30 min of drug administration. At intervals through 20 days random groups were killed, the liver and spleen of individual mice aseptically removed, and homogenized in 10 ml of cold saline. A 0.1-ml aliquot of the homogenate was transferred to tubes of thioglycolate broth and Trypticase soy broth. The animals were considered to be uninfected if no growth was observed in either tube after 3 days.

Preliminary data on spontaneous infection rates in protected and unprotected mice are presented in Table 17. Unprotected, irradiated animals showed a low spontaneous infection rate during the first 8 days after exposure. The percent of infected animals increased to 60% on day 11 and remained between 40 and 80% through day 18; no infected animals were found on day 20. The period of high spontaneous infection rates corresponds closely with the time of maximum death rates for unprotected animals. Sixty to 80% of the animals died between 10 days and 19 days after irradiation, with most deaths occurring in the 6-day period between 12 and 18 days. In contrast, animals treated with MEA or AET before irradiation had significant protection against spontaneous infection.

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On day 11, 20% of the AET-protected animals had positive cultures of livers and spleens while the remaining groups were negative; no positive MEA animals were found. No deaths occurred in the MEA-protected group; however, three deaths were recorded in the AET-protected animals. The cause of death in these animals is unknown but possibly deaths were a result of infection, since they occurred on day 14.

These preliminary data suggest that significant protection against postirradiation infection can be afforded by MEA or AET and possibly other radioprotective chemicals as well. More work is needed to understand the mechanisms by which these chemicals protect the host's natural defense systems.

TABLE 17

EFFECT OF MEA AND AET ON SPONTANEOUS INFECTION RATES IN MICE AFTER 800 R TBI (50 R/min)

Time postirradiation	Percent of animals with infected livers and spleens		
Days	Unprotected	MEA (200 mg/kg)	AET (200 mg/kg)
0	0	0	0
4	0	0	0
6	20	0	0
8	0	0	0
11	60	0	20
13	40	0	0
15	80	0	0
18	60	0	0
20	0	0	0

Transient Monoclonal Gammopathy Due to Cytomegalic Virus Infection

The cause of a patient's transient monoclonal gammopathy has been ferreted out through the cooperative effort of the ORAU Medical Division; University of Tennessee Memorial Research Center, Knoxville; and the National Communicable Disease Center, Chamblee, Georgia (Helen Vodopick, Stuart Chaskes, A. Solomon, and J. Stewart). The patient, a 13-year-old girl with acute leukemia, has been followed at the ORAU Medical Division since December 1969. After a rather stormy initial course, a remission of her leukemia has been achieved without relapse to the present time (January 1972). She has been on biweekly doses of oral methotrexate as maintenance antileukemic therapy. In May 1971, she noted painless lymphadenopathy in her left cervical chain. Slight tonsillar enlargement was also noted. Penicillin was given for *S. aureus* found on culture of throat secretions. In spite of antibiotic therapy the tonsils became further enlarged, exudate appeared on them, and submandibular salivary glands became

visibly enlarged. Because of lack of response to the 11-day course of penicillin, tetracycline was begun. Her temperature rose to 40°C orally, but she appeared less ill than her temperature would have indicated. Her blood count became abnormal with significant pancytopenia. However, marrow aspiration showed a cellular marrow with no evidence of relapse of the leukemia. Several other antibiotics were tried with no observable effects upon her febrile course. After the methotrexate had been withheld for 1 week, the blood count returned to normal, fever abated, and the tonsillar and lymph node enlargement disappeared dramatically during a 3-day period.

During the course of her illness several serum samples had been collected for immunoglobulin determinations, and for measurement of complement fixation and antibody (hemagglutination inhibition) titers to cytomegalic virus and herpes simplex. Immunoglobulin levels in her saliva were also measured. As seen in Fig. 10, the serum IgA level gradually rose to a peak of 2900 mg/100 ml on July 6 and in her saliva when tested 4 days later to 72 mg/100 ml (normal 9-10 mg/100 ml). These values returned to normal as her condition improved. The hemagglutination inhibition antibody titer to cytomegalic virus, 1 : 4096 on the initial sample examined (June 11), fell on subsequent samples tested. This

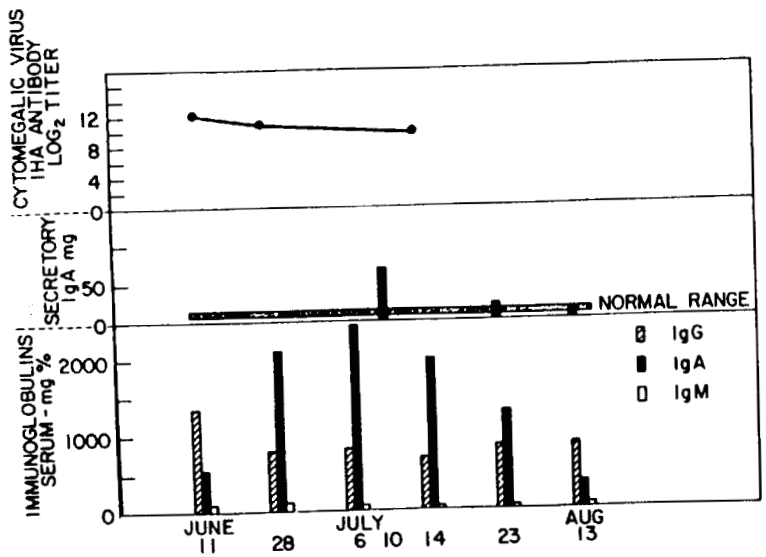


Fig. 10. Transient monoclonal gammopathy found during cytomegalic virus infection. The relationship of the laboratory determinations hemmagglutination inhibition antibody titer to cytomegalic virus, secretory IgA, and serum immunoglobulin levels is illustrated.

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falling titer suggested a recent cytomegalic virus infection, a finding in retrospect consistent with her clinical picture.

The increased concentration of IgA was unexpected, but can perhaps be explained: cytomegalic virus has a predilection for secretory organs, known sites of IgA production; if such an infection occurs in these glands, stimulation of IgA production would seem reasonable. Whether the augmented IgA level is nonspecific or represents a specific antibody response to cytomegalic virus is unknown. Current investigation of possible specific antibody to cytomegalic virus in this immunoglobin fraction is under way.

DOSIMETRY STUDIES AND MONITORING

Internal Dose Center Activities

A Center for Information on Internal Dosimetry of Radiopharmaceuticals supported by the Food and Drug Administration was established at the Medical Division in May 1971. The center's staff (Roger Cloutier and Evelyn Watson) specialize in the analysis of the modifying factors (Fig. 11) that influence the

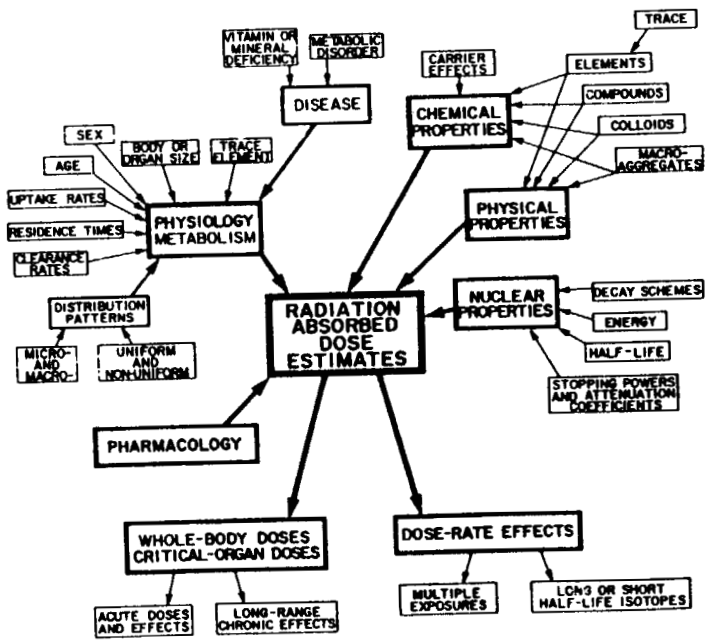


Fig. 11. Modifying factors that influence the patient's radiation dose from an administered radiopharmaceutical.

dose a patient receives from a radiopharmaceutical. Much of the retention, distribution, and excretion data are available from studies of ORAU patients.

An initial study of the center was the estimation of radiation dose to patients from the radiogallium isotopes. The results are being published by the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine. Retention and distribution values used for the dosimetry calculations are based on whole-body counting and autopsy data (27) from ORAU patients who had been given ^{67}Ga intravenously. Gallium retention in the human body appears to have two exponential components. Twenty percent of that administered has a biological half-time of 43 hr, and the other 80% has a biological half-time of 638 hr. Differences in patients' ages showed no statistically significant effect on retention and distribution; however, the data suggest that males retain a greater percentage of the administered gallium in the longer-lived component than females. Statistical differences related to patient's sex will probably be shown as more data are collected.

The center's staff developed a modified internal dose equation based on the MIRD schema for calculating the dose to a target organ from activity in other organs and in the total body. Previously the radiation dose to the target organ was overestimated because of the improper use of absorbed fractions in the equations. The modified equation is:

$$\bar{D}_t = \bar{D}_{np} + \sum_{k=a}^{t} \frac{1}{m_t} \left[\tilde{A}_k - \frac{m_k}{m_{rem}} \tilde{A}_{rem} \right] \sum \Delta\phi_{(t \leftarrow k)} + \frac{m_{TB}}{m_t} \left(\frac{\tilde{A}_{rem}}{m_{rem}} \right) \sum \Delta\phi_{(t \leftarrow TB)}$$

where $\bar{D}_t$ = mean dose to target organ from all sources
$\bar{D}_{np}$ = mean dose to target organ from nonpenetrating radiation
k = all organs influencing the dose to the target organ
m = mass in grams
$\tilde{A}$ = cumulated activity in μCi-hr
TB = total body

$$\tilde{A}_{rem} = \tilde{A}_{TB} - \sum_{k=a}^{t} \tilde{A}_k$$

$$m_{rem} = m_{TB} - \sum_{k=a}^{t} m_k$$

Δ = equilibrium dose constant
ϕ = absorbed fraction

The dose to a fetus from radioactivity in the mother's urine was also calculated during the past year. The calculation uses a computer model developed in collaboration with Dr. W. S. Snyder of the ORNL Health Physics Division. The computer program has been run for photon energies of 0.01, 0.03, 0.05, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, and 4.0 MeV and the center now has absorbed fraction tables available for each month of pregnancy. A synopsis of this study

1028026

was published in the 1971 ORNL Health Physics Division Annual Progress Report, ORNL-4720.

Personnel Monitoring with Film and Thermoluminescent Dosimeters for High Exposures

During the past few years many studies have compared the merits of film and thermoluminescent dosimetry (TLD) for personnel radiation monitoring for exposures below 10 R. Most of these comparisons were made under controlled conditions, such as constant temperature and humidity, unidirectional exposure, and fixed time from exposure to processing. This study compares TLD and film dosimeters at higher exposures and under conditions more likely to be encountered in a life-threatening radiation accident (W. L. Beck, Roger J. Cloutier, and Evelyn Watson).

Forty-two patients who varied in weight from 45 kg to 120 kg have worn both film badges and thermoluminescent dosimeters during their total-body irradiation in the low-exposure-rate total-body irradiation facility. These treatments provided a unique opportunity to compare these devices since the radiation's angle of incidence varied as the patient moved about the treatment room. Temperature and humidity were adjusted to suit each patient individually; typical values were 74°F and 50% RH.

After the exposure the badges were sent to the commercial film badge service along with those used for routine ORAU personnel monitoring; the company was not told which badges were given high exposures. The TLD dosimeters were evaluated in the ORAU Radiation Dosimetry Laboratory within 3 days after the end of the treatment. The TLD were calibrated with ^{60}Co gamma radiation (ORAU-107, p. 37).

Table 18 summarizes our experience with the two monitoring systems. For comparison, the exposures measured by dosimeters worn on the chest are expressed as a percentage of the exposure in air at the room center without a patient present in the room. The room-center exposure was calculated from the exposure rate measured by a standard ionization chamber and the total exposure time. Room-center exposures ranged from 10 R to 250 R.

Because the dosimeters on the chest are always partially shielded by the patient's body, depth-dose calculations predict that the chest exposure should be about 75% of the room-center exposure. The average chest exposure measured by TLD was 74% ± 5% while the average chest exposure measured by film for 36 patients was 95% ± 27%. The high values from the film measurements may be due to the film's greater sensitivity (density per roentgen) to low-energy photons. Although the ^{60}Co sources emit high-energy photons, many of those reaching the patient are of low energy because they have been scattered by the walls of the inner and outer rooms.

For six patients the film measurements were grossly in error as a result of a mistake by the supplier who discarded the insensitive film before evaluating the sensitive film. The estimates of exposure from these films were low by factors ranging from 3.5 to 13.

Even after excluding these six measurements, only 19% of the film measurements were within ±15% of the expected value compared to 98% for the TLD. While 56% of the film measurements differed from the expected by 30% or more, the worst TLD was only 17% greater than the expected value. Some of the variability in both systems can be attributed to differences in sizes of patients and their positions within the treatment room. However, other factors such as storage time, process conditions, energy and angular dependence, etc., must also be contributing to the variability in the films since their standard deviation is about 5 times that of the TLD results.

This comparison is not completely unbiased since the TLD were processed soon after their exposure and film processing occurred from a few days to as much as 4 months after exposure. Also the film processor was unaware that some film had received high exposures.

The results of this study indicate that the film badge is less reliable than TLD for monitoring high radiation exposures. We conclude that if accurate dosimetric information is to be provided after a life-threatening radiation accident, film dosimeters should be complemented with high-range dosimeters such as the lithium fluoride (LiF) TLD.

Dosimetry for Total-Body Irradiation Accident

On February 4, 1971, a technician performing seed irradiation experiments in the UT-AEC Variable Dose Rate Irradiation Facility unknowingly entered the exposure room and walked to within 60 cm of an unshielded 7,700-Ci ^{60}Co source. There he proceeded to change his samples assuming that the previous exposure had been completed and that the source was in its shield. When he returned to the control room, the facility operator realized that the source was still "on." A personnel monitoring dosimeter worn at the belt level by the technician was found later to have been exposed to 260 R.

The technician was interviewed to enable us to make time-motion studies of the tasks he recalled having performed while in the radiation field. Based on this information, we concluded that most of the exposure took place in about 40 sec while he was close to the source and that the exposure was essentially unidirectional to his anterior surface.

We determined the distribution of depth dose by irradiating an Alderson Rando phantom in the position estimated to "average" those positions occupied by the technician (W. L. Beck, T. R. Stokes, and L. Wade, Jr.*). Inside the phantom we distributed 520 LiF dosimeters in the volumes that represented the more radiosensitive organs. We also placed dosimeters at several sites on the surface of the phantom, including the site of the personnel monitoring dosimeter. After the phantom was irradiated, we processed the dosimeters by our standard techniques (Proc. 2nd Int. Conf. on Luminescence Dosimeters, USAEC Report CONF-680920, 1968, p. 976).

*University of Tennessee-AEC Agricultural Research Laboratory.

1028027

The results of this phantom's exposure of 40 sec at an average distance from the source of 60 cm indicated an exposure of 230 R, only 12% lower than the 260 R recorded by the technician's dosimeter. Although this agreement is excellent considering that both time and position were estimated, we chose to normalize all experimental surface and depth-dose measurements to the actual

TABLE 18

SUMMARY OF HIGH-DOSE MONITORING RESULTS

	Film		TLD	
	Number	%	Number	%
Number of patients monitored	36*	100	42	100
Number of measurements within ±15% of expected	7	19	41†	98
Number of measurements outside ±30% of expected	20	56	0	0
Number of measurements outside NSF‡ control limits of +160% to −63% of expected	4	11	0	0

*Six other patients wore film badges but the exposure estimates were made from sensitive film due to processing error (see text).

†One TLD measurement was 17% higher than the expected value.

‡National Sanitation Foundation.

260 R personnel dosimeter measurement. Except for the bone marrow, organ dose estimates were made by averaging the response of those dosimeters located in the volume corresponding to a particular organ (Table 19). We estimated the dose of 120 rads to the marrow by measuring the average dose in each of the marrow compartments of the body and weighting each dosimeter's response by the mass of marrow it represented. This weighting procedure was necessary because some marrow near the body surface will receive a high dose compared to other deep-seated marrow compartments surrounded by a thick bony cortex.

Organs near the anterior midsection received the highest dose since most of the ^{60}Co in the 85-cm long source is located in its lower 10-cm which was at about the same height as the technician's waist. Because of distance and attenuation of the body, organs nearer the posterior surface (kidneys, spleen) received only about half as large a dose. We estimated the average midline dose in the torso as 125 rads.

As in most accidents, the depth-dose distribution in this one was nonuniform due to the geometry of the unidirectional exposure. This variability imposes difficulties in relating clinical responses to dose in accident cases; most clinical experience with total-body irradiation has been derived largely from somewhat more uniform therapeutic exposures. These average organ and marrow doses when used prognostically on the basis of previous clinical experience resulted in

underestimating the level and severity of this man's blood responses. Their deep nadir and slow recovery (see p. 13) are unexpectedly severe for such a small exposure unless dose rate, which was unusually rapid in this case, has a more important effect than is generally accepted. Although this dosage information was therefore overridden by clinical considerations, it provides a well-documented set of dose-response correlations of great importance for treating such accident victims in the future.

TABLE 19

ESTIMATED DOSE TO HUMAN ORGANS FROM ACCIDENTAL EXPOSURE TO 260 R ^{60}Co RADIATION (AVERAGE MIDLINE-DOSE OF 125 RADS)

Organ	Average dose (rads)
Bone marrow	120
Gonads	200
Intestines	165
Kidneys	105
Lenses of eyes	155
Skin, front midchest	245
Skin, back midchest	150
Spleen	115
Stomach	165

Use of Whole-Body Counting to Individualize Radiation Dose Estimates in Total-Body Irradiation Therapy

In total-body irradiation (TBI) therapy the active bone marrow is generally considered the target organ and in hematopoietic neoplastic disease the marrow dose is probably the dose of most importance for correlating clinical signs and biological responses with radiotherapeutic effectiveness. Since it is not possible to measure this dose directly in a patient, we previously estimated the average marrow dose from phantom dosimetry (ORAU-113, p. 39). Thermoluminescent dosimeters were placed in the simulated marrow-spaces of an Alderson Rando phantom and the average dose measured by each dosimeter was weighted by the mass of marrow it represented. With this heterogeneous phantom and the weighting technique, the marrow estimate would seem to be realistic for patients of similar size and shape to the phantom. However, our patients have ranged from 19.8 to 122.7 kg whereas our phantom represents the 70-kg "standard man."

Since much of the marrow is located deep within the body (average depth of 5 cm), every patient's body serves as a marrow shield of some effective thickness which is related to body mass and cross section. Unfortunately this effective

1028028

thickness, or marrow self-shielding factor, is also a quantity that cannot be measured directly for a given patient. Considering how we might estimate this quantity, we realized that a similar problem arises in whole-body counting. There the body acts as a shield between the radiation emitted from radioisotopes within the body and the external detectors of a whole-body counter and so for an equal amount of radioisotope, a large patient's whole-body count is lower than that of a small patient.

In recent extensions of this work, we used an empirical approach to determine the self-shielding factors for the ORAU low-level whole-body counter (W. L. Beck, William D. Gibbs, Harold D. Hodges, Mildred Bailey, and C. C. Lushbaugh). Three aquaria-like phantoms representing in mass and length (or height) an adult, an adolescent, and a child were counted while filled with water containing a known amount of ^{137}Cs. The mass of each of these phantoms could also be reduced (to simulate a person of the same height but smaller mass) by simply using less total water. The data accumulated from counting each phantom with varied mass but equal amount of ^{137}Cs were then analyzed and expressed as mathematical functions relating the whole-body counter's relative counting efficiency (RCE) to average body thickness (T) computed from body mass and height. The equations for each of the three phantoms have been determined to be:

$$\text{Adult: } RCE = (0.9788)^T$$

$$\text{Adolescent: } RCE = (0.9753)^T$$

$$\text{Child: } RCE = (0.9690)^T$$

The T is given by: $T = \sqrt{\frac{\text{Weight (g)}}{\text{Height (cm)}}}$

Although T is an empirically chosen quantity, it has been shown to approximate the body thickness at the xiphoid (Clinical Uses of Whole-Body Counting, Proceedings of a Panel, Vienna, 1965. International Atomic Energy Agency, 1966, p. 15).

Since the RCE (or size correction) equations were obtained by counting the three phantoms with the ^{137}Cs distributed uniformly throughout them, we hypothesized that the same size-correction factors should apply to measurements of average whole-body dose to the same phantoms from external ^{137}Cs sources if their geometry is similar to that of detectors in the whole-body counter. In other words we are considering two situations: one in which the sources are in the body and the detectors outside at a distance and another in which the source positions and sites of interaction are the reverse; in the two situations the degrees of absorption are similar.

To test this hypothesis, we compared the RCE size-correction values measured from whole-body counting of the three water phantoms to the differences in average whole-body dose determined by Hayes et al. (Int. J. Appl.

Radiat. Isotop. 15, 313, 1964) in a dosimetry study for our medium-exposure-rate total-body irradiator (METBI). In the Hayes study the same three water phantoms were filled with a liquid chemical dosimeter that measured the average whole-body dose. Calculations showed that the geometry for the METBI source configuration is similar to the geometry of the detector arrangement in the whole-body counter. The RCE values were: adult 65%, adolescent 67%, and child 68%. These values do predict size differences like those seen in Hayes's results; the correlation is shown in Table 20 where the RCE values are multiplied by a constant of 1.09. This value was derived by assuming that the chemical dosimetry results were correct and consists of the best-fit standard value available for correcting the whole-body counting results so that they will coincide with the chemical dosimetry.

The close agreement in these data indicate that this whole-body counting technique could be used to determine size-correction factors for modifying whole-body dose estimates from phantom dosimetry to fit actual patients whose weights and heights (size) vary widely.

Since we have not yet completed this whole-body counting study nor developed a standard method for determining routinely an individual's marrow self-shielding factor for modifying estimates of marrow dose from heterogeneous phantom dosimetry, we have tried to simulate the results of such a dosimetry system and to evaluate its importance for clinical radiation therapy. We first applied the size-correction factors for whole-body dose determined above to correct the phantom estimates of marrow dose for each of our TBI patients based on their computed T. Obviously, T is not equal to the marrow self-shielding factor but since both quantities are dependent upon body mass and shape, we have assumed that they are proportional. Thus, the size-correction factors used are the ratio of the whole-body correction factor for a particular patient to the whole-body correction factor for our standard-man phantom. Dose estimates were computed by the following equation:

$$\text{Patient's average marrow dose (rad)} = \frac{\text{Patient's body-size correction factor}}{\text{Phantom's body-size correction factor}} \times \text{Phantom's marrow dose (rad/R)} \times \text{Total exposure (R)}$$

The estimates of the dose to patients are summarized in Table 21 and show that for a given exposure, dose to the marrow may vary by as much as ±15% depending on the size of the patient. Because of this variation, comparisons of the clinical or hematologic responses of two patients given the same *prescribed exposure* can lead to unwarranted conclusions. While these results show that there is little difference between the dose to the marrow in the phantom estimated by thermoluminescent dosimetry and the *average* size-corrected estimates, individual patients, unusually large or small, may experience deviations in absorbed dose that are highly significant. In addition, the range of these marrow doses in our two main irradiation facilities has been significantly

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TABLE 20

COMPARISON OF CHEMICAL DOSIMETRY AND WHOLE-BODY COUNTING FOR DETERMINING SIZE CORRECTIONS FOR WHOLE-BODY DOSE ESTIMATES

Phantom	Average whole-body dose (Rads/100 R)		
	Chemical dosimetry	Whole-body count*	% Difference
Adult	70	71	+1.4
Adolescent	74	73	−1.4
Child	75	74	−1.3

*RCE × 1.9.

TABLE 21

MARROW DOSE ESTIMATES WITH SIZE CORRECTIONS

Total-body exposure (R)		No. of patients	Active marrow dose estimates (rads)	
Prescribed	Actual		70-kg phantom	Patients with size correction (range)
		METBI		
150	145-154	12	95.1 (93.99)	93.8 (88-100)
180	174	2	111	109.5 (109-110)
250	259	1	166	149
333	353	1	226	248
		LETBI		
100	100	16	57	56.6 (55-59)
150	150	12	86	85.3 (82-88)
200	200	2	114	114 (113-115)
250	250	10	143	140 (129-151)

different at the same exposure; for example, patients exposed to 150 R in METBI have had average bone marrow doses from 88 to 100 rads compared to 82 to 88 rads in LETBI. On this basis, it would seem valid to compare directly the hematologic responses of only the two patients with the 88-rad doses to marrow and obviously questionable to compare the LETBI patient with 82 rads with the METBI patient who received the 100-rad dose. The close agreement of the average corrected and uncorrected doses in the same irradiator might lead us to question the necessity of the body-mass correction at least for TBI in the range of 100 to 150-R exposures. It would seem, however, that before differences in observed human responses can be considered with certainty to be dose-rate effects (or those of variable radiosensitivity) that the best target-organ dose estimates must be used in such dose-response studies.

A Study on Radiation Sickness: Cumulative and Conditional Probabilities of Responding in Patients Receiving Portal (Local) Irradiation.

In past years data were gathered on the systemic responses of patients given portal irradiation for the treatment of malignant conditions. These responses are: anorexia, nausea, and vomiting. Further analysis (F. V. Comas,* C. C. Lushbaugh, and Edward Frome†) of those data indicates that the patterns of response can be well described by a Weibull distribution of the form

$$f_{(t)} = \alpha\beta t^{\beta-1} e^{-\alpha t^{\beta}} \qquad (1)$$

Although these same data can be fitted to a logit or probit distribution (last years research report) the Weibull distribution has been used here because of somewhat greater mathematical simplicity, and especially because the beta exponent in equation 1 indicates, without further calculations, the rate of change in probability of response as a function of time. When beta is less than 1 (as in most of our data) the probability of response decreases with time.

In a Weibull distribution the cumulative probability of response is given by

$$e^{-\alpha t^{\beta}} \qquad (2)$$

where alpha and beta are parameters of the equation and t represents days after beginning of treatment. Figure 12 shows that the values calculated by equation 2 fit well the experimental data.

Of greater interest than the cumulative probabilities are the conditional probabilities defined as the probability that a response will occur at a certain time, provided that it has not occurred before. The conditional probabilities can be calculated by subtracting the cumulative probabilities of occurrence in two successive days, and dividing by the cumulative probability that the response has not yet occurred in the earlier of the 2 days being compared. Equation 2 is used to compute the cumulative probabilities. Figure 13 shows the conditional probabilities for the onset of anorexia, nausea, and vomiting. It clearly shows that an irradiated patient is more apt to respond on the very first day of treatment than at any other time. If no response occurs on the first day, the risk of responding on the next 10 to 20 days steadily decreases and beyond that time it is almost constant.

This analysis can also be carried out for responses other than the first one. For instance one may wish to determine what is the probability that a certain response will occur provided it has already occurred once and only once before. Figure 14 shows the conditional probability for an irradiated patient to develop

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†U. S. Public Health Service Fellow; Consultant, Department of Statistics, Emory University, Emory University, Georgia.

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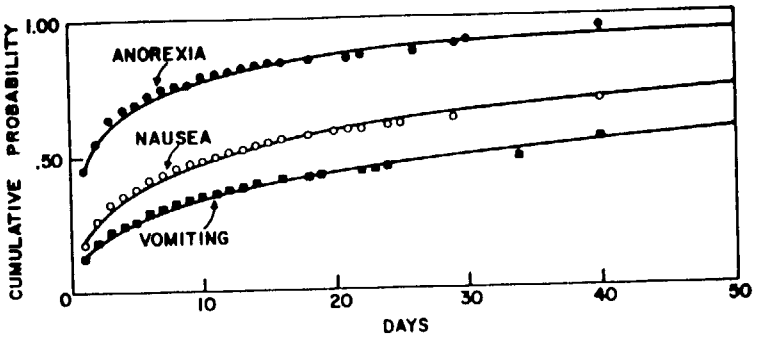


Fig. 12. Cumulative probability of onset of anorexia, nausea, and vomiting in patients receiving portal irradiation.

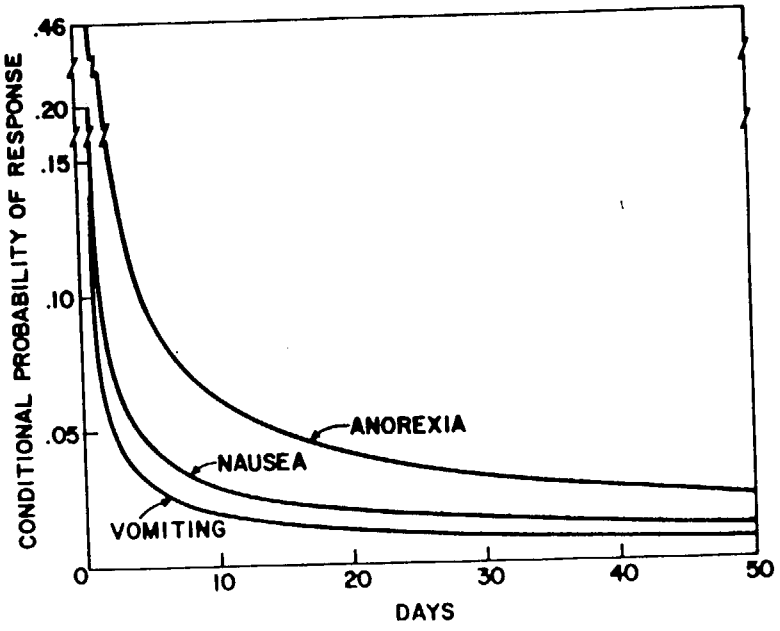


Fig. 13 Conditional probability of onset of anorexia, nausea, and vomiting in patients receiving portal irradiation.

anorexia, nausea, or vomiting for the second time during a radiation treatment course. It is clear that if the patient previously responded he is at a much greater risk to respond again than if he had not had anorexia, nausea, or vomiting once before. Yet, the conditional probability of responding for the second time also decreases with time. In general, this is true for any response number, either first, second, or nth.

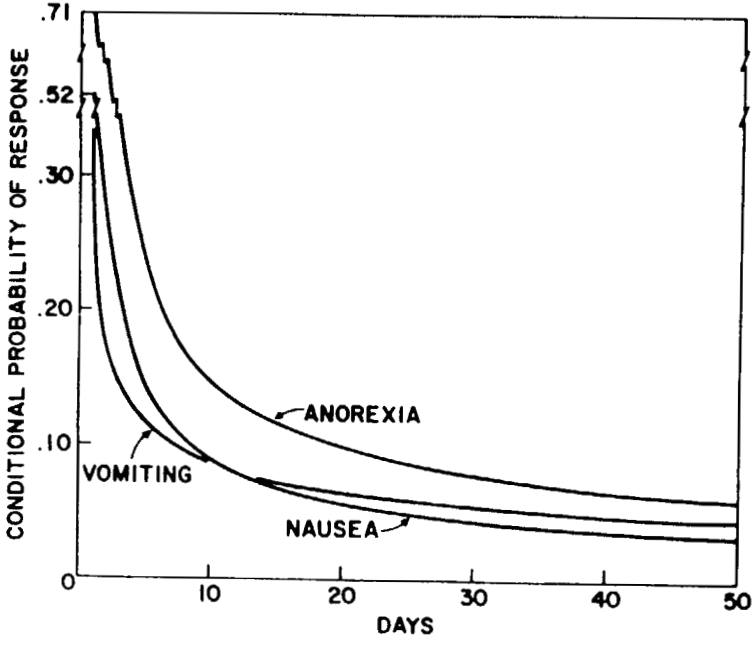


Fig. 14. Conditional probability, in patients receiving portal irradiation, to become anorectic or nauseated or to vomit for the second time.

The overall pattern of responses in a population receiving radiation therapy is shown in Fig. 15, where the conditional probabilities of responding for the first, second, nth time have been summated for each day, weighing those conditional probabilities by the relative number of people that at any one day are responding for the first, second, nth time. This figure shows that the overall probability of response decreases little with time. This is not in contradiction with the earlier conclusion that a patient's risk of manifesting a response steadily decreases with time. That is true so long as we consider a certain response number (first, second, nth) by itself. However, with the passage of time, patients are exposed to new risks which increase the overall probability of responding.

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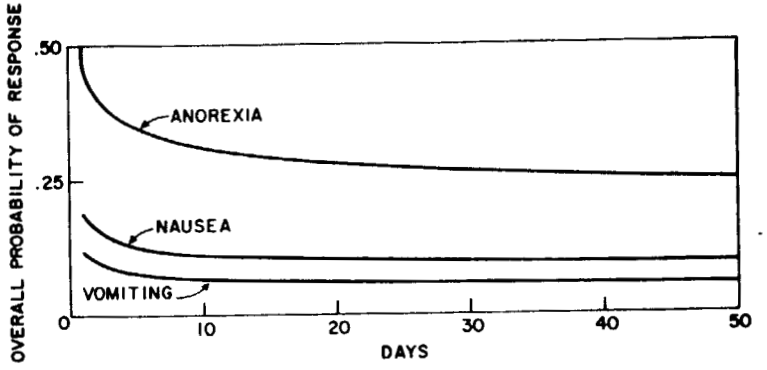


Fig. 15. Overall probability of three kinds of responses in patients receiving portal irradiation.

For instance, the risk of vomiting for the 10th time does not begin until the 10th treatment day, and only for those who have vomited daily for the past 9 days. For these patients the risk of vomiting again is very great, although overall this is an uncommon occurrence. To put it another way, after a few days of radiation treatments, almost all the patients who vomit consist of a rather small population of patients who have vomited several times earlier, and who have high risks of vomiting again.

Changes in Respiratory Efficiency, Cardiac Efficiency, and Creatine Phosphokinase during Exercise Stress after Total-Body Irradiation

Last year we reported successful measurement of radiation-induced performance decrement using pulmonary-impedance power-spectral analysis (Robert C. Ricks, Ann Sipe, C. C. Lushbaugh, and Edward Frome). We had previously shown that this method of analysis could be used to recognize induced (radiation or drug) gastrointestinal distress. Radiation-induced performance decrement was detected in two patients who received total-body irradiation (TBI) therapy (1.5 R/hr) protracted at 30 R/20-hr day (ORAU 113, p. 42). During the exposure period (100–150 R) the changes in average variance of pulmonary impedance measured before, during, and after controlled exercise stress (bicycle ergometer) were similar to those seen in nonpatient, nonirradiated, exercising volunteers; that is, only a low level of increased respiratory demand (deep breathing) was shown during and immediately following exercise as reflected by increases in the average variances of the power spectra. These increases were amplified after radiation exposure; this was evident at 3 days postexposure and lasted for 10 days to 2 weeks.

Recently, we have studied the decrement in performance induced by radiation in two patients receiving fractionated exposures of 10 R daily (1.5 R/hr for 6.7 hr) who were stressed by controlled exercise levels on a bicycle ergometer. The results are graphed in Fig. 16. Subject A previously had received protracted radiation therapy of 100 R and had shown increased fatigability on the third and tenth postexposure days. In this fractionated exposure, he also received 100 R, while Subject B received 140 R (10 R/day for 14 days at 1.5 R/hr). The latter patient developed a thrombophlebitis that prevented postexposure exercise testing. However, slight increases of the variance in pulmonary impedance occurred during the exposure period in both subjects. The magnitude of these changes is more obvious when the data are plotted against time as the average respiratory variance before exercise, the highest respiratory variance during exercise, and the average respiratory variance during the

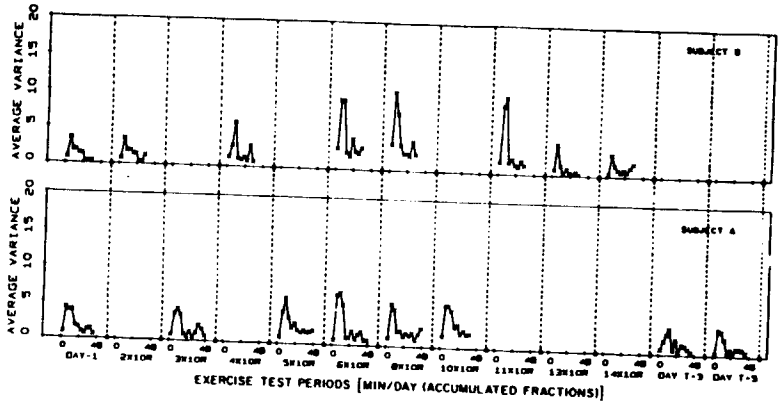


Fig. 16. Pulmonary-impedance variance obtained from two patients before, during, and after daily 10 R fractions of ^{60}Co TBI at 1.5 R/hr. Subject A received a total exposure of 100 R; subject B 140 R. Each patient exercised periodically on a bicycle ergometer before and during exposure. Subject A exercised after exposure. The work loads for A and B were 50 watts, 60 RPM and 40 watts, 40 RPM, respectively for 5 min each exercise period.

postexercise recovery period (Fig. 17). These case studies indicated that progressively increased fatigability (increased respiratory demand) occurred after 40 R were accumulated. Near the end of the radiation exposure period, however, the same exercise work load no longer caused this increased respiratory variance. During the period of increased respiratory demand (fatigability) the before- and after-exercise levels of respiratory effort also increased. The reason for elevated baselines is not clear; they may indicate that the test subjects did not fully

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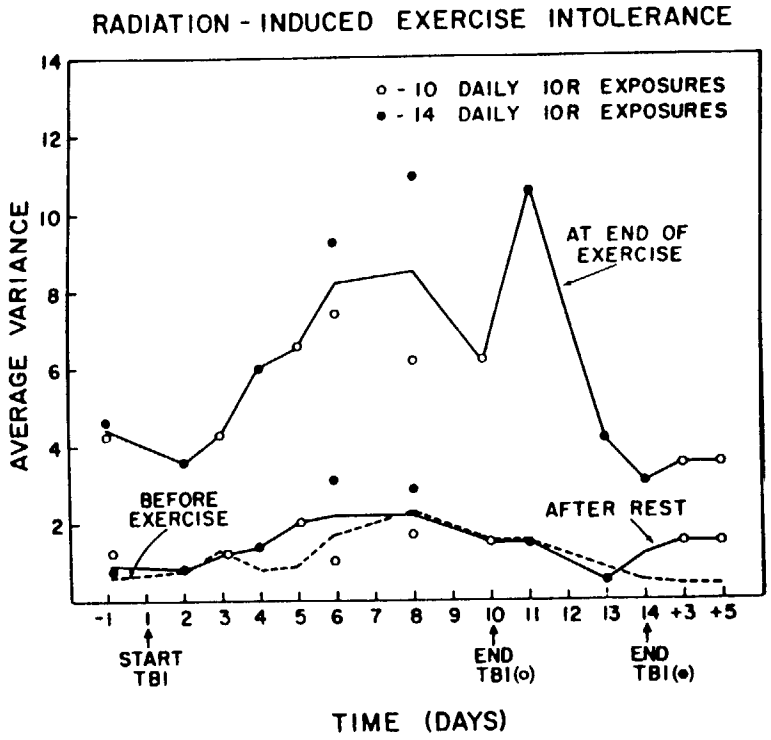


Fig. 17. Graphic representation of performance decrement obtained by averaging (where possible) data points from values shown for each respective test period in Fig. 16.

recover from the exercise stress and that some unknown physiologic change had been induced by irradiation.

A similar time-course study of radiation-induced performance decrement was made using this method after the accidental TBI of a man to 260 rem of ^{60}Co gamma rays for approximately 40 sec at 350 R/min. His estimated bone marrow depth-dose based on thermoluminescent dosimetry was 115–155 rads. Pulmonary-impedance measurements during controlled exercise were obtained commencing 3 days postexposure and on a regular basis for a total of 60 days. Initially he exercised to his subjective tolerance against a work load of 50 watts. There was 1 min of exercise on the third day but tolerance progressively

increased to 30 min until on the 7th day postexposure the work load was doubled to 100 watts. At this exercise level, his exercise tolerance time remained relatively constant at ~6 min. The zenith of variance in pulmonary impedance was reached within the first 6–8 min of each exercise test period. The results summarized in Fig. 18 represent the same kind of data reduction as illustrated in Fig. 17. Decrement in performance occurred at days 7 through 13 and then at

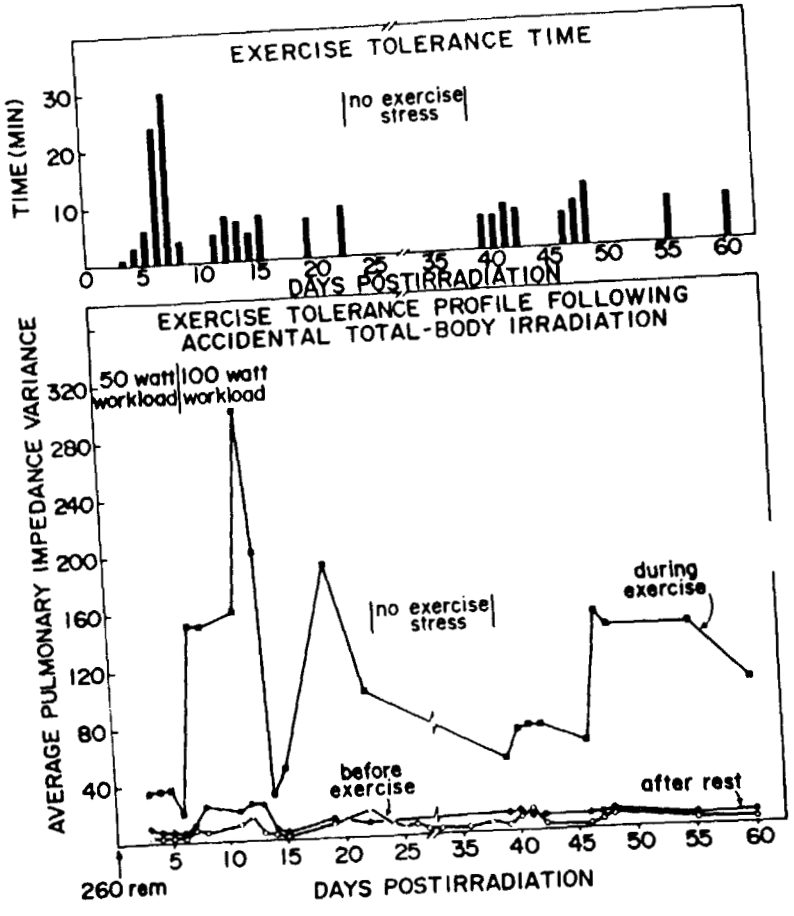


Fig. 18. Graphic representation of exercise tolerance profile and tolerance time in a normal man accidentally exposed to 260 rem gamma radiation. Due to thrombocytopenia no exercise stress was applied from days 23 through 38 although physiologic monitoring was continued.

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unpredictable times thereafter. Surprisingly, during the first week after exposure, we failed to demonstrate any significant changes in the respiratory variance of this man even though he said he was exercising to tolerance. His subjective symptoms of fatigue were apparently not respiratory as in the radiation-induced fatigue of our irradiated patients. This observation suggests that radiation-induced fatigue was not always directly related to shifts in the pulmonary-impedance power spectra and their variance. Perhaps in persons in good physical condition, respiratory reserves are too large for our system of exercise stress to elicit signs of performance decrement even though respiratory or vascular changes have been induced by irradiation. We are presently attempting to obtain an explanation of this dichotomy in our results by studying related biochemical and physiologic systems such as serum creatine phosphokinase (CPK) levels, respiratory CO_2 output, and cardiac rate regularity during the exercise stress test.

Creatine phosphokinase is responsible for the following reaction in muscular tissue:

$$\text{creatine phosphate} + \text{adenosine diphosphate} \xrightarrow{\text{CPK}} \text{creatine} + \text{adenosine triphosphate}$$

and is therefore important in sustained muscular activity. Recent studies (*Amer. J. Physiol.* 220, 100, 1971) have shown that during or after exercise increased plasma levels of various enzymes (including CPK) occur that seem to be due to hypoxia in the active muscles.

The CPK values of serum samples obtained from the accident victim before each exercise test period, as well as on the 2 days before the first exercise period, are shown in Fig. 19. Before regular ergometric exercising began, the victim's basal CPK level was 25 μU/ml. Following the first and second exercise periods (on days 4 and 5) the enzyme level rose by a factor of 13.7 and 18.2, respectively. When CPK levels were elevated (days 4, 5, 6) changes in pulmonary-impedance variance were minimal but when enzyme levels returned to the normal range, changes in this variance increased. This inverse relation of changes in pulmonary impedance are enigmatic in view of our previous studies. Experiments are in progress to determine changes in CPK levels after exercise in volunteers and after a combination of exercise and irradiation in patients undergoing TBI therapy. In one patient fractionated exposures of 10 R/day (1.5 R/hr) caused a slight but sustained rise in CPK values while protracted exposures of 30 R/day (1.5 R/hr) failed to do so (Fig. 20). The greatest change was seen during the period immediately following fractionated irradiation when CPK levels rose 2.5 times. However, this was within the range considered normal for serum CPK levels.

Recent software modifications have been made by our computer group to compute the frequency distribution and variance of cardiac rate (R–R interval), recorded on analog tape, simultaneously with pulmonary-impedance trace obtained by our physiologic monitoring system. In initial results during exercise

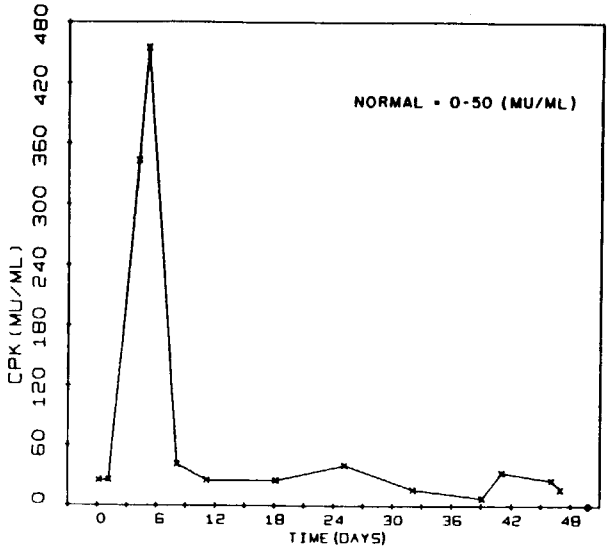


Fig. 19. Serum CPK enzyme levels obtained from a radiation accident victim exposed to 260 rem TBI.

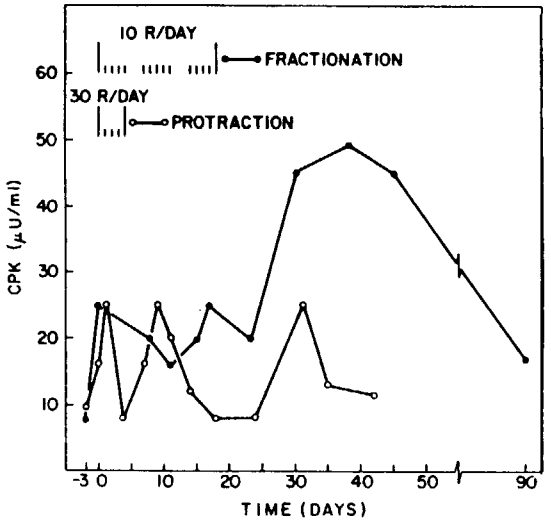


Fig. 20. Serum CPK enzyme levels obtained from a nonexercising patient who received both protracted (150 R, 30 R 20-hr day) and fractionated (15 daily 10 R fractions) TBI.

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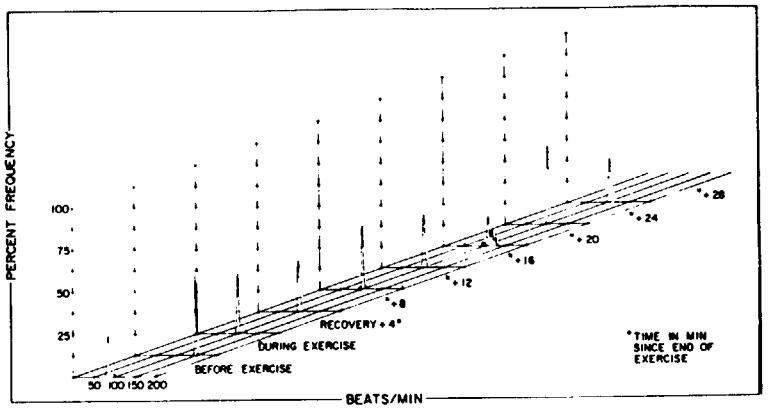


Fig. 21. Frequency histograms of cardiac rate (averaged over 4 min) over a time period commencing before exercise on a bicycle ergometer and ending 28 min postexercise. The test subject exercised for 4 min against a work load of 50 watts; pedaling speed was 60 RPM.

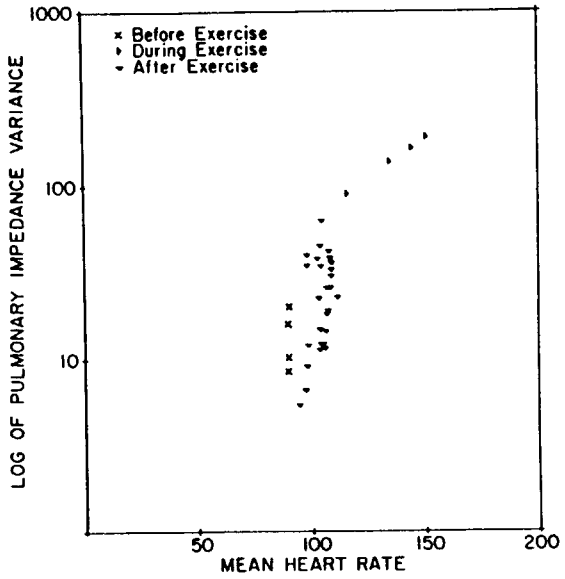


Fig. 22. Graphic correlation of heart rate and average variance of pulmonary impediance from a volunteer exercising on a bicycle ergometer for 4 min against a work load of 50 watts; pedaling speed 60 RPM.

the variance of heart rate decreases as rate increases. Both return to pre-exercise levels within 30 min depending upon individual cardiac status (Fig. 21). When changes in mean cardiac rate are graphed with the simultaneous changes in pulmonary-impedance variance before, during, and after exercise, the alterations in the variance of pulmonary impedance exceed those in heart rate during exercise and recovery periods. Cardiac rate and pulmonary impedance correlate well only during exercise (Fig. 22). Similar cross correlations of data obtained from the radiation accident victim (see above) have not yet been completed. The data obtained so far indicate that his periods of increased fatigability were associated with elevated basal pulmonary variance (i.e., pulmonary-impedance variance increased prior to exercise) but not with an increased resting cardiac rate. These preliminary results suggest that analysis of pulmonary function may be a more sensitive measure of performance decrement than cardiac function as measured by R–R interval. Experimentation currently is underway to test clinically a model of performance decrement based upon pulmonary-impedance variance, cardiac rate, serum enzyme levels, and expired CO_2 levels. With it we hope to recognize performance decrement by remote monitoring facilities.

Erythrocyte Production, Hemoglobin Synthesis, and Iron Kinetics Under Standardized, Protracted, Life-Long Radiation Exposures of Mice

Previously in our studies of the recovery mechanisms involved in surviving TBI in continuous low-dose-rate, gamma-ray fields, we have shown that the biologic systems at risk at ~1.5 and 3.2 R/hr (35 and 70 R/day) seem to vary from species to species. In man our therapeutic studies show that patients with a greatly expanded bone-marrow organ [as in polycythemia rubra vera (PRV)] can repair the effects of 1.5 R/hr level of exposure for up to 10 days but that patients with a diminished marrow as in chronic lymphocytic leukemia (CLL) can withstand this exposure for no more than 4 to 5 days. As with prompt exposure, the threatened systems at these protracted exposures are myelo- and thrombopoietic, so that undesirable hematologic effects are most frequently related to infection from granulocytopenia and hemorrhage from thrombocytopenia. Evidence of a radiation effect on the human erythropoietic system exposed at 1.5 R/hr is seen only in the postexposure red blood cell (RBC) production rates of patients with PRV. Patients with other diseases rarely show either any acute or chronic fluctuation in their RBC. The chronic exposure of dogs to 1.5 R/hr for 50 days by Fritz et al. (*Proc. Vth Int. Symp. on Comparative Leukemia Research*, Padua, Italy, September 1971, to be published) has been shown recently to kill 100% by septicemia; whereas in mice (C57BL/6) we have shown that death by this dose rate after 150 days of exposure is attributable to anemia following the failure of erythroblasts to replenish RBC lost through senescence. Fritz et al. in their report showed that smaller daily exposures of dogs obtained by reducing the exposure rate progressively from 1.5 to 0.8, 0.5, and 0.3 R/hr no longer cause septicemia from an agranulocytosis but cause a fatal anemia. This changeover in mode of lethality

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occurs in dogs, in contrast with C57BL/6 mouse, at 10 R/day; average duration of exposure required for this anemia to reach fatal proportions is 260 days (2600 R). Like our mice, these dogs survive this radiation-induced anemia by developing extramedullary erythromyeloid metaplasia but later develop a myeloid proliferative syndrome that in many instances has had clinical and morphologic features of acute and subacute myelogenous leukemia. This observation relates a late consequence of radiation exposure (leukemia) with an early acute lethality syndrome (septicemia) on a dose-rate scale for a large animal species, as Sacher and Grahn have done systematically for chronically irradiated mice. It raises again the question of whether there may be a causal relation between hematologic recovery mechanisms and leukemogenesis; and extramedullary erythromyelopoietic activity with myelogenous leukemia. The superficial histologic similarities of the two processes often make the differentiation of these pathologic entities difficult unless one cell line is outstripping the others and failing to differentiate properly, and replacing normal tissues in an aggressive manner. The early stages of conversion of obvious "metaplasia" to "neoplasia" are often impossible to prove morphologically, and transplantability is often the only sure criterion that the irradiated marrow is malignant.

We have not yet encountered leukemia in any of our chronically irradiated mice but extramedullary erythromyelopoiesis is extreme. At the two dose rates we use, the marrow expands into the spleen, liver, and other "metaplastic" sites. We have concluded tentatively that survival depends upon how extensive this extramedullary hematopoiesis becomes and how long it endures, but our attempts to prove this hypothesis have so far been unsuccessful because quantitating the size of the marrow compartment has proven difficult. So far the continuing increase in body weight is our best quantitative criterion of successful adaption of the mouse to its radiation environment. Blood leukocyte levels become remarkably low but have no prognostic usefulness. Levels that would be alarming clinically have had no infectious consequences at 0.8, 1.5, or 3.2 R/hr (17, 35, or 70 R/day). The rate of fall in RBC count and hematocrit assesses the success or failure of erythropoiesis but changes in hematocrit are offset by the production of macrocytes that increase in size and number with increase in size of accumulated exposure.

Splenic weight, which seemed most likely to be the quantitative observation needed to measure the marrow response objectively, was found to increase as exposure accumulated, but in our experiments fluctuated so widely that obviously some uncontrolled biologic variable was present. We then tried to quantitate this recovery effort by spleen-colony counting. Here we could show again only a general trend: numbers of countable colonies increased for a time as splenic weight increased, but then, as splenic weight reached the maximum, the counting technique failed because many colonies coalesced when erythroblastic hyperplasia became widespread. Extremely large but discrete (and therefore countable) colonies then reappeared as the result of continued irradiation at 70 R/day, since it caused atrophy of intervening red pulp. Although not quantitatively adequate, these studies along with histologic studies of the tissues

helped to define several aspects of the low-dose-rate irradiation/recovery syndrome: that survival was accompanied by extramedullary expansion of marrow function; the major marrow effort was erythropoietic; when cell numbers declined, adequate blood cell mass was maintained temporarily by a macrocytosis that was accompanied by production of an (electrophoretically rapid) abnormal hemoglobin of unknown oxygen-exchange capabilities. Loss or failure to gain in total-body weight was accompanied by progressive loss in RBC mass that preceded death. At death, however, many spleens were still enlarged and nodular. Histologically, the peripheral nodules in these spleens were composed of erythroblastic tissue surrounded by a cell-poor stromal connective tissue containing unusually large amounts of hemosiderin and diffuse, Prussian-blue positively stained, iron. The central areas of these spleens, normally occupied by large lymphoid follicles which disappeared under chronic irradiation, contained at death large masses of hyperplastic reticular lymphocytes, many with nuclear characteristic immunoblasts and other primitive lymphopoietic cells. These findings suggest that an abnormal reticular proliferation might be occurring.

To help interpret these complex events we tried this year to see if they might be quantified by iron measurements (T. R. Stokes, C. C. Lushbaugh, Lydia Corrill, and Dorothy Lushbaugh). These were made by assaying radioactive iron localization in spleen, liver, and bone marrow. In these studies, 0.25 μCi of ^{59}Fe citrate of high specific activity was injected intraperitoneally at intervals in mice irradiated continuously at 1.6 and 3.2 R/hr (35 and 70 R/day). After 24 hr we measured the percent of the initial dose of radioactive iron retained by the whole spleen, whole liver, and whole femur. In addition, mice were used in this study 30 days after splenectomy and sham splenectomy to determine whether the mouse spleen might play a unique role in this radiation-recovery syndrome. Some of the mice in this part of the experiment were from the ancestral stock (C57Black) of the C57BL/6 mouse (originally bred out by W. Russell) usually used in these experiments. This mouse strain is more radiosensitive than the C57BL/6 mouse and since it succumbs at lower accumulated doses, it seemed possible that it might accentuate any marginal deleterious effect of splenectomy.

Splenic ^{59}Fe uptake for C57BL/6 unirradiated control mice was ~2% of the total dose of ^{59}Fe. At 1.6 R/hr splenic ^{59}Fe uptake was elevated to ~3% when 1130 R had accumulated (Table 22). It continued to increase linearly with accumulating dose to ~6% at 4900 R. At 1130 R splenic weight was reduced to ~80% of normal (Table 23). It then increased linearly to ~130% of normal at 4900 R. Good correlation was found between splenic ^{59}Fe uptake and splenic weight, uptake expressed on a per weight basis being constant for all samples at this dose rate. At 3.2 R/hr ^{59}Fe uptake was normal up to an accumulated dose of 800 R, was increased to a maximum of 6% by 3300 R, and had declined to 5% by 4000 R accumulation. Splenic weight was ~65% of normal at 800 R point. It increased to 110% of normal by 2700 R but then had declined to ~80% of normal by 4000 R. Good correlation was found between splenic ^{59}Fe uptake and splenic weight up to 2700 R after which increasing accumulated dose

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TABLE 22
PERCENT OF NORMAL (AND PERCENT OF TOTAL) TRACER ^{59}Fe DOSE IRON UPTAKE OF ORGANS OF TWO STRAINS OF MICE CONTINUOUSLY IRRADIATED

		Accumulated doses				
		At 1.6 R/hr		At 3.2 R/hr		
Organ	Normal (%)	1100 R	4900 R	800 R	2700–3300 R	4000 R
		C57BL/6 Mice				
Spleen	100 (2.0)	150 (3.0)	300 (6.0)	100 (2.0)	300 (6.0)	250 (5.0)
Liver	100 (6)	100 (6)	165 (10)	100 (6)	210 (13)	417 (25)
Marrow	100 (1.0)	160 (1.6)	160 (1.6)	200 (2.0)	200 (2.0)	70 (0.7)
"Total"*	100 (22)	140 (31)	175 (38)	165 (36)	215 (47)	200 (44)
		At 1.6 R/hr				
		2000 R	3200 R	3700 R	4000 R	
		C57Black Mice—Sham Splenectomized				
Spleen	100 (2.0)	160 (3.3)	250 (5.0)	260 (5.3)	140 (2.8)	
Liver	100 (6)	100 (6)	100 (6)	200 (12.0)	560 (28)	
Marrow	100 (1.0)	170 (1.7)	150 (1.5)	140 (1.4)	70 (0.7)	
"Total"*	100 (22)	150 (33)	145 (32)	170 (37)	125 (41)	
		C57Black Mice—Splenectomized				
Spleen	---	---	---	---	---	
Liver	100 (6)	135 (8)	350 (21)	315 (19)	---	
Marrow	100 (1.0)	150 (1.5)	150 (1.5)	190 (0.8)	---	
"Total"*	100 (20)	145 (29)	210 (42)	230 (46)	---	

*"Total" = Computed total tissue taking up iron = (marrow x 14) + spleen and liver.

resulted in pronounced decrease in splenic weight with only slight reduction in percent uptake of ^{59}Fe. These divergent changes resulted in an increased iron uptake/mg of spleen at 3.2 R/hr after 2700 R.

Femoral ^{59}Fe uptake for the normal controls was ~1%. At 1.6 R/hr femoral uptake remained constant ~1.6% from 1100 to 4900 R accumulation. At 3.2 R/hr marrow uptake was constant at ~2% until 2700 R accumulated exposure, when it began a gradual decline to less than 1% at 4000 R.

Hepatic ^{59}Fe uptake for the normal controls was ~6%. At 1.6 R/hr hepatic uptake remained normal until it increased to ~10% at the 4000 and 4900 R levels. Liver weights were ~90% of normal throughout the complete exposure. At 3.2 R/hr hepatic ^{59}Fe uptake remained normal only until 2700 R had accumulated, then it increased ~13% at 3300 R and ~25% at ~4000 R. Liver

weights were ~90% of normal from 800 to 2700 R, then declined to ~70% of normal at 4000 R at 3.2 R/hr.

No significant differences appeared in any ^{59}Fe uptake level, peripheral blood values, or body weight changes between splenectomized and sham splenectomized C57BL/6 mice under these exposure regimes. However, in parallel studies using the C57Black mouse irradiated at 1.6 R/hr, a difference was found between splenectomized and sham splenectomized animals. Body weight changes were essentially the same for both splenectomized and sham splenectomized mice. However, after 3600 R, only 1 of 25 (4%) sham splenectomized mice had died while 12 of 71 (71%) splenectomized mice were dead. Iron uptake assays were made in both groups at 2000 R, 3200 R, and 3700 R, accumulated doses and in the sham splenectomized animals that survived, to the 4000 R level. Femoral ^{59}Fe uptakes were the same for both groups (~1.6%) at all sacrifice times but marked differences were found in the hepatic ^{59}Fe uptake levels, while in the sham splenectomized mice hepatic ^{59}Fe uptake was normal (6%) at 2000 R and 3200 R, increased to 12% at 3700 R and to 28% at 4000 R; in splenectomized mice it was ~8% at 2000 R, and increased to ~20% at 3200 R and 3700 R. Splenic ^{59}Fe uptake in the sham splenectomized mice increased linearly from ~3.5% (normal 2%) at 2000 R to ~5% at 3700 R, then decreased to ~4% at 4000 R. Splenic weight peaked (130% normal) at 3200 R, then declined to a minimum of 50% normal at 4000 R.

TABLE 23
PERCENT OF NORMAL WEIGHTS OF TWO STRAINS OF MICE IRRADIATED AT 1.6 AND 3.2 R/hr

	Accumulated Doses				
	At 1.6 R/hr		At 3.2 R/hr		
Organ	1100 R	4900 R	800 R	2700–3300 R	4000 R
C57BL/6 Mice					
Spleen	80	130	65	110	80
Liver	90	90	90	130	70
Total body	100	83	93	86	72

	At 1.6 R/hr			
	2000 R	3200 R	3700 R	4000 R
C57Black Mice—Sham Splenectomized				
Spleen	120	160	115	50
Liver	96	88	82	60
Total body	97	87	75	68

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The survival histogram, ^{59}Fe uptake pattern, peripheral blood values, and splenic weight changes in the splenectomized adult C57Black mouse exposed to 1.6 R/hr corresponded to those of the C57BL/6 mouse at 3.2 R/hr, indicating a two-fold difference in radiosensitivity. In both C57BL/6 and C57Black mice the level of hepatic ^{59}Fe uptake rose as the hematocrit fell. Those animals with high hepatic iron uptake (~25%) had hematocrits of 20 to 25%.

Predictably, these iron uptake studies reveal the large extramedullary erythroblastic activity of the liver that develops in this chronic low-dose-rate irradiation syndrome. While this activity, in terms of the percent of injected tracer, was less than that of the spleen at the lower dose rate it was three to five times that of the spleen at the higher dose rate. Both splenic and marrow iron uptake had declined in the period between 3000 and 4000 R (3.2 R/hr) a period in which hepatic iron uptake doubled. These observations indicate that our previous hypothesis based principally on splenic and marrow morphology needs to be modified to explain how the mode of death in this syndrome can be due to erythroblastic failure per se when erythroblastic iron uptake continues to increase rather than fail. In Table 22 we have compared the computer total-body "marrow," 24-hr iron uptake in percent of normal. This computation, based on the work of others who show that a single femur contains approximately 7% of the total marrow in the mouse, does not take into account that under these experimental conditions the peripherally located marrow compartments such as the sternum are extremely atrophic. We will avoid this computation by direct measurement of total marrow within bone by whole-body counting of the carcass, in the future using increased levels of radioactivity. The computed values show a progressive increase proportional to both accumulated exposure and exposure rate that suggests that the ability of the erythroblastic tissue to incorporate iron is not finally surpressed as we formerly thought. In light of the terminal anemia that develops, this observation could mean that either RBC production becomes "ineffectual" or that RBC life-span or destruction increased. To measure these aspects of the problem, the size of the tracer dose of ^{59}Fe will be increased to allow us to measure the 24-hr RBC production and total RBC mass by counting aliquots of peripheral blood. In addition, the survival of the RBC entering the peripheral blood, particularly during the terminal phase of this syndrome, will have to be determined. The terminal, splenic germinal center hyperplasia that we find occurring at this time could cause increased destruction of RBC through autoimmune process seen in some human myeloid proliferative syndromes. Proof of this would establish that 30 to 70 R/day levels of chronic radiation exposure are the threshold levels in the mouse production of myeloproliferative disorders after accumulated exposures in excess of 3000 R. In the context of space exploration, where exposures to less than 50 millirem/day are anticipated and at this rate, total exposures of 35 R (total marrow dose of ~20 rads) might result. The large accumulations necessary for the production of this described kind of disease in the mouse seem to place present space radiation constraints an order of magnitude or more on the side of safety.

Radiation Immunology and Marrow Transplantation

The immunology program's primary theme has been the understanding of antigen-antibody reaction in transplantation of cellular elements. Specifically, we have been concerned with procuring marrow transplants in heavily irradiated recipients with the ultimate goal the successful extrapolation of animal experimentation to clinical situations. A key observation made some years ago in our laboratory was the importance of radiation exposure rates in achieving optimum immunosuppression for graft acceptance. In brief, we found that exposure rates of 40 R/min or more were required to obtain maximum immunosuppression for marrow graft acceptance. Total-body irradiators for marrow grafts in patients, however, have routinely used rates of 0.5 R/min to 9.0 R/min, not by choice but because of geometric restrictions. Applying this information last year to a clinical situation, we successfully eradicated a leukemic process and obtained an allogenic marrow transplant. Although the patient subsequently died due to complications unrelated to the graft procedure itself, this initial success has encouraged future trials not only for the treatment of malignant diseases but also other blood dyscrasias that may be life limiting. One of the persisting, serious problems in achieving therapeutic marrow transplants in man and the experimental animal has been the graft-versus-host (GvH) reaction. Preventing this process is still to be accomplished; indeed, little is still known about its kinetics and primary tissue sites. Previously we had reported the adaptation of the Jerne hemolytic plaque technique which permitted unequivocal identification of the ongoing GvH reaction in the spleen of irradiated-marrow-treated animals. This has now been extended by the use of immunofluorescent reagents, which not only verifies the hemolytic focus as a site of GvH activity, but also permits identifying the donor immunoglobulin-producing cells in other selected tissues of the grafted animal. These GvH foci suggest that at least a humoral component is involved in the secondary disease phenomenon but whether it is the lone etiologic agent in the pathogenesis is yet to be determined. With modifications, we hope the technique can now be used for clinical studies since at present diagnosis of GvH reaction in humans is subjective.

In addition to marrow transplant studies, the dose-rate effects on immune processes have been documented extensively by measuring humoral antibody formation to heterologous antigens in irradiated mice. We find both total dose and quality of radiation (^{60}Co gamma, 250 kVp X ray, and fission neutrons) to be important variables in the rate effect. Some immune response studies also led to the observation that regardless of dose rate, we get maximum suppression 24 hr after exposure. Since most marrow transplants have been attempted with 2–4 hr after exposure, it was important to determine whether a simple delay of marrow injection would obviate the need for high exposure rates for a marrow graft. Parallel studies of this problem were conducted with both ^{60}Co gamma radiation and 250 kVp X irradiation; since the latter is used extensively by most investigators for exposure of small animals, it was important to know whether rate effects were discernible with X rays in studies involving tissue transplantation. We have extended our interests in radiation effects on the immune process to include one of the lowest rate chronic radiation exposure units available for man, the low-exposure total-body irradiation (LETBI) facility of ORAU designed for the total-body exposure of patients for periods of several weeks. Current studies with this unit showed a significant influence on the immunologic capabilities of mice exposed to radiation delivered at rates of 1.6 R/hr to 6.0 R/hr. Surprisingly, even at these low rates, a differential rate-effect was noted on the immune response. Potential application of similar studies to patients treated in the LETBI facility now seem warranted.

1028038

Allogeneic Marrow Transplantation in Man: A Final Evaluation of Case No. 1

The importance of radiation exposure rates on the degree of immunosuppression has, as noted above, been documented extensively. A primary reason for these dose-rate studies has been marrow transplantation, which depends on completely eliminating any host immune competence that may interfere with graft acceptance. We applied these laboratory findings clinically last year, and successfully grafted marrow in a patient with acute granulocytic leukemia (ORAU-113, p. 62). The present report is a final evaluation of this case study (N. Gengozian, C. L. Edwards, Helen Vodopick, and K. F. Hübner).

The patient had been prepared for allogeneic marrow graft from his brother by immunosuppression with antilymphocyte gamma globulin (H-AHTGG) and 500 R total-body irradiation delivered at 40 R/min. HL-A antigen typing had indicated identical phenotypes existed between patient and donor; however, blood group and immunoglobulin data provided two potential markers for detecting a graft. Further, cytogenetically the patient had a stem-cell line diagnostic for the leukemic cells, i.e., hypodiploid cells with a chromosomal rearrangement involving the group D and G chromosomes, permitting us to definitely know whether the treatment eradicated the leukemic cell population.

As shown in Fig. 23, complete repopulation of the erythropoietic cell line by donor-type elements had occurred within 100 days posttreatment. Although definitive markers were lacking for the platelets and granulocytes, their increase occurred much too early for spontaneous recovery from radiation injury of 500 R. Further, the aberrant cell lines diagnostic for the leukemic cell population disappeared after the third day posttransplant (Table 24). Possibly these normal granulocytes were of host, not donor, origin, but characteristic radiation-induced lesions in the metaphases of the cells were not found beyond the fourth day after irradiation. In contrast, he had no evidence for transplant of donor-type lymphocytes; indeed the low lymphocyte levels and the absence of donor-type immunoglobulins in the serum throughout the period of study indicate that this did not occur. The availability of only one marker to denote lymphocyte grafting, i.e., immunoglobulin allotypes, may limit this interpretation since there is evidence that engraftment of either thymus-derived (T)- or bone-marrow-derived (B)-type lymphocytes can occur independent of each other. Several factors may have prevented a transplant of B (and also possibly T)

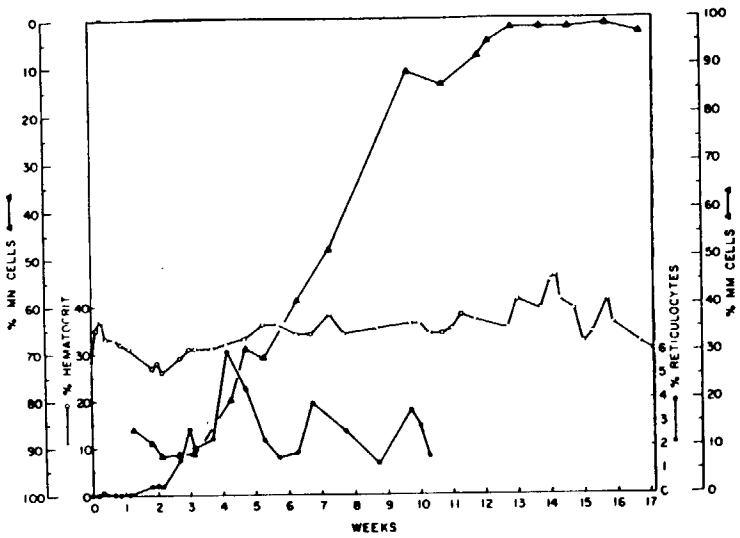


Fig. 23. Erythropoietic parameters in patient after immunosuppression and marrow infusion. Increase in percentage donor-type MM cells (right ordinate ▲——▲) offers definitive evidence for graft "take."

lymphocytes: (1) prednisone given daily for some weeks after marrow infusion; (2) methotrexate given immediately and at frequent intervals after marrow infusion; and (3) a cytotoxic or suppressive effect of the H-AHTGG on the lymphocytes of the donor marrow. The suppressive qualities of both prednisone and methotrexate on hemopoietic systems are well known. Although the patient's serum 24 hr after the last injection of H-AHTGG had no agglutinating or cytotoxic activity, growth inhibitory factors in the serum or fixed to lymphoid tissues are a distinct possibility. The extent to which any of these three, alone or in combination, affected donor lymphocyte repopulation (T or B) can only be speculative at this time and requires further laboratory study. In any event, establishing a complete erythropoietic graft (and probably myelopoietic and thrombocytopoietic grafts) in the presence of persisting host lymphocytes indicates that conditions were not only conducive to immunosuppression but also to inducing tolerance to allogeneic antigens. We need additional studies utilizing similar combined immunosuppressive regimens to determine whether the "split engraftment" observed here will occur consistently. Depending upon the specific hemopoietic disorder to be treated, this may or may not be a desirable outcome.

The patient did extremely well through the first 9 weeks after transplantation. Discharged from the hospital 44 days after the transplant, he resumed many of his recreational and avocational activities although he did not go back

1028039

to work. In the tenth week, he had a fever of undetermined etiology and complained of aching all over, headaches, and dyspnea. There were rales in both lungs and a diffuse infiltrate on the chest X ray. A lumbar puncture showed no abnormality. The urine contained *Pseudomonas aeruginosa* and upon treatment with Carbenicillin and Garamycin, he improved and his fever subsided. A marrow aspirate showed no leukemic cells and the patient went home returning for outpatient visits. At 14 weeks he returned with weakness, severe dyspnea, and dizziness when sitting up. Both lungs had rales and edema was apparent in the lower extremities. At this time, he had phlebitis in the left lower leg. Lung infiltrates were again present on chest X ray. He was hospitalized for close observation and treatment with antibiotics. In the second week of hospitalization his fever subsided, his condition stabilized, and he appeared to be gradually improving. One week later, however, he coughed up blood-tinged sputum, and

TABLE 24
CYTOGENETIC ANALYSES FOR ABNORMAL METAPHASES DIAGNOSTIC FOR THE LEUKEMIC STEM-LINE IN DIRECT MARROW PREPARATIONS FROM PATIENT BEFORE AND AFTER MARROW INFUSION*

Days Relative to Marrow Transplant							
−12	0	+3	+8	+26	+44	+68	+122
21/24†	39/50	4/8	0/9	0/22	0/100	17/100	0/2

*Data from L. Gayle Littlefield.
†Denominator: Total metaphases scored.
Numerator: Abnormal metaphases (chromosomal rearrangement in G group, 2N = 45).

became severely dyspneic with moist rales in both lungs. There was virtually no urine output over the next 2 days. He gradually became somnolent and died 122 days posttransplant.

Postmortem microscopic findings indicated that clinically observed respiratory insufficiency was caused by an atypical interstitial pneumonitis due to infection with *Pneumoncystitis carinii.* A primary difficulty experienced in most clinical marrow transplants has been the susceptibility of the patients to infection with a wide variety of microorganisms. The risks incurred in such studies often relate to the underlying disease of the patient, the immunosuppressive therapy, and the occurrence of GvH reactions following transplant of the donor's lymphoid cells. We feel that confining the patient to the ultraclean environment of the laminar-air-flow (LAF) room during the early weeks was of significant benefit in view of the persisting lymphopenic state and the administration of immunosuppressive drugs during this time. Although a GvH reaction did not complicate our case, continuing the maintenance level of drugs

following discharge from the hospital and the poor lymphocyte reconstitution may have predisposed him to an opportunistic infection with *P. carinii.*

Autopsy Findings after Marrow Transplantation

Postmortem studies (Bill Nelson) failed to disclose evidence of recurrence of acute granulocytic leukemia. The marrow at autopsy appeared relatively normal, with considerable hemopoietic hypoplasia that might be attributed to treatment with methotrexate. A search for histologic features of a GvH reaction revealed none that were diagnostic, although a mild GvH could not be excluded. Focal hepatic necrosis, a mild granulocytic and lymphocytic infiltration of portal areas, and hepatocellular abnormalities were present, but might also be attributed to a viral or toxic hepatitis. The intestinal mucosa was near normal, with many plasma cells, eosinophils, and a few small lymphatic nodules. The lymph nodes and spleen had similar lymphatic nodules with no more lymphocytic depletion than expected after the irradiation and chemotherapy. Sections of skin showed only rare lymphocytes and were even less suggestive of GvH than the biopsies taken at the time of the rash.

The cause of death was complex: Clinical manifestations of respiratory insufficiency were caused by an atypical interstitial pneumonitis that might be explained by infection with the "opportunistic" protozoon *Pneumocystis,* demonstrated morphologically in scattered foci. Other considerations include a viral pneumonitis or a toxic effect of methotrexate. Irradiation with 500 R would not be expected to have this effect on the lungs. Organizing thrombi in pulmonary arteries, presumably embolic from veins of the left leg, undoubtedly contributed to respiratory distress although only one small pulmonary infarct was found.

Despite severe coronary artery atherosclerosis and the history of two serious myocardial infarctions, only slight areas of acute myocardial necrosis were found adjacent to an old left ventricular aneurysm. Hypoxia from pulmonary insufficiency could account for myocardial ischemia and cardiac failure and indirectly contribute to the renal failure and anasarca noted terminally. Histologically the epithelium of convoluted tubules had much degenerative change, with focal necrosis and other foci of regeneration. The abundance of eosinophilic casts suggested hemoglobinuric nephropathy, which was excluded by stains for hemoglobin. Degenerating granulocytes filled a few tubules but no bacteria or fungi could be demonstrated with special stains. Cells in tubular epithelium suggesting cytomegalic inclusion disease were rare and less typical than such cells in the mucosa of the alimentary tract; viral infection does not seem to provide a direct explanation for renal insufficiency, even in conjunction with respiratory and circulatory failure. Gentamycin given for pneumonia may have resulted in toxic injury to the kidney but the histologic findings were nonspecific.

1028040

Matching the Donor and Recipient

The transplant was performed in August 1970. Although we have given only one additional patient a marrow transplant (December 1971), we have continued our search for suitable candidates. In Table 25 are tabulated the patients who were potential marrow recipients. While these patients required a great deal of clinical and nursing care and laboratory studies, only a few can be expected to eventually fulfill the requirements of our marrow transplantation protocol. One of the prime prerequisites is histocompatibility between the recipient-donor pair.

The two methods used for testing of transplant candidates (Helen Vodopick and Elizabeth Rupp) were the in vitro mixed leukocyte culture (MLC) and the HL-A typing. In both tests we use peripheral blood lymphocytes believed to carry histocompatibility determinants.

The in vitro MLC as originally described by Bach and Voynow uses a mixture of two lymphocyte populations obtained from the persons to be matched as well as from unrelated normal volunteers. Various combinations of

TABLE 25
STUDIES ON MARROW TRANSPLANT CANDIDATES

Patient	Sex	Age yr	Diagnosis†	No. tested/ No. siblings	MLC‡	HL-A Match Grade§
1*	M	51	AGL	1/1	–	A
2	M	20	ALL	2/10	–, +	A, C
3	M	17	ALL	1/1	+	C
4	M	34	Hodgkin's disease	1(twin)/7	–	B
5	F	23	AGL	3/7	–, +, +	A, C, D
6	M	16	ALL	2/2	–, +	Not satisfactory
7	M	35	CGL (blastic phase)	1/1	Not satisfactory	Not satisfactory
8*	F	53	AGL	3/5	–, –, +	A, A, C
9	F	50	CGL	1/5	±	C
10	F	43	CGL	1/6	Not satisfactory	Not satisfactory
11	F	16	ALL	3/3	+, +, not done	E, E, C
12	M	30	Aplastic anemia	2/8	Not satisfactory	C, E

*Received marrow transplants.

†Abbreviations: AGL = acute granulocytic leukemia; ALL = acute lymphocytic leukemia; CGL = chronic granulocytic leukemia.

‡These are results of mixed lymphocyte cultures. Commas separate results for separate siblings tested. The order is the same in the next (HL–A) column. Symbols: – = no significant reaction between patient and sibling; + = significant reaction noted; ± = equivocal reaction

§Symbols: A = identical; B = no group mismatch; C = group mismatch; D = 2 mismatched groups; E = 2 allele difference

the cells are used. One population of the mixture is inhibited with a mitotic inhibitor, mitomycin C, and the other is uninhibited. Thus the stimulating and responding capabilities of the lymphocytes can be assayed. The index of compatibility between the two populations of cells was equated with the amount of radioactive-labeled thymidine incorporated by the stimulated cells, little incorporation suggesting compatibility and increased uptake indicating incompatibility. Reciprocal studies between the same lymphocyte populations were also performed. The results of this test done on the candidates for marrow transplantation and their siblings are shown in Table 25. On the basis of this test six patients had a suitable donor. No interpretation was possible in three cases because of the interference of granulocytes.

In the last three patients studied (patients 10–12), a newer micromethod of MLC devised by Bach and co-workers has been used. Since this method requires fewer lymphocytes in the test system, much smaller volumes of blood are required from both patient and volunteers, allowing for the possibility of testing a greater panel of prospective donors.

The other test for selection, the HL-A typing, is performed by Dr. Paul Terasaki, UCLA, California. Of the 12 patients studied, four had a sibling with identical HL-A types, referred to as an "A" match. Granulocyte contamination in the samples of three other patients interfered with the test system.

By the two methods cited, we are able to select the suitable donor-recipient pair for marrow graft and to exclude those without a satisfactory match. In addition to the two patients who have received marrow transplant, two other patients each with a donor who was histocompatible by both testing systems, were found, but developed an intercurrent, fatal infection before the procedure could be completed.

Immunofluorescent and Hemolytic Visualization of a Graft-Versus-Host Reaction

Although the clinical characteristics of GvH disease are well known, we have not identified with certainty the tissue and time course of the initial activity of grafted immunologically competent cells. Previously (ORAU-113, p. 66), we described a technique for demonstrating GvH reactivity in spleens of irradiated mice after injecting allogeneic and xenogeneic spleen or marrow cells. In the procedure, a modification of the hemolysis in agar technique, we placed either recipient red blood cells or thymocytes as target cells on cryostat sections of the test spleen; complement-dependent hemolytic or cytolytic foci could be detected within the first few days after grafting. Maximal focal activity was reached 6 days after xenogenic spleen-cell injection while mice given allogeneic spleen cells reached peak activity 5 days posttransplant. Xenogeneic marrow cells also yielded GvH foci, although the peak activity, reached a day later, was quantitatively less than with xenogeneic spleen cells. Gross lesions characteristic of a GvH reaction were obvious within the first week in mice grafted with xenogeneic spleen cells; also, hemolytic anemia and mortality occurred when

1028041

focal activity was maximal. In contrast, no mortality or anemia was observed after marrow grafting during the same period. The results indicated that foreign, immunologically competent cells react early against the host and that the GvH reaction identified, i.e., splenic foci, occurs either in the presence or absence of clinical evidence of a GvH reaction.

This investigation (John R. Kateley* and N. Gengozian) has been extended by combining the fluorescent antibody technique with the hemolytic focal assay to visualize in situ the cells mediating the GvH lytic reaction. In the procedure, recipient spleens were tested initially for focal activity. Once we saw foci, we outlined the lysed areas with a glass-marking pencil on the reverse side of the microscope slide and then removed the agar-red-cell layer covering the tissue sections by gentle agitation in balanced salt solution. Then we fixed the spleen sections in acetone, staining with fluorescein-conjugated antisera specific for rat IgM. Focal areas were easy to locate microscopically since they had been outlined on the slide. Fluorescent spleen follicles varied in size and shape and sometimes ran together as was often observed when scoring hemolytic foci [Fig. 24 (A,B)]. Identification of rat IgM-producing cells beneath the hemolytic foci suggests that the in vitro reaction was mediated by immune globulin of rat (donor) origin. Furthermore, inhibition of hemolytic and fluorescent reactions occurred when spleen sections were first treated with an unconjugated antirat IgM.

To implicate an antibody as a mediator in the pathogenesis of a GvH reaction and to relate the in vitro assay (hemolytic foci) with in vivo events (hemolytic anemia and mortality), we designed experiments to utilize chimera spleen cells in an in vivo situation. Lethally X-irradiated mice were injected with 50×10^6 chimera spleen cells, i.e., spleen cells from irradiated mice that had been injected with 50×10^6 normal rat spleen cells 6 days earlier. Day 6 was chosen for the transfer of chimera spleen cells since this was the time of peak hemolytic foci activity. The results are shown in Fig. 25. Both anemia and mortality were accelerated in mice injected with chimeric spleen cells compared with mice receiving lethal radiation only and mice irradiated and injected with normal rat spleen cells. In the experimental group, mortality began on day 5 and the red cell count then was 30% less than control groups suggesting that the anemia was immunologically mediated by the chimera spleen cells. Further studies are needed to substantiate this interpretation.

In other experiments, we studied time course of rat mononuclear cell infiltration into mouse lymphatic, intestinal tract, and liver tissues after lethal irradiation and marrow injection using specific immunofluorescent reagents. By day 7, both IgM- and IgG-containing cells were clearly evident in the spleens and mesenteric lymph nodes of grafted mice. We first saw fluorescent cells in the liver and colon of radiation chimeras during the third week after marrow grafting. In the liver cells were intralobular within periportal spaces and around bile ducts; in the colon fluorescent mononuclear cells infiltrated the mucosa and

*Predoctoral Research Fellow, National Institutes of Health.

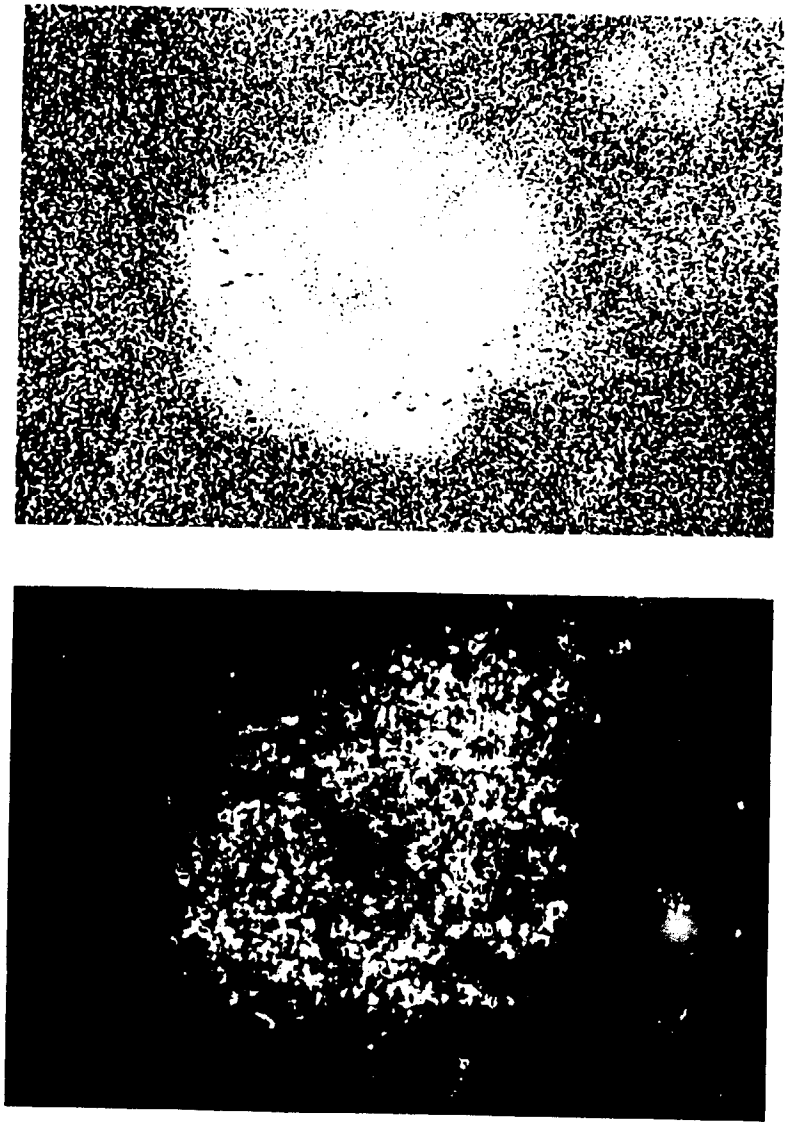


Fig. 24. *Hemolytic and fluorescent focus in spleen of mouse lethally irradiated and injected with rat bone marrow. (A) Hemolytic focus developed by addition of mouse red blood cells and complement on cryostat section of chimera spleen 7 days posttransplant. (B) Same area of spleen section of (A) stained with immunofluorescent reagent specific for rat IgM.*

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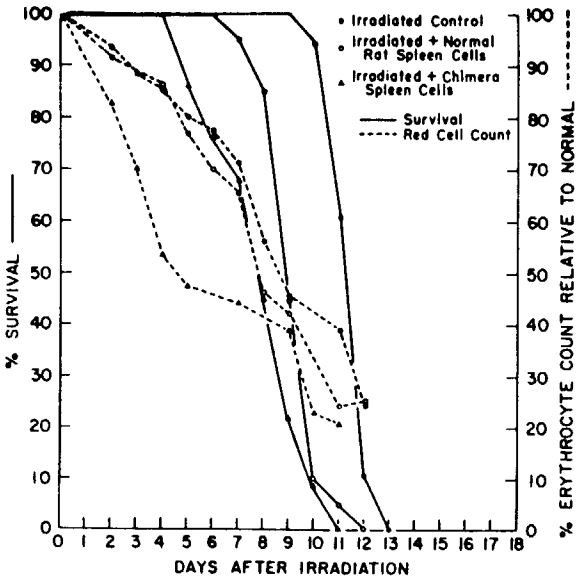


Fig. 25. *Mortality and erythrocyte values of mice lethally (950 R) irradiated and injected with either rat spleen cells (50 x 10^6) or chimera spleen cells (50 x 10^6).*

lamina propria. The majority of fluorescent cells in all tissues from the second until the ninth week postgrafting contained IgG, but relatively few cells stained for IgM. Since donor immunoglobulin-containing cells can be detected in host tissues after grafting and anemia and mortality are accelerated after transfer of chimeric spleen cells into irradiated recipients, we believe a humoral component operates in the pathogenesis of secondary disease.

Influence of Radiation Dose, Quality, and Rate in Xenogeneic (Rat) Marrow Engraftment in Mice

Some of our current studies have been concerned with the importance of dose rates in obtaining therapeutic foreign marrow transplants in mice exposed to ^{60}Co gamma radiation (ORAU-107, p. 260; ORAU-110, p. 63; ORAU-113, p. 70). In brief, we found that the higher the dose rate, the greater the probability of permanent graft. That these results were due primarily to the greater degree of immunosuppression achieved with the higher exposure rates was subsequently documented in extensive studies on humoral antibody formation in mice challenged with heterologous red cell antigens. With both ^{60}Co gamma and 250 kVp X rays, we saw pronounced effects of exposure rates on antibody formation. Although neutron radiation was also found to be highly

immunosuppressive, rate effects were not discernible with this quality of radiation.

The present report (N. Gengozian and John R. Kateley) which extends our knowledge of the importance of dose rate in transplantation, considers the time of marrow administration relative to the time of radiation. In humoral response studies, we found that regardless of dose rate, maximum suppression occurs when the animal is injected with the antigen 24 hr after exposure. Since in our previous work we injected the animals within 2 hr after irradiation, it was important to know whether a simple delay in marrow infusion would obviate the dose-rate effect. Another question was the effect on transplantation of dose rate with X-ray irradiation. All previous work with marrow transplants had involved ^{60}Co gamma radiation which has a lower linear energy transfer (LET) value of (0.3 keV/μ) than 250 kVp X rays (3.0 keV/μ). The dose-rate effect for antibody response was not as pronounced with X rays as it was with ^{60}Co gamma rays. In view of the overwhelming laboratory use of X-ray irradiators to expose small laboratory animals, we felt it important to know what role dose rates played with this quality of radiation in tissue transplantation.

We gave groups of mice either ^{60}Co gamma radiation or X rays ranging from 800 R to 1300 R. For each level and each quality of radiation, two rates were used, 4 R/min and 50 R/min. Rat marrow (50×10^6) cells were injected intravenously either 2 or 24 hr after exposure. The mice were tested at varying intervals for several parameters denoting graft persistence and/or host immune competence. Tests were made for: rat erythrocytes in the blood, rat granulocytes in the spleen and marrow of animals dying within 7 to 20 days, and agglutinating antibodies to rat erythrocytes. To simplify the results, data for only the 800 R and 900 R X-ray groups will be presented. The information from these two groups reveals the importance of delayed injection and also the definite rate effects with X-ray irradiation in tissue transplantation.

When marrow treatment was delayed 24 hr after 800 R at the high rate, donor erythrocytes appeared in only a slightly greater percentage of recipients 10 days posttransplant, 30% to 26% respectively; however at 30 days the graft take and recipient survival differed distinctly (Table 26 and Fig. 26). For animals treated 2 hr after irradiation only 4/12 animals were positive for rat red blood cells (RBC) while 15/19 animals were donor RBC chimeras when marrow was injected at 24 hr. In the latter group, the donor-type RBC constituted between 50% and 100% of the cells while animals treated with marrow immediately after irradiation revealed only 25% to 50% rat RBC. During the second month posttransplant, three of the four chimeras in the 2-hr treatment group reverted to recipient RBC but without additional mortality. In contrast, servere mortality occurred in the 24-hr treatment group; 12/15 animals died during the second month. At 60 days, only 3/27 animals survived irradiation and the immediate infusion of rat marrow. One surviving animal receiving the delayed marrow injection reverted to host-type erythrocyte between 50 and 60 days.

For animals exposed to 800 R at the low rate and treated with rat marrow 24 hr later, 4/22 animals were positive at 30 days, but three of these chimeras

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TABLE 26
TESTS FOR RAT ERYTHROCYTES IN X-IRRADIATED MICE TREATED WITH RAT MARROW

Exposure (R)	Rate (R/min)	Injection time (hr)	No. of animals	Days after treatment					
				10	20	30	40	50	60
800	4.0	2	30	0/29*	0/29	0/29	n.t.†	n.t.	n.t.
	4.0	24	30	1/30	3/25	4/22	0/18	n.t.	n.t.
	50.0	2	24	6/23	7/14	4/12	4/12	3/12	1/12
	50.0	24	27	8/27	17/23	15/19	10/13	3/4	1/3
900	4.0	2	30	0/25	0/15	0/15	n.t.	n.t.	n.t.
	4.0	24	30	4/30	5/18	0/8	0/6	n.t.	n.t.
	50.0	2	30	21/29	27/27	16/16	6/6	3/3	3/3
	50.0	24	30	28/30	27/27	22/22	17/17	9/9	7/7

*Numerator: number of animals positive for rat erythrocytes in blood.
Denominator: number of animals tested.
†n.t. = not tested.

died and the other reverted to host-type RBC during the next 10 days. Animals treated immediately after irradiation never showed evidence of donor red cell repopulation.

At a near lethal exposure of 900 R delivered at 50.0 R/min, more animals were positive for donor erythrocytes on day 10 when marrow treatment was delayed 24 hr, 93% to 72.5% respectively (Table 26). By 20 days, however, all animals in each group were positive for donor erythrocytes (50% rat RBC). Between the third and sixth weeks, many deaths occurred in animals irradiated and treated with marrow 2 hr later. For animals treated with rat marrow 24 hr after irradiation this early mortality was delayed but did occur between the fifth and seventh weeks. At 60 days, 7/30 animals treated with a delayed marrow injection had survived and were complete chimeras while 3/30 animals survived when treated with rat marrow at 2 hr (Table 26). Two of the survivors were complete chimeras, but the other contained only 25% donor-type RBC.

For animals irradiated with 900 R X ray at 4.0 R/min, only those receiving a delayed marrow injection accepted a graft, but at 30 days, all were negative (Table 25). One mouse positive for donor RBC on day 20 died while the other four reverted to host-type RBC before day 30. A high mortality occurred in this group between the second and fourth week; at 30 days only 8/30 animals survived.

Tests for a donor graft in the marrow and spleen of animals dying within 20 days also revealed a dependence on exposure, exposure rate, and time of marrow treatment (Table 27). Higher exposure and exposure rate along with a delayed marrow injection, increased the possibility of detecting rat granulocytes in these

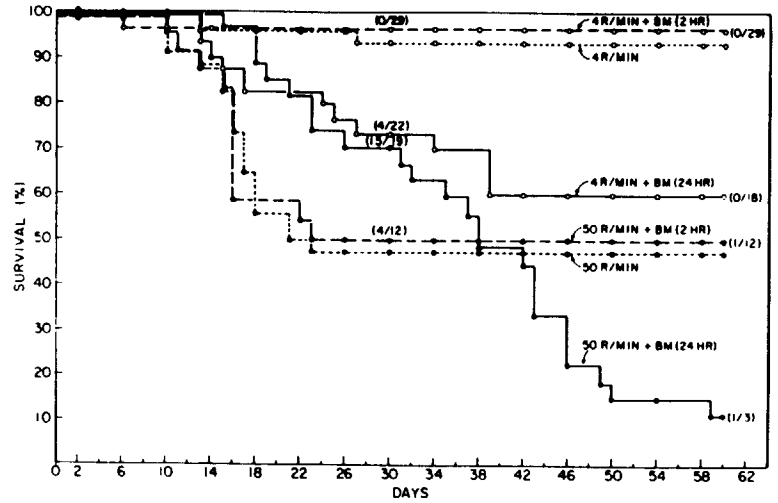


Fig. 26. Survival of mice X irradiated with 800 R at either 4 R/min or 50 R/min and injected with rat marrow 2 hr or 24 hr after exposure. Fractions indicate number of animals showing positive test for rat erythrocytes of total number tested at 30 and 60 days posttreatment.

TABLE 27
TESTS FOR DONOR GRANULOCYTES IN MARROW AND SPLEEN OF IRRADIATED MICE DYING WITHIN 20 DAYS AFTER TREATMENT WITH RAT MARROW

Exposure (R)	Rate (R/min)	Injection time (hr)	Tissue tested	
			Marrow	Spleen
800	4.0	2	---*	---*
	4.0	24	2/5 (40%)†	0/5 (0%)
	50.0	2	5/10 (50%)	1/10 (10%)
	50.0	24	3/4 (75%)	1/4 (25%)
900	4.0	2	0/15 (0%)	0/15 (0%)
	4.0	24	7/12 (58%)	2/12 (17%)
	50.0	2	3/3 (100%)	2/3 (67%)
	50.0	24	3/3 (100%)	3/3 (100%)

*None died.

†Numerator: number of animals whose tissue(s) showed positive tests for rat granulocytes.

Denominator: number of animals tested.

1028044

two hemopoietic tissues. In all groups, animals with donor granulocytes in the spleen also had donor granulocytes in the marrow, but not the converse.

Consequently we have reached the following conclusions; (1) a delayed injection of marrow is more apt to lead to engraftment of foreign marrow than one given shortly after radiation exposure; (2) a high exposure rate is more conducive to a marrow graft than a low-exposure rate, even when the marrow injection is delayed; and (3) rate effects are also apparent with X-ray irradiation in tissue transplantation. Points (1) and (2) have as their basis the degree of immunosuppression achieved under the conditions stated, i.e., immunosuppression is more apparent when challenge with the foreign antigen (graft) is delayed for 24 hr and when the radiation is delivered at a high rate.

Immunosuppression of Mice Exposed to Chronic, Low-Dose-Rate Gamma (^{60}Co) Irradiation

Our studies concerned with humoral antibody formation and marrow transplantation as a function of radiation-exposure rate used exposure times that are generally considered to be acute, i.e., ranging from 4 R/min to a maximum of approximately 72 R/min. The present study (K. F. Hübner, Thomas Gilman, and N. Gengozian), continuation of our dose-rate work, involves more chronic exposures with rates of 1.6 R/hr and 6 R/hr. These rates resemble the conditions currently being used in the ORAU LETBI program for treating patients with varying blood disorders. Therefore, two questions were asked about the immunologic consequences of such exposures to these patients: (1) are total-body doses of 233–2819 R given at 1.6 to 6 R/hr immunosuppressive, and (2) are dose-rate effects detectable under these conditions?

Mice were exposed continuously to gamma rays (^{60}Co) in the LETBI facility at a dose rate of 1.6 R/hr (36 R/day) or 6 R/hr (144 R/day). The total exposure time per day varied from 18 to 24 hr. Six groups for each of the two dose rates were irradiated. The only difference in the treatment was the total-body dose acquired; 233 R, 468 R, 1005 R, 1579 R, 2138 R, and 2819 R.

As soon as they had accumulated the predetermined doses, they were removed from the LETBI facility. Approximately 19 hr after completing the irradiation each animal was injected with sheep red blood cells (SRBC) intravenously. Four days later we killed the animals, collected their serum to determine circulating antibody, and removed the spleens to measure the plaque-forming-cell (PFC) response by the Jerne plaque technique. Control groups were normal, nonirradiated mice injected with SRBC, irradiated but noninjected mice, and normals injected with isogeneic red cells.

Continuous total-body irradiation at 1.6 R/hr definitely suppressed the PFC response at approximately 1000 R (Fig. 27). With increasing dose up to 2138 R there was only a sustained suppression but upon increasing the total dose to approximately 2800 R the suppression was further enhanced. Figure 27 also shows the immunosuppressive effect on spleen cells from chronically irradiated mice receiving different total-body doses at a dose rate of 6 R/hr. In this group

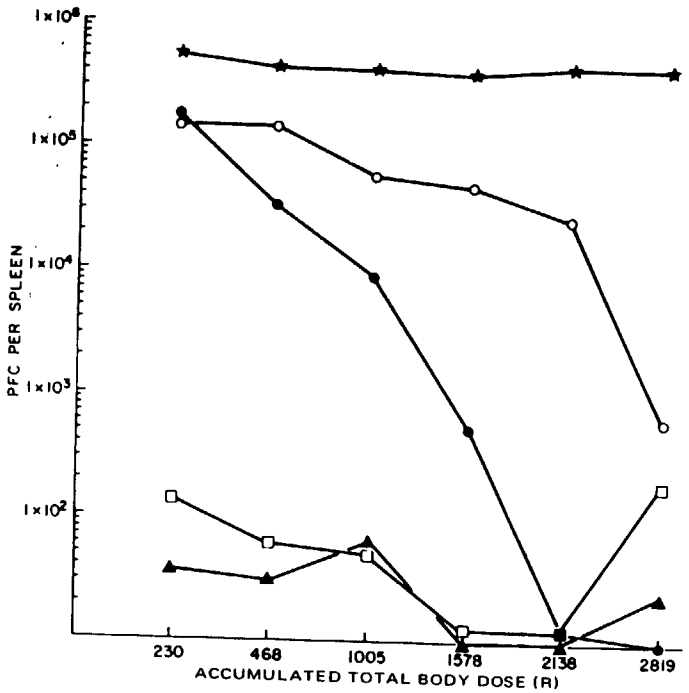


Fig. 27. Effect of irradiation at either 1.6 R/hr or 6 R/hr on the ability of mice to respond to SRBC antigen measured by PFC in the spleen.

**——* normal, unirradiated control*
o——o irradiated at 1.6 R/hr and injected with SRBC
•——• irradiated at 6 R/hr and injected with SRBC
□——□ normal, unirradiated mice injected with isogeneic RBC
▲——▲ irradiated at 1.6 R/min with no antigen injection

the suppression of the PFC is already quite obvious after a total dose of 468 R; the decrease continues in an almost linear fashion with increasing doses. At 2138 R the PFC response is nearly zero. On comparing the two PFC response curves, we conclude that even at such low-dose rates as 1.6 R/hr and 6 R/hr one sees differences which are dependent on dose rate. Definite immunosuppression is seen after a total dose of 1005 R in the 1.6 R/hr group and at 468 R at the 6 R/hr group.

We compared the PFC immune response with the circulating antibodies. The average serum titers against SRBC after different radiation exposures at different dose rates are shown in Fig. 28. With total doses of 230, 468, and 1005 R, we found no immunosuppressive effect and no convincing dose-rate-dependent

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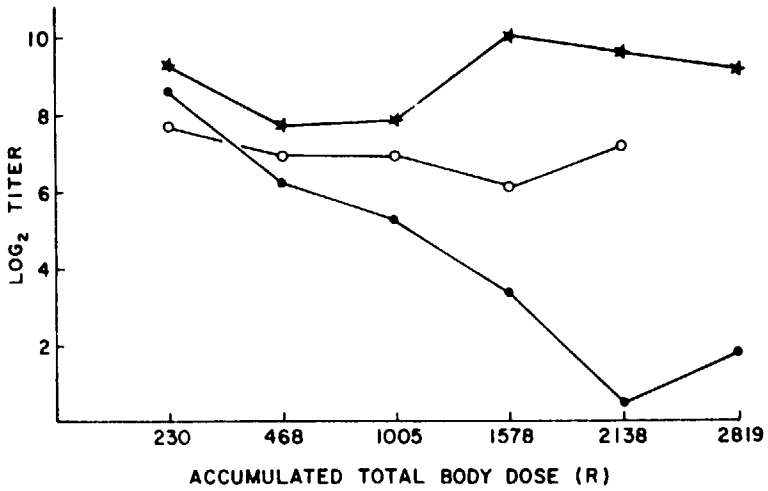


Fig. 28. Effect of irradiation at either 1.6 R/hr or 6 R/hr on the ability of mice to respond to SRBC antigen as measured by circulating humoral antibody titers.

**——* normal mice injected with SRBC*
o——o irradiated at 1.6 R/hr and injected with SRBC
•——• irradiated at 6 R/hr and injected with SRBC

difference between the 1.6 R and 6 R/hr group. At higher doses (1578 R and 2138 R) a dose-rate-related reduction of the amount of circulating antibodies against SRBC is seen.

These data indicate that the PFC technique which measures antibody production at the cellular level is more sensitive for the detection of dose-rate-dependent immunological effects than the detection of circulating antibodies in the serum.

Our results have demonstrated that, even with chronic radiation, dose-rate differences exist in immune response capabilities. Whether such effects would occur in patients receiving similar treatments in the LETBI facility is unknown. The level of radiation at which significant reductions occurred (at least a 468 R accumulated dose) is noteworthy. Treatments of patients in LETBI have most often involved total doses of 100 R or 150 R at rates ranging from 0.4 R to 1 R/hr. Further, based on our study, the best way to assess the human's immunologic system would be to use methods measuring the cellular response rather than serum antibody concentrations.

RESEARCH IN NUCLEAR MEDICINE

The mission of this group is to advance the diagnostic and therapeutic applications of radionuclides. Oak Ridge has special advantages for such research because of the unusual facilities for producing radioactive and stable nuclides, and the available expertise in many fields, especially in instrument development. The study of gallium-67 has continued to occupy a major portion of our resources.

SUMMARY OF ACCOMPLISHMENTS

Further studies of subcellular gallium localization (by means of ultracentrifugation associated with special techniques) have shown the gallium to be in the lysosomal fraction. Evidence has been obtained indicating that it is in the form of a gallium-macromolecular complex. We have been unable to confirm the studies of other workers that a significant share of gallium is localized in nuclei.

Of a series of polyvalent cations given with gallium and studied for their effect on gallium distribution, only scandium and iron had an appreciable effect. Scandium, as has been previously shown, causes an increased removal of gallium from nontumor tissue, giving a greater relative concentration in cancer. An extensive study has been made of the toxicity of stable scandium. The results show that there is apparently a large margin of safety for doses of the magnitude needed to influence the distribution of gallium.

Sex hormones appear to play a significant role in the retention and distribution of gallium. In pregnant mice the breasts have a high gallium concentration and in lactating mice the milk has significant amounts.

The Cooperative Group to Study Localization of Radiopharmaceuticals has worked actively on the gallium study. Progress has been made on standardizing interpretation and reporting of scans. Shipments of gallium supplied by ORNL Isotope Development Department and processed into a pharmaceutical partly through resources at the Medical Division have provided material for a large number of clinical observations.

At the Medical Division a further analysis was made of studies of 140 patients done here before the Division entered the group protocol. Extensive data on normal distribution have been summarized as well as data on the localization in various types of cancer.

Instrument work has included further development and clinical trials of the computer-interfaced four-probe counter and on the development of a mechanism for computer-acquisition of data from the Anger camera. Other significant progress has been made in computer applications to specific problems in nuclear medicine.

1028046

RADIOPHARMACEUTICAL DEVELOPMENT

The Subcellular Binding Sites of Gallium in Normal and Malignant Tissues

Our studies of the nature of gallium-binding granules (GBG) has continued (D. H. Brown, B. L. Byrd, J. E. Carlton, and R. L. Hayes). Previously we had determined that the preferential binding of gallium in tumor cells was associated with a small granule fraction isolated by density gradient zonal centrifugation. These studies indicated that the particles are found in a number of different soft-tissue tumors. From autoradiographic studies it was predicted that the GBG were lysosomal in character (20). Assays of the zonal gradients for acid phosphatase activity (a lysosomal enzyme) supported this contention.

Subsequent zonal centrifugation experiments with poorly differentiated rat tumors (RFT) (*Science* 167, 289, 1970), a mouse lymphosarcoma, and a well differentiated hepatoma (Morris 7794-A) revealed that in each case the GBG sedimented to the same zone in the gradients in the same integral. The optical density profile in Fig. 29 shows the particle distribution for these three different tumors. The gradient profile of the ^{67}Ga activity indicates that the GBG are primarily located in the sixth stage particle zone.

The sedimentation profile of a hepatoma homogenate (Morris 7794-A) in Fig. 30 shows the distribution of two lysosomal enzymes, acid phosphatase, and N-acetyl-β-D-glucosaminidase. In the gradient the highest specific enzyme activities appear in those fractions with the highest radioactivity. We could not detect β-galactosidase, another lysosomal enzyme, in the tumor fractions but found it in the GBG fractions of corresponding liver experiments.

Previously, using normal liver homogenates, we had shown that the zone in which the GBG sediment also contains the majority of the peroxisomes. This was determined by assaying the gradients for catalase activity. Peroxisomes are not the organelles responsible for binding gallium since little or no catalase activity was detected in tumors having very high gallium binding capacities. This conclusion was further supported by the fact that no microbodies having the characteristic peroxisomal morphology were found in electron micrographs of the specific particle zones in question.

Some questions exist in the literature as to the nature of the distribution of gallium in the subcellular millieu. Higasi et al. (*Jap. J. Nucl. Med.* 6, 217, 1969) have reported that 40% of the gallium was bound to the nuclear fraction of Ehrlich cancer tissue homogenates. Ito et al. (*Radiology* 100, 357, 1971) have also reported finding 33% of the ^{67}Ga activity in the nuclear fraction from VX-2 rabbit carcinoma homogenates. The results in each case were obtained by differential centrifugation studies. In our procedure we first centrifuge the homogenate to remove unbroken cells, debris, and nuclei. In the process we lose from 20 to 40% of the radioactive gallium; however, we believe the activity is not associated with the nuclei but trapped in the cell debris, since only 8% of the gallium appears to be associated with the nuclei as determined by electron microscopic autoradiograms (20). In three different tumors zonal centrifugation

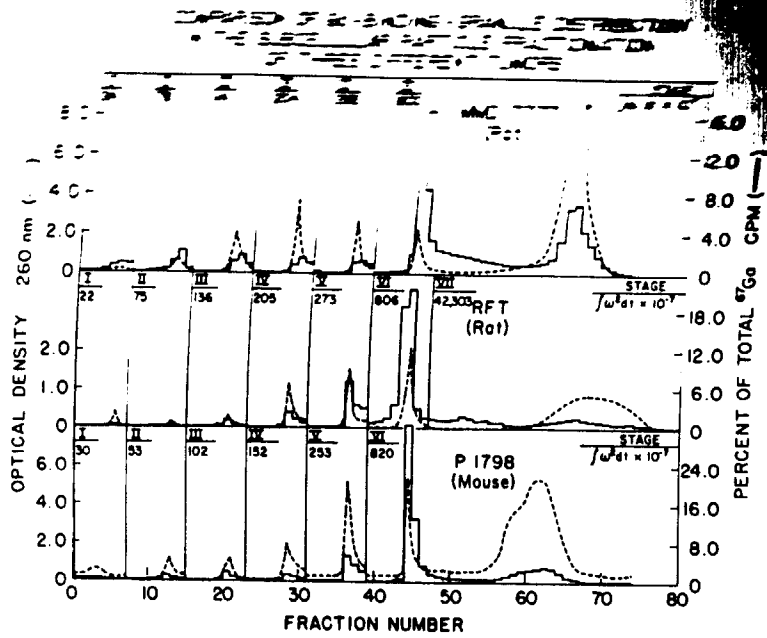


Fig. 29. Comparison of GBG distribution in the subcellular fractionations of three different tumors. The particles were separated by a discontinuous rate-zonal centrifugation procedure in an edge-unloading zonal rotor (B-XXIX). The first five particle zones (stages) for the MMD 7777 and P-1798 tumors and the first six stages of the RFT tumor fractionation were obtained by removing the cushion (45% sucrose outboard of the sucrose density gradient) and the accompanying particles at the edge of the rotor. The centrifugal integrals ($\omega^2 t$) are noted at the time of each stage in the figures. After each retrieval, a new cushion was introduced into the rotor. At the final stage, the rotor was unloaded at the edge by introducing water at the center of the rotor. (The direction of sedimentation is from right to left.) The light scattering profile (260 nm) of the rotor effluent is shown as a dotted line. The histograms (solid lines) show the distribution of the ^{67}Ga. The peaks at the right of the figures represent those components that remain in the starting zone. To determine if any residual particulate-bound ^{67}Ga would leave the starting zone a seventh step in the procedure was performed in the RFT tumor fractionation. After removal of the sixth stage GBG in the RFT tumor study, the rotor was accelerated to 25,000 rpm and run overnight. Very few counts were deposited on the cushion indicating that the majority of the GBG were bonded in the sixth stage zone after $\sim 800 \times 10^7 \omega^2 t$.

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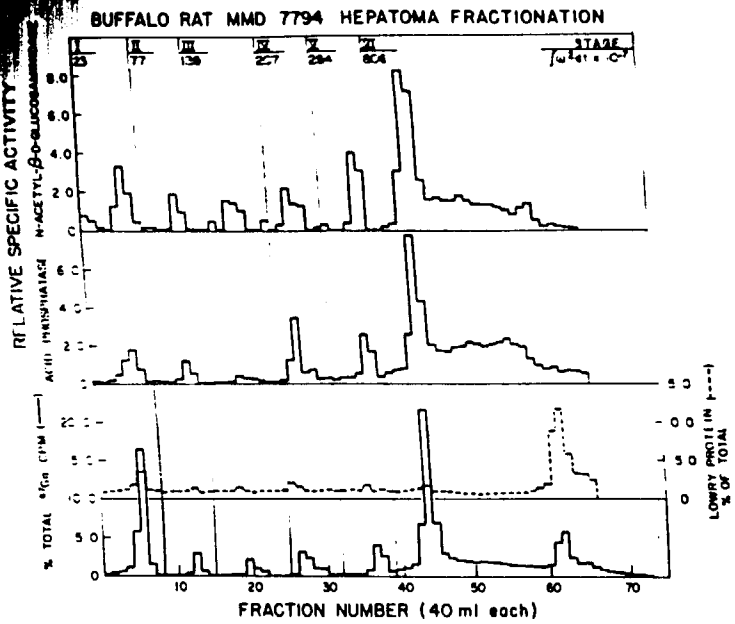


Fig. 30. Rate-zonal particle profile of a rat hepatoma. The relative specific activities (% of total enzyme per fraction divided by % of total protein per fraction) of the lysosomal enzymes, N-acetyl-β-D-glucose-minidase and acid phosphatase, were determined. Protein was determined by an automated Lowry procedure. Gallium-67 radioactivity was measured for each fraction in a well-type gamma counter. The details of the zonal centrifugation procedure are described in the legend to Fig. 29.

experiments designed specifically to separate nuclei showed only 6 to 11% of the radioactivity sedimenting in zones that contained the nuclei and cell debris. Figure 31 shows one such experiment.

Since these experiments did not resolve the nuclei from the cell debris, we still cannot conclusively state that the nuclei are free of gallium. The radioactivity of course may have been associated only with the intact cells. Studies are in progress to further purify the nuclei with essentially complete recovery of the radioactivity.

To characterize the GBG further, we began a study to isolate lysosomes by the method of Leighton et al. (*J. Cell Biol.* 37, 482, 1968). Their procedure intrinsically depends upon modifying the lysosomes in the animals from which they are isolated. The detergent Triton WR 1339 injected in the animals is taken up selectively by lysosomes causing them to become less dense than the

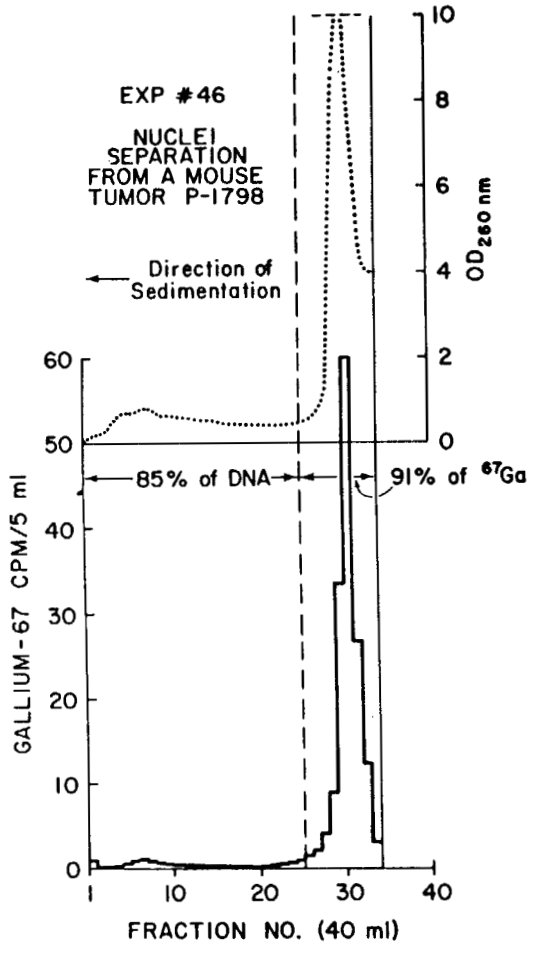


Fig. 31. Isolation of nuclei from a homogenate of P-1798 mouse lymphosarcoma. Nuclei were isolated in a 1-liter 12 to 60% sucrose gradient using the reorienting Sorval SZ-14 zonal rotor. The gradient was loaded while the rotor was at rest; subsequent to reorientation, a 20-ml sample of the tumor homogenate was introduced into the rotor while operating at 1000 rpm. The rotor was then accelerated to 1500 rpm for 10 min and then reoriented for unloading. The gradient was recovered by pumping the contents from the bottom of the rotor through a flow cell of a Gilford spectrophotometer to obtain the particle distribution trace shown by the dotted line at the top of the figure. Deoxyribonucleic acid (DNA) was determined by an automated procedure adapted from the method of Burton (Biochem. J. 62, 315, 1956). The majority of the nuclei as determined by microscopy and DNA analysis were in fractions 1–20. Only 9% of the ^{67}Ga radioactivity was found in these nuclear fractions.

1028048

mitochondria so that they can be easily separated by isopycnic-zonal centrifugation. Tumors from animals injected with this detergent and ^{67}Ga were fractionated by means of isopycnic-zonal centrifugation and the sedimentation profiles examined for radioactivity and acid phosphatase activity. The results are shown in Fig. 32. Clearly the greatest relative specific activity for the lysosomal enzyme is in those fractions comprising peak #3. The greatest relative specific activity of ^{67}Ga also is found in that peak. The density of these particles was 1.13, precisely that obtained by Leighton et al. for their "light" lysosomes. However, a large portion of the radioactivity is in the mitochondrial peak (#2) and activity in peak #4, the soluble or starting zone, is greater than we normally find in our differential rate-zonal centrifugation procedure. Leighton et al. indicated that frequently in their procedure the mitochrondrial zone was contaminated to a small extent with lysosomes that had not taken up the detergent. Since peak #2 (Fig. 32) contains acid phosphatase as well as ^{67}Ga activity, we suspect that the radioactivity in that zone is associated with lysosomes. The high level of ^{67}Ga in the starting zone (peak #4) probably was a result of greater breakage of the lysosomes in the homogenization procedure. Leighton et al. noted that lysosomes containing the detergent were more labile than untreated ones. These additional data further suggest to us that GBG are lysosomes.

Lysosomal enzymes have a structure-linked latency, i.e., acid hydrolase activities are increased in tissue homogenates by disrupting membranes. We have found the ^{67}Ga content of different experimental tumor tissues (1 to 3 days after intravenous administration of ^{67}Ga to the host animal) to be released into the supernatant fraction after centrifugation for 6×10^6 g-min following similar treatments (Table 28). The ^{67}Ga in these supernatant fractions has also been shown by dialysis and ultrafiltration to be in a bound macromolecular form. We have been exploring such preparations for the nature of this gallium-macromolecular complex, since we believe that this material derives mainly from

TABLE 28
PERCENT ^{67}GA IN ULTRACENTRIFUGE SOLUBLE FRACTION AFTER VARIOUS HOMOGENATE TREATMENTS

Treatment	Tumor Type				
	RFT Tumor	SAD_2 Fibrosarcoma	P-1798 Lymphosarcoma	7777 Hepatoma	5123C Hepatoma
0.25M Sucrose	11	–	16	21	5
0.25M Sucrose 0.2% Triton X-100 0.25M Sucrose	84	–	78	84	83
5% butyl alcohol	76	–	67	73	77
Distilled water	70	66	58	68	56
Distilled water 0.2% Triton X-100	81	77	83	84	88

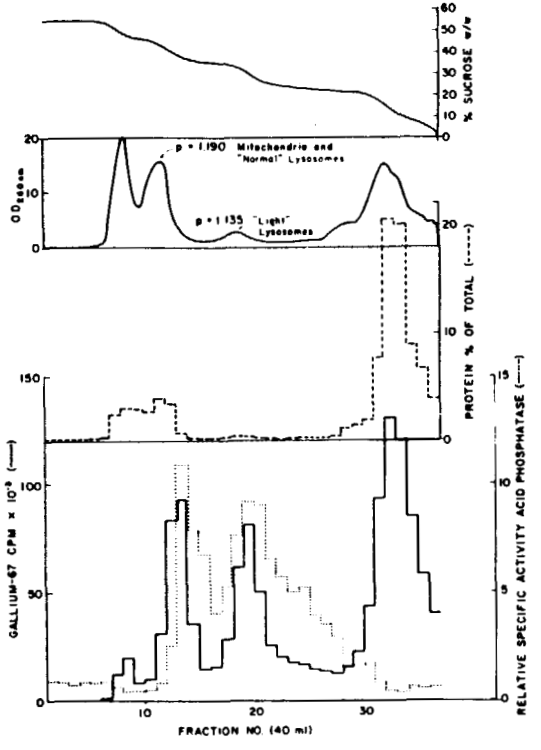


Fig. 32. Isolation of "light" lysosomes by isopycnic-zonal centrifugation. The method used was a modification of Beaufay's "B" procedure described in Lysosomes in Biology and Pathology 2, 534, 1969. The tumors from animals injected with Triton WR-1339 2 days before excision were disrupted in a Potter-Elvejhem homogenizer and centrifuged for 10,000 g-min in a Sorval GLC-1 centrifuge to remove debris and nuclei. A 100-ml sample of the supernatant was layered over a 500-ml 20 to 25% sucrose gradient. Outboard of the gradient there were three steps consisting of 300 ml of 35%, 200 ml of 45%, and 227 ml of 55% sucrose. The centrifuge was run at 25,000 rpm for a total of 4.3 x 10^6 g-min or 3750 x 10^7 $\omega^2 dt$. The upper trace in the figure shows the concentration of sucrose in the rotor as determined from refractive index measurements of the collected samples. Sedimentation of the particles was from right to left. The optical density trace (260 nm) shows three major particle peaks on the left and a slow moving zone of particles (presumably the endoplasmic reticulum vesicles) just to the left of the peak of those subcellular components that had not left the starting zone. The protein profile was determined by an automated Lowry protein procedure. The relative specific activity of acid phosphatase is shown by the dotted histogram at the bottom of the figure (refer to Fig. 30 legend for details). The profile shows two major regions in the gradient representing two classes of lysosomes. The total ^{67}Ga radioactivity of each fraction is shown as a solid line histogram.

1028049

the GBG identified in the zonal ultracentrifugal studies already cited (R. L. Hayes and J. E. Carlton).

The ^{67}Ga activity in these supernatants is precipitated by the protein precipitants ammonium sulfate and Rivanol (6,9-diamino-2-ethoxyacridine lactate); however, attempts with proteolytic enzymes to render the ^{67}Ga dialyzable and ultrafilterable have not given clear cut results. The gallium-macromolecular complex is sensitive to both heat and pH. Table 29 shows one experimental test of the heat sensitivity of the complex as judged by dialysis. Figure 33 shows the effect of pH on the ultrafilterability of the ^{67}Ga activity in two different tumor extracts. When aqueous extracts of various tumor tissues are subjected to gel filtration in the cold (5°C) on superfine Sephadex G-200 we get somewhat similar elution patterns (Fig. 34). A small amount of the ^{67}Ga activity in the extract appears in the void volume; however, the major portion is associated with two peaks at elution volumes corresponding to globular molecular weights of approximately 100,000 and 50,000. These two peaks are greatly reduced (60°C) or completely abolished (95°C) by heating the extract before gel filtration. Although these results do not furnish any definitive answers as to the nature of the gallium-macromolecular complex, obviously it is composed of more or less discrete labile fractions.

TABLE 29
EFFECT OF TEMPERATURE ON THE GALLIUM-MACROMOLECULAR COMPLEX EXTRACTED FROM RFT TUMOR

Treatment	^{67}Ga Dialyzable (24 hr, 5°C)
5°C for 3 hr	5
~25°C for 3 hr	15
60°C for ¼ hr +	28
5°C for 2¾ hr	–
60°C for 1 hr +	41
5°C for 2 hr	–

Factors Influencing the Tissue Distribution of Gallium

The tissue distribution of gallium in experimental animals is affected by a number of factors. Among those previously identified are dose level, anionic form, age, and sex. We have continued these studies as part of the overall effort to determine the mechanism involved in the localization of gallium in soft-tissue tumors (R. L. Hayes, B. L. Byrd, and J. E. Carlton).

When gallium in microgram quantities is administered intravenously to animals it is initially bound by plasma proteins. Present evidence indicates that the rate of excretion and tissue deposition are then controlled to a great extent by the rate at which this bound form dissociates. If milligram amounts of gallium are administered, the plasma binding sites are saturated and the excretion rate is greatly increased, resulting in a pronounced decrease in the

percent deposition of gallium in both normal and malignant soft tissues. Aluminum and indium, neighboring elements in the IIIB periodic group, produce a similar effect on the deposition of gallium in normal soft tissues, again with a corresponding decrease in the concentration of gallium in tumor tissue (Table 30).

The effect of a number of polyvalent cations (with ionic radii similar to that of gallium) on the tissue distribution of gallium has been studied (CoII, CrIII, CuII, FeIII, NiII, ScIII, and ZnII). All were without appreciable effect except for scandium and iron. Some enhancement of the relative concentration of gallium in tumor tissue occurred when iron was administered, but a much more dramatic effect was noted with scandium (Table 31). Table 31 also shows that scandium

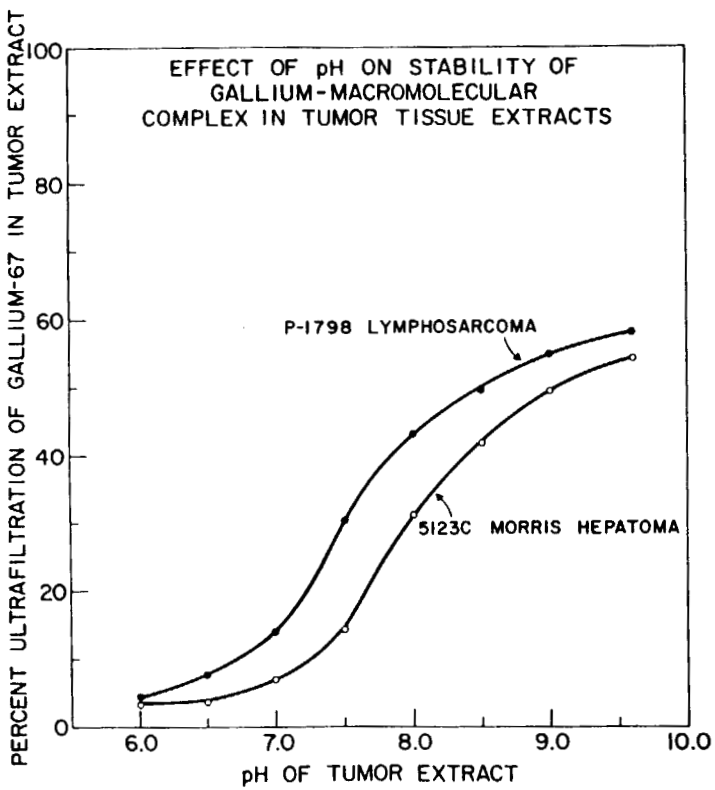


Fig. 33. Effect of pH on stability of ^{67}Ga-macromolecular complex from aqueous tumor extracts of P-1798 mouse lymphosarcoma and Morris 5123C rat hepatoma. Homogenates were spun in the ultracentrifuge for 6 x 10^6 g-min and the supernatant mixed with Michaelis's barbital sodium-acetate buffers; the preparations were then filtered through PM-30 Amicon Diaflo ultrafiltration membranes after standing at 5°C for 24 hr.

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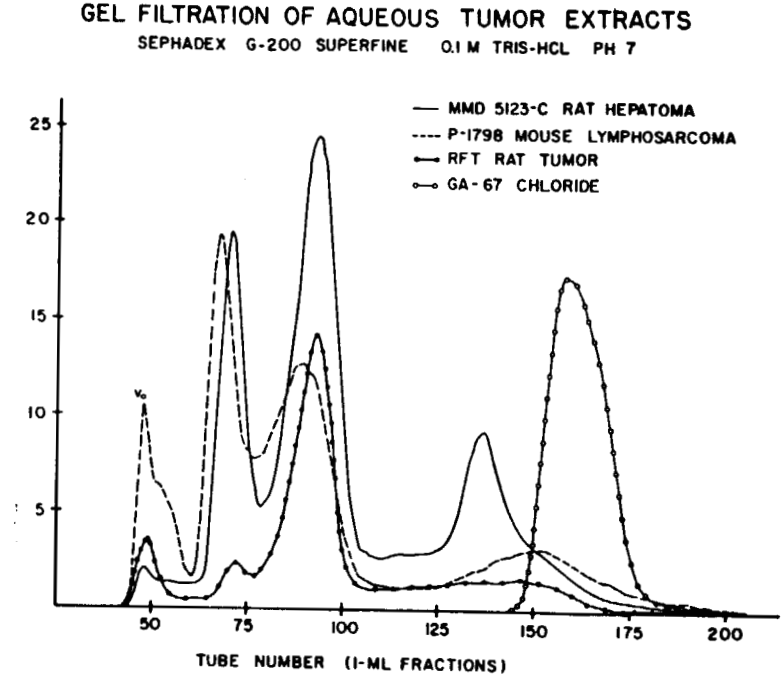


Fig. 34. Gel filtration at 5°C of aqueous extracts of various transplanted tumor tissues. Host animals received ^{67}Ga 3 days before harvest of tumors. Tumors were homogenized in distilled water and the homogenates spun for 6 x 10^6 g-min. Supernatants were concentrated by ultrafiltration through Aminco PM-30 Diaflo membranes (<10% of ^{67}Ga activity was ultrafilterable). The peak of ^{67}Ga Cl_3 elution occurred at the column bed volume.

can be administered up to 2 hr before gallium without loss of this effect. A level of approximately 0.5 mg Sc/kg appears to produce an optimum effect in the rat (Table 32). Tumor tissue does not show an affinity for high specific activity ^{46}Sc nor is the tissue distribution appreciably affected by the presence of stable scandium through a period of 24 hr (Table 33).

With whole-body retention studies we find that gallium excretion is greatly increased during the first few hours after scandium administration. We believe that the effect of scandium on gallium distribution in tissue is produced by its competition for plasma-protein binding sites of gallium. Gallium is forced into an ionic state or into a protein-gallium complex with a much weaker stability constant. In this form the kidneys are able to excrete gallium much more rapidly. Tumor tissue is capable of competing with the kidney excretory process whereas the normal soft tissues are not, so that the concentration of gallium in

TABLE 30
EFFECT OF ALUMINUM AND INDIUM ON THE 24-HOUR TISSUE DISTRIBUTION OF GALLIUM-67 IN THE RFT TUMOR RAT

Tissue	^{67}Ga	^{67}Ga + 5 mg Al/kg	^{67}Ga + 1 mg In/kg
	Percent administered dose per gram*		
Liver	1.07	0.37	0.29
Spleen	1.75	0.45	0.65
Lung	0.23	0.08	0.08
Muscle	0.21	0.04	0.01
Blood	0.15	0.05	0.08
Tumor	2.87	1.20	1.03

*Normalized to body weight of 250 g.

TABLE 31
EFFECT OF TIME OF SCANDIUM (0.5 mg/kg) ADMINISTRATION ON 4-HOUR TISSUE DISTRIBUTION OF ^{67}GA IN THE MORRIS 7777 HEPATOMA RAT*

	No Sc with ^{67}Ga	Sc with ^{67}Ga	Sc 2 hr before ^{67}Ga	Sc 4 hr before ^{67}Ga
Tumor conc. (%/g)†	3.03 ± 0.11	5.76 ± 0.31	6.04 ± 0.35	6.72 ± 0.35
Ratio tumor conc. to:				
Liver	4.3 ± 0.2	38.6 ± 0.6	35.6 ± 4.1	17.5 ± 1.9
Spleen	3.5 ± 0.3	47.4 ± 3.6	34.9 ± 4.1	18.5 ± 1.6
Lung	4.3 ± 0.4	29.6 ± 2.3	16.0 ± 1.9	11.8 ± 3.1
Muscle	13.6 ± 1.0	199 ± 33	188 ± 27	94 ± 15
Blood	2.8 ± 0.2	21.6 ± 0.8	14.7 ± 1.7	10.8 ± 3.5
Marrow	2.7 ± 0.2	39.0 ± 9.5	26.5 ± 3.7	13.8 ± 1.7
Femur	3.2 ± 0.1	3.7 ± 0.2	4.5 ± 0.1	4.5 ± 0.2
Kidney	5.1 ± 0.3	5.4 ± 0.4	7.7 ± 0.2	8.0 ± 0.9

*Four animals per group.
†Percent administered dose/g normalized to body weight of 250 g.

tumor tissues remains approximately the same, while that in the normal tissues drops thus producing a marked enhancement in the relative concentration of gallium in tumor tissue. This suggests that either the mechanism by which gallium concentrates in tumor tissue is different from that in normal tissue or that tumor cells are "hyperpermeable" to gallium.

We previously reported that the tissue distribution of gallium differs significantly in the male and female rat, particularly in the spleen, muscle, and

1028051

TABLE 32
EFFECT OF SCANDIUM DOSE LEVEL ON 4-HOUR TISSUE DISTRIBUTION OF GALLIUM-67 IN RATS BEARING RFT TUMORS*

Scandium dose (mg/kg)	0.10	0.25	0.50	0.75	1.00
Tumor conc. (%/g)†	1.1 ± 0.13	1.4 ± 0.3	1.6 ± 0.1	1.6 ± 0.2	0.9 ± 0.1
Ratio tumor conc. to:					
Liver	2.6 ± 0.3	6.5 ± 0.6	10.6 ± 0.3	9.5 ± 1.4	5.1 ± 0.6
Spleen	2.0 ± 0.3	5.9 ± 0.6	14.4 ± 0.7	11.9 ± 1.7	5.9 ± 0.6
Lung	2.0 ± 0.2	5.1 ± 0.6	11.4 ± 0.8	12.3 ± 1.7	6.1 ± 0.6
Muscle	6.0 ± 0.8	21.7 ± 3.9	74.4 ± 4.6	69.4 ± 8.1	36.4 ± 8.0
Blood	1.3 ± 0.1	2.9 ± 0.3	8.7 ± 0.5	9.4 ± 1.1	5.3 ± 0.6
Marrow	2.4 ± 0.2	6.8 ± 0.8	17.6 ± 1.4	13.9 ± 1.5	8.2 ± 1.0
Femur	0.9 ± 0.1	1.1 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	0.5 ± 0.1
Kidney	2.1 ± 0.1	2.3 ± 0.5	2.3 ± 0.1	1.9 ± 0.4	1.3 ± 0.1

*Four animals per group; 3:1 molar ratio of citrate to scandium.
†Percent administered dose/g normalized to body weight at 250 g.

TABLE 33
EFFECT OF TIME AND ADDED STABLE SCANDIUM ON THE TISSUE DISTRIBUTION OF SCANDIUM-46 IN RATS BEARING RFT TUMORS

	Time after administration (hr)			
	4	4	24	24
Added Sc (mg/kg)	0	0.5	0	0.5
	Percent administered dose/g*			
Liver	1.21 ± 0.03	1.17 ± 0.04	1.73 ± 0.12	2.59 ± 0.08
Spleen	1.54 ± 0.06	1.13 ± 0.03	2.22 ± 0.24	2.20 ± 0.13
Lung	1.32 ± 0.04	1.13 ± 0.03	0.45 ± 0.03	0.51 ± 0.01
Muscle	0.19 ± 0.01	0.22 ± 0.01	0.17 ± 0.03	0.20 ± 0.01
Blood	2.03 ± 0.06	2.00 ± 0.07	0.31 ± 0.02	0.28 ± 0.01
Marrow	1.83 ± 0.06	1.13 ± 0.06	2.18 ± 0.13	1.63 ± 0.20
Femur	0.57 ± 0.02	0.57 ± 0.01	0.84 ± 0.06	0.96 ± 0.03
Kidney	1.21 ± 0.04	1.06 ± 0.02	1.30 ± 0.09	1.24 ± 0.03
Tumor	0.21 ± 0.02	0.21 ± 0.02	0.35 ± 0.03	0.26 ± 0.02

*Four animals per group, normalized to body weight of 250 g.

bone marrow. We have also investigated the effect of castration and hormone administration on this difference. Table 34 shows that castration of the male rat significantly lowers the concentration of gallium in the spleen and muscle but not in the bone marrow. The gallium in the spleen and muscle of the castrated male (Group 3) was, however, still significantly higher than that in the female (Group 1). Doses of testosterone in most instances increased the gallium concentration in spleen and bone marrow.

In keeping with observations on differences in tissue concentration, by measuring whole-body retention we find gallium is excreted more rapidly by the

TABLE 34
EFFECT OF CASTRATION AND TESTOSTERONE ADMINISTRATION ON GALLIUM-67 (24 HR) DISTRIBUTION IN THE FISCHER-344 RAT

Group*	Spleen	Muscle	Bone Marrow
	Percent administered dose per gram		
1. Female	1.35	0.081	0.71
2. Male	2.13	0.286	1.22
3. Male-C	1.76	0.202	1.09
4. Male-CT	2.64	0.203	1.56
5. Female-T	1.49	0.108	0.95
6. Male-T	2.63	0.262	1.51
	Significance of Differences (P)		
1 vs 2	<0.001	<0.001	<0.001
2 vs 3	<0.02 >0.01	<0.001	–
3 vs 4	<0.001	–	<0.01 >0.001
1 vs 5	–	<0.01 >0.001	<0.05 >0.02
2 vs 6	<0.05 >0.02	–	<0.1 >0.05
1 vs 3	<0.01 >0.001	<0.001	<0.01 >0.001

*C = castrated; T = 10 mg testosterone enanthate/kg 7 days before ^{67}Ga administration; 5 animals to a group.

female rat. Whole-body retention of the female at 1 day was 68.8 ± 0.6% while that of the male was significantly higher at 76.1 ± 0.4%. At 12 days the values were 31.1 ± 0.5% and 41.6 ± 0.3%, respectively. Analysis of the retention data (Evelyn Watson and R. Cloutier) indicates that three compartments appear to be involved (Table 35). The female has a higher percentage of the administered dose associated with the two short half-time compartments.

Following Larson and Schall's report (*J. Amer. Med. Assoc.* 218, 257, 1971) of a high concentration of ^{67}Ga in the breasts and milk of a woman who received a ^{67}Ga scan 3 weeks postpartum we studied the tissue distribution of gallium in pregnant hamsters and of its transfer to the offspring through the

1028052

TABLE 35
GALLIUM-67 RETENTION IN THE FISCHER-344 RAT

Compartment	Males		Females	
	Percent in compartment	Biological half-time (hr)	Percent in compartment	Biological half-time (hr)
1	6	2.0	12	3.5
2	35	25.2	41	26.3
3	59 ± 0.4	575 ± 42	47 ± 0.2	469 ± 16

TABLE 36
TISSUE DISTRIBUTION OF GALLIUM-67 (24 HR) IN THE PREGNANT HAMSTER

Tissue	Day of pregnancy		
	0	10	14
	% Administered dose/g		
Liver	1.31 ± 0.08	2.01 ± 0.26	1.15 ± 0.14
Spleen	1.02 ± 0.07	0.97 ± 0.10	1.07 ± 0.15
Kidney	2.07 ± 0.10	1.50 ± 0.11	1.54 ± 0.18
Lung	0.53 ± 0.04	0.98 ± 0.23	0.20 ± 0.02
Muscle	0.10 ± 0.01	0.05 ± 0.01	0.02 ± 0.01
Femur	2.07 ± 0.10	1.85 ± 0.13	1.37 ± 0.08
Marrow	1.89 ± 0.12	1.70 ± 0.18	1.46 ± 0.28
Blood	0.47 ± 0.07	0.05 ± 0.01	0.05 ± 0.01
Mammary gland	0.29 ± 0.04	2.64 ± 0.46	6.69 ± 0.72
Fetus	–	2.60 ± 0.28	1.23 ± 0.13

mother's milk (B. L. Byrd, E. L. Candler,* and R. L. Hayes). Table 36 shows that gallium was readily transferrred to the fetus and that the mammary glands, shortly before term (15 days), became the organ of highest concentration. We interpret the drop in concentration in the fetus to be a dilution effect produced by the increasing size of the fetuses coupled with a competition by the mammary glands for the activity administered. The amount of ^{67}Ga associated with the mammary glands is more graphically shown in Fig. 35. The maternal whole-body retention and transfer of ^{67}Ga from mother to infants following gallium administration to the mothers 1 day postpartum is shown in Fig. 36. In view of our finding that gallium localizes in lysosomes, it is noteworthy that lysosomal enzymes in the mammary gland have been reported to be increased during pregnancy, lactation, and involution.

*Molecular Anatomy Program, Oak Ridge National Laboratory.

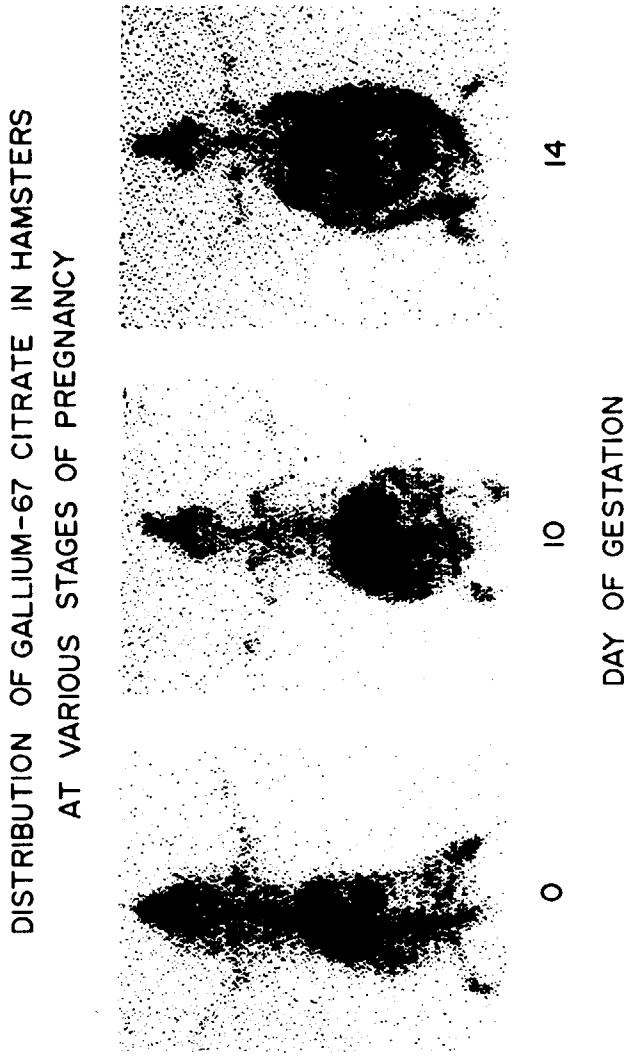


Fig. 35. Gallium-67 scans of hamsters during different stages of pregnancy. All three animals were scanned with similar instrument settings 24 hr after administration of equal doses of ^{67}Ga.

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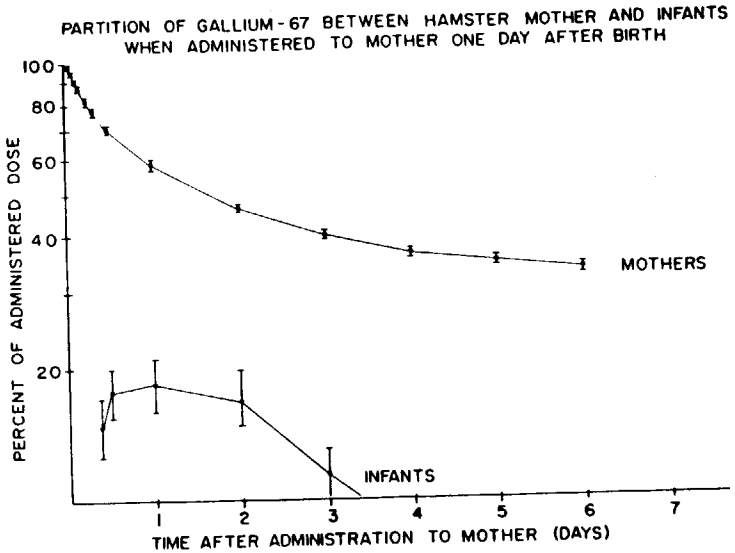


Fig. 36. Whole-body retention of ^{67}Ga administered to female hamsters 1 day postpartum. Offspring were counted at same times as mothers.

Toxicity of Scandium

In animal studies we have found that scandium administered in conjunction with ^{67}Ga may offer considerable benefit in tumor scanning, since it enhances the relative concentration of ^{67}Ga in tumor tissue (Table 31). We have now completed a study of the toxicity of scandium in animals (R. L. Hayes, J. J. Rafter, B. Nelson, C. L. Edwards, and F. A. Goswitz) and plan to test its use in humans.

We have studied the acute intravenous toxicity of scandium citrate in the female New Zealand white rabbit at six different dose levels and in the dog at one level. (Scandium was administered in a citrate-to-scandium molar ratio of 1.5:1 with calcium chloride added to counteract the hypocalcemic effects of the excess citrate.) Based on results of the rabbit study (Table 37) we estimate the 7-day LD_{50} in the rabbit to be 33 mg Sc/kg. In 10 mongrel dogs (6 males, 4 females) given 25 mg Sc/kg, no deaths occurred through 30 days indicating that the $LD_{50/30}$ in the dog exceeds 25 mg Sc/kg.

Most of the deaths in the rabbits occurred within 1 day; none were observed after 3 days. Autopsies and histopathological examinations were performed (10 of 20 deaths) where autolysis had not rendered tissues unsuitable for study. A survivor from each of the 10, 20, 30, and 45 mg Sc/kg groups was also killed and studied histologically.

In seven animals dying within 3 hr after 20 to 45 mg Sc/kg, pulmonary congestion, usually moderate was present and perhaps attributable to left heart failure, findings consistent with the experience reported by Haley et al. in the cat (*J. Pharm. Sci.* 51, 1043, 1962). No emboli or foreign bodies attributable to the injection were seen, and with light microscopy no foreign material was detected in histologic sections in the rabbits dying early. With hemotoxylin and eosin stains, foreign material was first recognized in macrophages in the rabbit found dead 20 hr after injection (30 mg Sc/kg). Macrophages were plainly engorged with foreign material in both rabbits found dead 43 hr after injection (20 mg Sc/kg), and in all four rabbits killed at 7 days. Aside from the bulk of the foreign material in reticuloendothelial cells and the resulting splenomegaly, no lesions could be associated with its presence.

TABLE 37
SEVEN DAY ACUTE TOXICITY OF SCANDIUM CITRATE IN THE FEMALE NEW ZEALAND RABBIT*

Sc dose (mg/kg)	No. survivors	No. deaths	Time of deaths (hr)
45.0	1	7	0, 1, 1, ~10, ~10, 25, ~40
37.5	2	6	0.5, 3, 8, ~40, ~60, ~60
30.0	6	2	1, ~20
25.0	6	2	0, 6
20.0	5	3	2, ~40, ~40
10.0	8	0	

*Molar ratio of $ScCl_3$: Sodium citrate: $CaCl_2$ = 1:1.5:0.5.

In a study of the tolerance of the New Zealand white rabbit to multiple doses of scandium citrate, we gave weekly or twice weekly intravenous doses of 5 mg Sc/kg (10 times the effective dose in the rat, as shown in Table 32) to 8 females and 8 males. The present status of this study is shown in Table 38. One rabbit (#1) died at a cumulative dose of 95 mg Sc/kg, at which time three others (#8, 11, and 5) were killed for histopathological comparison. Administration of scandium was then terminated and the rest of the rabbits were observed during the next 3 yr. Two (#4 and 14) died within 2 months of the termination of dosing and two others (#13 and 15) at approximately 2 yr. All deaths occurred in male rabbits. To date six more animals (#2, 3, 6, 7, 10, and 16) have been killed for morphologic study (2 to 38 months after termination of dosing).

All rabbits in the study with multiple doses became anemic as indicated by lower hematocrits. Some recovery from the anemia occurred after treatment was ceased. Approximately 1½ yr after termination of dosing, we made chemical and hematologic measurements on blood samples from the survivor and a group of controls. Except for somewhat lower red cell values in the treated rabbits, we saw no differences from controls.

One of the elderly females (#6) was found to have a well-differentiated adenocarcinoma of the endometrium, said to be the most common, spontaneous

1028054

malignant neoplasm in the rabbit. Otherwise, no neoplasms have been recognized. Aside from the splenomegaly and other morphologic alterations related to the storage of foreign material in macrophages, no significant morphologic alterations are conclusively attributed to the experimental procedure in autopsies of the rabbits living many months afterwards. A few of the elderly rabbits had slight portal fibrosis in the liver in the vicinity of the greatest concentration of the foreign material, but it is not clear that this cirrhosis resulted from the scandium injections, or whether the macrophages containing the material tended to aggregate in fibrous interstitial tissue. A similar problem is posed by the macrophages found in small fibrous foci in the myocardium. Fibrosis was never seen in the spleen, which had by far the greatest concentration of the foreign material, nor in the marrow, which had large deposits of material in phagocytes especially concentrated about venous sinuses.

The cause of the anemia remains obscure; both daily observations of the rabbits and autopsy with its histologic examination failed to provide evidence of gastrointestinal bleeding. Histologic studies of the marrow from the rabbit (#1) dying shortly after the last dose of scandium showed an absolute decrease in hemopoietic cells. Areas of marrow necrosis might be attributed to a combination of the severe anemia and compromise of blood flow by the many

TABLE 38
STATUS OF LONG-TERM STUDY OF MULTIPLE DOSE (5 mg Sc/kg) INTRAVENOUS ADMINISTRATION OF SCANDIUM CITRATE IN NEW ZEALAND WHITE RABBITS*

No.	Sex	Cumulative Sc/kg dose (mg)	Doses/wk	Time after last dose (months)	Comments
1	M	95	2	0	Died
14	M	90	2	1	Died
4	M	105	1	1½	Died
13	M	90	2	17½	Died
15	M	50	1	23	Killed
11	F	135	2	2 days	Killed
8	F	70	1	2 days	Killed
5	F	65	2	2 days	Killed
2	M	95	2	11½	Killed
10	F	90	1	20½	Killed
16	M	50	1	31½	Killed
3	M	105	1	33	Killed
6	F	95	2	36½	Killed
7	F	135	2	38	Still alive
9	F	90	1	37	Still alive
12	F	90	1	37	

*Molar ratio $ScCl_3$: sodium citrate : $CaCl_2$ = 1:1.5:0.75.

large macrophages containing foreign material. The cause of death was not established as other than the severe anemia. Moderate extramedullary hematopoiesis was seen in this rabbit and another male (#5) killed 2 days after the last dose. The latter also had marrow *hypoplasia* and focal marrow necrosis. Iron stores were abundant in reticuloendothelial cells and in hepatic cells in both aimals. The two females killed 2 days after the last dose (#8 and 11) had *hyperplastic* marrow and much iron.

A male (#14), dying 1 month after the last dose, had pronounced marrow *hyperplasia* with many red-cell precursors, despite the abundance of foreign material in phagocytes. The kidneys had focal calcifications and probably focal necrosis (considerable postmortem autolysis). The cause of death was not shown.

The bulk of the foreign material in phagocytes in samples from the femur did not amount to a third of the volume of marrow, and usually it was considerably less. Even in animals showing the most foreign material and with severe anemia (where a strong stimulus for erythropoiesis would be expected) the marrow retained considerable adiposity. Nevertheless, erythropoietic elements were apparently not greatly reduced. These observations lead us to speculate that scandium in such massive doses interferes with iron metabolism or with the homeostatic control of erythropoiesis. Scandium reportedly inhibits the enzymatic activity of ferroxidase. Although the function of histiocytes as the "nurse cells" for erythropoietic clones is poorly understood, we might postulate that scandium engorging the marrow histiocytes causes the loss of this function.

To define the onset, extent, and rate of recovery from the anemia occurring after multiple large doses of scandium citrate to the rabbit, we expanded our hematologic and biochemical protocol to study the early and late phases of the anemia. Four groups of rabbits (6 animals each) of young males and females matched for age were used. One group of males and one of females were given weekly doses of 5 mg Sc/kg intravenously as the citrate (molar ratio $ScCl_3$: sodium citrate: $CaCl_2$ = 1:1.5:0.5). The other groups received weekly doses of the same amount of sodium citrate and $CaCl_2$ and served as controls. On the day following each dose, small amounts of blood were drawn from the ear vein for hematologic measurements. A larger volume for various tests was taken from each animal before and at the termination of the dosing (40 mg Sc/kg cumulative dose). No significant changes took place in white blood cell and platelet counts. However, the hematocrit, hemoglobin, and red cell counts were greatly decreased. Figure 37 shows these measurements during the course of the treatment and for approximately 10 months thereafter. Lowered hematocrits and hemoglobins became apparent in both males and females at a cumulative dose of approximately 20 mg Sc/kg. Significant lowered red blood cell counts occurred at a cumulative dose of 35 mg Sc/kg. At termination of doses (40 mg Sc/kg) all three values showed a highly significant depression in both males and females. At 10 months both males and females appeared to have recovered, although the values for males might be borderline.

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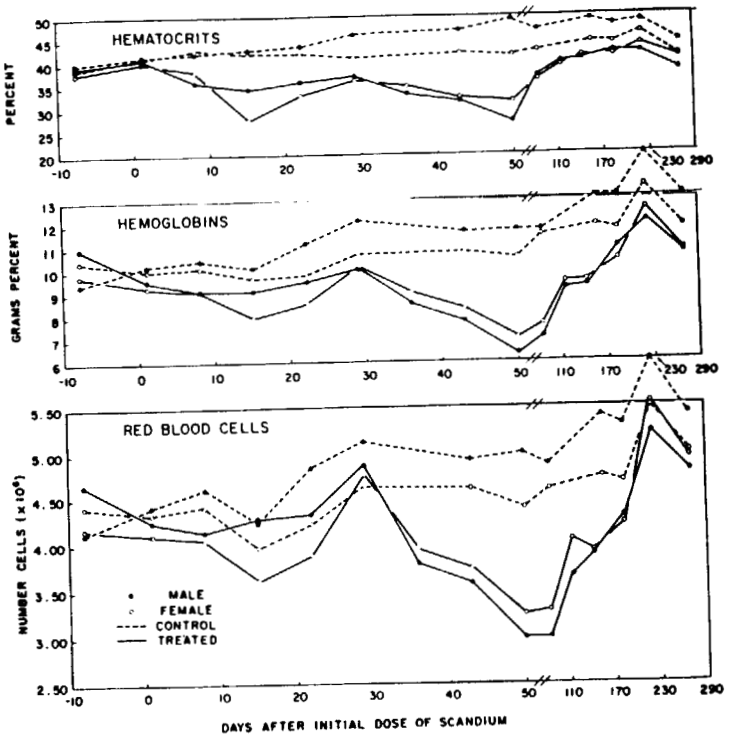


Fig. 37. Effect of prolonged intravenous administration of scandium citrate (5 mg Sc/kg at weekly intervals) on hematocrit, hemoglobin, and red cell counts of male and female New Zealand white rabbits during and after treatment. The total cumulative dose of scandium was 40 mg Sc/kg; the last 5 mg Sc/kg dose was administered on day 49.

No significant differences in blood chemistries between treated and control were observed except for a decreased plasma iron in the female. The plasma half-clearance time for ^{59}Fe was determined in the male groups 1 month after terminating the scandium doses, and although in the treated group it was lengthened approximately 30% over that of the controls, this was not statistically significant. None of the animals in the study have died and we continue to observe them.

Marrow samples aspirated from 5 treated and 6 control males showed a distinct reduction in adiposity 6 days after termination of dosing (cumulative dose of 40 mg Sc/kg). Aside from the accumulation of foreign material in phagocytes an abnormality was conspicuous in only one treated rabbit; this was

a pronounced hyperplasia of erythropoietic and granulocytic elements. In none of the other treated rabbits was marrow hypoplasia evident.

In view of the known anticoagulant effects of rare earth salts including scandium, it was important to measure bleeding time, clotting time, plasma fibrinogen, prothrombin time, and partial thromboplastin time. We gave the animals greater levels of scandium than that proposed for human use, and only transient effects were produced on bleeding time and clotting time with levels as high as 20 mg Sc/kg. We detected no major effects on fibrinogen concentration, prothrombin time, and partial thromboplastin time. Minor elevations of the plasma fibrinogen and partial thromboplastin time at 1 day postadministration at the 20 mg Sc/kg level were recorded but were contrary to any anticipated anticoagulant effect.

In summary, we conclude on the basis of our studies and other reports in the literature that the doses of scandium proposed for phase I and II of human trials (0.5 mg Sc/kg) are safe and we have accordingly filed an Investigational New Drug Application with the Food and Drug Administration.

1028056

CLINICAL INVESTIGATIONS IN NUCLEAR MEDICINE

Cooperative Group to Study Localization of Radiopharmaceuticals

As noted in last year's report, the Cooperative Group to Study Localization of Radiopharmaceuticals was formally constituted in 1970 under the sponsorship of ORAU. Initial support has come jointly from the US AEC and the National Cancer Institute for a protocol designed to answer specific questions about the usefulness of ^{67}Ga in managing lung cancer and lymphoma. In November 1971 a second grant was received, this one from the American Cancer Society, for a broader project, *Gallium-67 as a Tumor-Localizing Radioindicator;* here answers will be sought in the usefulness of gallium in diagnosis and management of a wide spectrum of cancers other than lymphoma and lung cancer.

The members of the Executive Committee are: Chairman, Robert Greenlaw, Marshfield Clinic; Vice Chairman, C. Lowell Edwards, ORAU; Secretary, Ralph M. Kniseley, ORAU. Members at Large: John K. McBain, University of Louisville; David E. Preston, University of Kentucky; Morton B. Weinstein, University of Miami; A. B. Brill, Vanderbilt University. Ex-officio member: Gerald Johnston, National Institutes of Health.

The Cooperative Group embarked on its first protocol in May of 1971. Recognizing a primary problem concerning comparable results from the diverse instrumentation and procedures of the individual members, P. R. Bell and D. Ross, Medical Instrumentation Group, ORNL, designed a plastic phantom which would severely stress the ability of an instrument to discriminate a small source when placed near a large source. Each member in the gallium protocol received a phantom and scanned samples under a standard set of conditions. Additionally as a test of recording equipment, Dr. Bell designed a method for determining uniformity of performance and linearity of response.

The Cooperative Group also recognized a significant problem in uniformity of interpretations both between observers and by the same observer on rereading the scan. Variations also significantly depend on whether the interpreter has the benefit of clinical information, diagnosis, radiologic data, etc. The following classification of scans has been proposed, to be applied separately to each known or suspected lesion: P = positive, definite abnormal focus of activity at a site; N = negative, no abnormal foci of activity; and U = uncertain. The "uncertain" category is subclassified as follows: (1) uncertain whether the degree of concentration is significant; (2) definite increased activity which may be physiologic organ uptake; (3) uncertainty, nonspecific.

The Cooperative Group at its fall work meeting also reviewed the results of an exercise in which independent observers without clinical information interpreted the scans on 49 patients from 4 institutions. The Cooperative Group concluded that (a) scans should be interpreted by individual members, rather than by a central panel. The results will more closely represent the real-life situation that exists for practitioners of scanning even though a bias is

introduced by knowledge of the patient's status; (b) subjective differences will be minimized if the interpretation represents the consensus of two or more observers.

As a part of each future work meeting, random samples of scans will be brought by every member. All participants will score them individually and the results will be analyzed to detect trends. Any member with scorings disparate from the normal will be able to modify his criteria. The objective will be to achieve a common basis for interpretations and minimize individual differences. The problem of scan interpretation is common to all imaging procedures and not confined to tumor localization.

Our preliminary experience with the initial data form demonstrated that it would not work well with the computer. In a second approach we used the Standard Nomenclature of Pathology (SNOP) with modifications needed for our own particular use. The scheme included the four-digit code for region and organ systems, and morphology code for lymphomas and lung cancer. A field test of this code, together with a computer-compatible data encoding form, unroofed further difficulties in the minds of the members. The Cooperative Group recommended that we reorganize the code by anatomic regions and simplify it to fewer named organ sites. The outcome is a new code that identifies in a step-wise fashion (a) regions, (b) subregions, and (c) tissues by system, permitting an observer to use "levels of abstraction" more readily, yet providing for a specific coding possibility. More than 200 scan studies were performed in the first 3 months. With a series of problems during this initial shake-down phase, the usage experience from institutions has been somewhat spotty. A number of noteworthy problems, some completely unanticipated, did emerge and many of these have been surmounted and are not expected to be major hangups in the future.

Because the standardization procedures, field testing of data sheets, and the experience in interpreting scans have developed concurrently, no analysis of data is yet available. However, all participants have been able to demonstrate neoplasms in their subject patients and all have had some false-negatives. The work sessions have underscored the difficulties in interpreting scans with this agent, and have moved the Cooperative Group into a more uniform approach to this problem.

Processing of Gallium-67 for Clinical Studies

The Medical Division processed 24 batches of ^{67}Ga during the last half of 1971. A total of 239 shipments were made to the other 15 cooperating institutions; 2,165 mCi were distributed. The processing is a joint venture of the Medical Division and the Isotopes Development Group at ORNL. The ORNL produces the ^{67}Ga and prepares and packages it in the citrate form. The Medical Division supplies the solutions and packaging materials, tests for pyrogenicity and sterility, and monitors the assay. No reactions of patients to the preparations have been noted by any of the participants in the study.

1028057

Tumor Scanning with Gallium

During 1971 we administered 88 doses of ^{67}Ga and scanned 62 additional patients. Of these, 52 doses were provided through the Cooperative Group's ^{67}Ga protocol. These cases will be analyzed according to the protocol for the Cooperative Group and pooled with those from other members (C. L. Edwards, R. L. Hayes, and B. Nelson).

Of the total group of 140 patients studied here before we began to receive ^{67}Ga as a part of the protocol of the Cooperative Group, about half showed evidence of tumor localization on the initial scans. Some with positive scans initially, later had negative scans, and vice versa.

Table 39 lists the patients by broad diagnostic categories showing the number of positive and negative initial scans in each group.

Although all the patients were thought to have active tumors at the time of the scans, the subsequent courses or other information in nine of them led us to believe that they probably were free of tumors at the time of the scan. Tables 40, 41, and 42 show the histologic classification of those diseases showing the highest percentage of positive scans. These show that among the lung cancers squamous carcinoma has the greatest affinity for gallium, and among patients with lymphoma, those with well-differentiated lymphocytic lymphomas have the lowest percent of positive scans. The cases are too few to be statistically valid but as yet no clear differences are seen between the different subgroups of Hodgkin's disease.

Table 39

INITIAL GALLIUM-67 SCANS

	Positive	Negative	Totals
Hodgkin's disease	19	8	27
Other malignant lymphomas	14	10	24
Carcinoma of the lung	11	9	20
Carcinoma of the breast	10	4	14
Miscellaneous malignant tumors	18	28	46
Probably no tumor	2	7	9
	74	66	140

Any listing of cases as "positive" and "negative" is a crude and certainly misleading expression of the effectiveness of gallium in locating occult tumors. In such a listing a scan of a patient with a single positive lesion and multiple lesions that failed to concentrate gallium, some or all of which may be unknown to the investigation, would still be regarded as positive; we still seek a more meaningful way of expressing the results in this heterogeneous group of patients.

Like others, we have seen ^{67}Ga concentrated in nonmalignant lesions (false-positive scans for tumor localization). One was a woman with a

pseudolymphoma of the stomach thought to be due to cytomegalic inclusion virus. Another was a man with an abdominal mass that was probably an abscess that was subsequently irradiated. He later had a colostomy and died of postoperative complication. By the time of autopsy no tumor was found. The third was a young man with Hodgkin's disease who developed a pulmonary infiltration clinically thought to be a pneumonitis at the site where the scan showed increased gallium localization.

Table 40

GALLIUM-67 SCANS OF PATIENTS WITH CARCINOMA OF THE LUNG

	Positive	Negative	Totals
Squamous	7	1	8
Undifferentiated	2	5	7
Adenocarcinoma	1	2	3
Unspecified	1	1	2
	11	9	20

Table 41

GALLIUM-67 SCANS OF PATIENTS WITH MALIGNANT LYMPHOMA

	Positive	Negative	Totals
Lymphocytic, well-differentiated	2	6	8
Lymphocytic, poorly differentiated	4	1	5
Histiocytic	3	0	3
Mixed lymphocytic-histiocytic	3	3	6
Undifferentiated, pleomorphic	1	0	1
Undifferentiated, Burkitt	1	0	1
	14	10	24

One of the most difficult problems in analyzing scans is knowing what the "normal" scan should look like. Ideally, one would like to see a large number of scans of patients without tumors. There are obvious prohibitions, however, and so the concept of normal must be pieced together from the data available. Upon reviewing a series of scans one is impressed with the dissimilarity among the scans of individuals. Yet some tissues and organs regularly accumulate more gallium than others, even when not directly involved with tumors, and must be distinguished from abnormal concentrations of the activity. In most whole-body scans there is a diffuse body-background of activity which varies a great deal

1028058

from patient to patient but is usually high enough that a body silhouette can be detected, yet low enough that the liver, spleen, kidneys, and skeleton stand out above it. From the assays of biopsies and tissues taken at autopsy, we know the gallium concentrates mostly in the spleen, liver, bone marrow, kidneys, and bones. Not surprisingly therefore, these normal structures are regularly seen with varying intensities in most scans.

Table 42

GALLIUM-67 SCANS IN HODGKIN'S DISEASE BY HISTOLOGIC TYPE

	Positive	Negative	Totals
Mixed cell	11	4	15
Nodular sclerosis	4	2	6
Lymphocyte predominant	1	1	2
Lymphocyte depleted	0	0	0
Not classified	3	1	4
	19	8	27

Other tissues giving focal increases of activity, sometimes asymmetrically, are the salivary glands and the lacrimal glands. Usually also a hot spot appears on the anterior view of the head which corresponds to the nasopharynx. However, when viewed laterally, only a diffuse area with a slightly anteroposterior orientation is seen rather than a hot spot. The breasts, especially in women, are apt to show on scans as areas of increased activity. The greater variable, and consequently the most difficulty in interpreting, is due to the isotope in the bowel. Despite enemas and laxatives, we frequently find the activity here to be greater than in any other area on the scan. There may also be activity low in the midline of the pelvis. Some is probably due to staining of the perineum with radioactive feces or urine but some appears to be in the penis or vulva.

As yet we do not understand why gallium localizes in some tumors and why it does not in others, but it is clear that in an individual with multiple metastases of the same tumor, some may be seen on scans and others not. In the past we have emphasized that some of this difference is due to the amount of fibrosis or necrosis in the individual tumor sites. Size is certainly a key factor as is the location, e.g., a tumor of only modest concentration of gallium (mCi/g) may be readily detected if located in the midst of low-density tissues like the lung, or superficial as in a cervical lymph node; but a tumor with the same concentration of gallium may be undetected in the epigastrium near the liver. The site of the tumor may be important in other ways too. In at least three patients we failed to detect tumors of the central nervous system. One was a young boy with Burkitt type lymphoma readily demonstrated in its extradural locations. These cases raise but do not answer the disconcerting questions as to whether gallium will cross the blood-brain barrier to reach and concentrate in intradural lesions.

Until we understand the vagaries of gallium localization in tumors, scanning can play only a limited role in decision making that may involve the patient's life or death, e.g., the staging of Hodgkin's before deciding on curative radiation therapy. However, even now gallium scans are proving especially useful in the follow-up evaluation of some of these same patients. The ease with which the scans can be made allows for periodic studies following therapy designated for cure; thus one might detect recurrences early, allowing further therapy to be given and perhaps achieving additional cures.

During 1971, we saw two such cases. Both were women with Hodgkin's disease treated with extensive ^{60}Co teletherapy to all the lymph-node-bearing areas above and below the diaphragm. Both were thought to be stage III but following completion of therapy one developed pain in the left lower extremity. No explanation could be found for the pain until a ^{67}Ga scan showed a small focus of intense activity in the ilium. X-ray studies focusing on this area then detected a small lesion in the bone. Radiation to this lesion completely relieved the pain without adding appreciable marrow injury to that already received from her primary therapy.

The other patient had received the radiation above the diaphragm first, later followed with radiation below the diaphragm without splenectomy or splenic irradiation. One year later she developed systemic symptoms of Hodgkin's disease. A scan detected a focus of activity precisely between the two radiation-treatment fields, where the radiotherapist had allowed a gap to prevent too much overlapping. Removal of this mass of nodes along with a splenectomy followed by radiation produced a complete remission of her symptoms.

COMPUTER METHODS APPLIED TO NUCLEAR MEDICINE RESEARCH

The computer center provides a service function for the Medical Division's research effort. The progress reported here will concentrate on the methods applied to the research effort; the interpretation of the computer results in various projects will appear under the appropriate research headings.

Significant improvements were added to the computer hardware and software systems. The core cycle time was increased from 4 μsec to 2 μsec and the core storage capacity was decreased from 16K to 24K words. These core improvements have allowed us to double our through-put capabilities and expand our on-line real-time system work. This expansion has increased machine availability because the computer system can operate all the on-line programs at the same time that batch processing is in operation. The random-access storage facilities of the computer have been expanded by the addition of an IBM 2311 disk drive which made possible the realization of many of our objectives for the clinical laboratory system. Without this disk the current and expanded clinical system would not be practical.

The ability to store and retrieve data from the ORAU clinical data system has been demonstrated (Mona Smith, Paulette Aaron, and A. E. McDow, Jr.). A researcher can request specific information on a particular patient or he can request information under a general heading. For example the results of a patient's whole-body count for a specified period of time can be retrieved (Fig. 38). On a more general approach a physician can request a plot of selected blood values (Fig. 39).

The original method of analysis of a 4-min segment of low-exposure total-body irradiation (LETBI) pulmonary-impedance data required 3 hr. The method reported last year reduced the time analysis to 1 hr; and now, using an improved technique and some new computer equipment, the current system requires only 40 sec for the same segment of data (A. E. McDow, Jr., C. C. Lushbaugh, Robert C. Ricks, and Edward Frome*). In addition to the reduction

WHOLE BODY COUNTS

TAPE NO 13262 PERSON OR SAMPLE COUNTED
DATE 1/19/71 AMOUNT OF DOSE 0.027001SOTOPE 5313100 DOSE NO 3076
TIME OF COUNT 1015 LENGTH OF COUNT 1 · GROSS/NET 0 STANDARD NO. 3013
HOSPITAL/S.S. HEIGHT 165.0 WEIGHT 59.2 SEX 1 TYPE 3

DATE	HOUR	INTERVAL	PERCENT DOSE RETAINED	MISCELLANEOUS
1/19/71	1015	0 HR	100.000	
1/19/71	1415	4 HR	86.813	
1/20/71	1055	1 DA	40.793	
1/21/71	1115	2 DA	26.528	
1/22/71	1120	3 DA	23.570	
1/25/71	1005	6 DA	17.827	

COMPUTATION COMPLETED BETWEEN 13258 AND 13287

Fig. 38. Computerized calculation of whole-body retention.

in analysis time, the new system provides better analytical results of the pulmonary-impedance data and includes analysis of the R-R interval from the electrocardiogram (Figs. 40 and 41). The program is currently being modified to acquire data from a CO_2 gas analyzer and to further reduce the pulmonary-impedance analysis time by another factor of two. The structure of the program provides for this change without adding any additional time to the data acquisition phase. All of the original data and the analyzed results are maintained on a tape file as a portion of the clinical data system.

The interfacing of the Pho/Gamma camera to the computer has also allowed us to use the camera as a whole-body scanner (Fig. 42). To test the feasibility of this idea we used a simple motor arrangement that pulled a phantom over a

*Emory University, Georgia (PHS Fellow)

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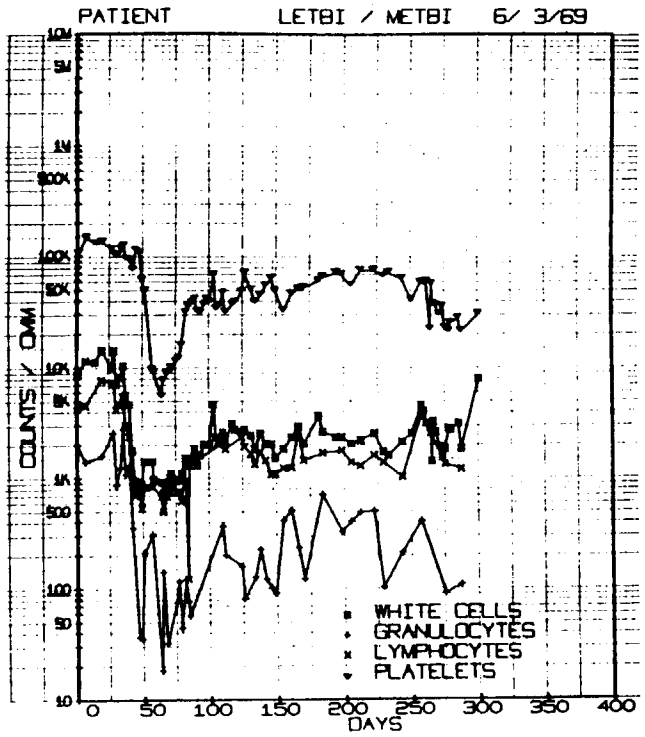


Fig. 39. A computer plot of the blood values for a patient undergoing treatment in LETBI and METBI.

narrow slit between two lead plates placed over the camera's crystal (William D. Gibbs and A. E. McDow, Jr.). The phantom was a cardboard square with a simple house structure painted on it with ^{131}I in solution. Sequential counts from the camera were acquired by the computer, then reassembled in time sequence to produce a scan image of the phantom. Based on the results of these tests, the design of a new whole-body scanner using the above principle is being studied.

We have completed a set of two programs that read paper tape and analyze the gamma-count data from a well gamma-counter in the radiopharmaceutical development laboratory. The first program, HGRED, reads paper tape, converts the data to a computer usable code, prints the data on the 1053 printer for verification purposes, and writes the converted data into one of four disk-files. The second program, HGAMA, calculates the "percent/g" and "percent/g per 250 g" for each of the tissues counted. Also, it prints on the 1443 printer the group heading, animal heading, tissue name, tissue weight, net counts/min,

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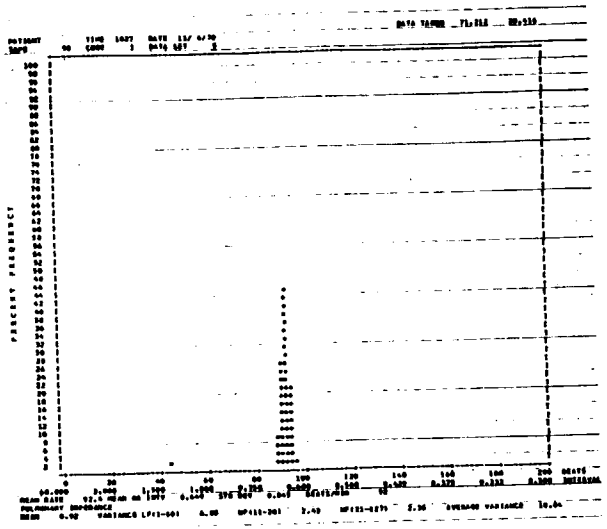


Fig. 40. Computer analysis of the pulmonary impedance and R-R interval data from a patient in LETBI.

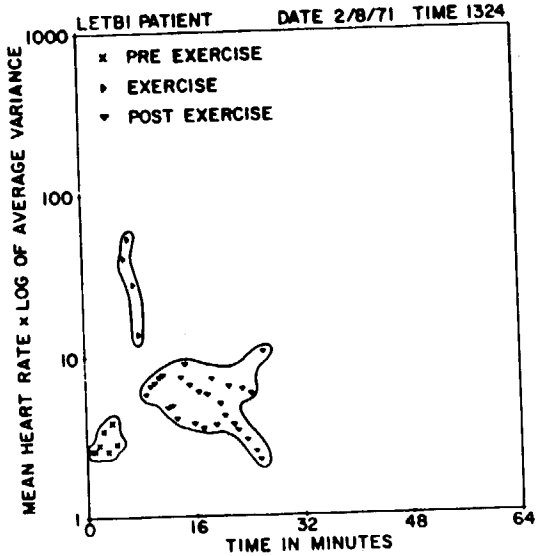


Fig. 41. Computer plot of the mean heart rate times the log of the average pulmonary-impedance variance as a function of time for a LETBI patient undergoing exercise.

percent/g, and percent/g per 250 g for each tissue counted for each animal in a group. It is optional to calculate the ratio of tumor to tissue for each animal. It is also optional to calculate the average of the percent/g per 250 g values and the ratio values for the animals in a group and the standard deviation of the mean of these averages (Paulette Aaron).

Also completed for the radiopharmaceutical development laboratory is a modification of an IBM OS/360 program to calculate the sedimentation coefficient in zonal centrifuges. This coefficient indicates the distance an ideal particle has travelled in the given suspending medium for a finite time period. The program contains the equations for only one suspending medium, sucrose. With some minor changes, the program could support other suspending media (Barbara Bishop* and Paulette Aaron).

Work on the NASA retrospective studies data bank has concentrated on gastrointestinal effects of the portal radiation studies (Frank V. Comas, Edward Frome,† and Paulette Aaron). Using preliminary response data for nausea, anorexia, and vomiting, analyses are being made, using the Weibuell function, to aid in determining the probability of the incidence of positive response for each separate response.

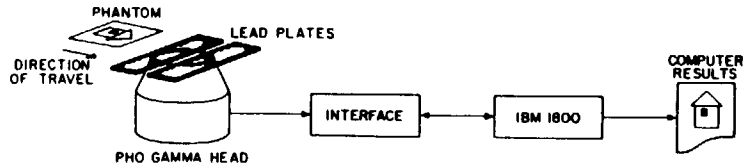


Fig. 42. The Pho/Gamma IBM 1800 set up to test the feasibility of using the Pho/Gamma as a whole-body scanner.

*Computing Technology Center, Oak Ridge Gaseous Diffusion Plant
†Emory University, Georgia (PHS Fellow)

1028061

Computer Acquisition of Gamma-Ray Camera Images

An important objective this year has been the connection of a Pho/Gamma III scintillation camera to the IBM 1800 computer. Other researchers (viz., Brownell, Brill, Hill, Monahan, Carlson, etc.) have made similar connections between such cameras and computers. Our work has been directed toward producing an economical, uncomplicated interface that can be reproduced at other medical institutions with minimum hardware and labor cost. The coordinate conversion unit and the acquisition program for this interface are completed and operating (A. E. McDow, Jr., Thomas R. Barclay, and A. C. Morris, Jr.). Figure 43 illustrates in block form the camera-computer connection. Three signals are transmitted from the camera to the interface circuitry: the X and Y analog position voltages, plus the camera's unblanking pulse. Under control of the software support, the interface unit converts each analog value to a 10-bit binary number. When the analog-to-digital conversion is complete the data is transferred into the computer memory from the interface unit. As each set of coordinates enters memory, a time of entry is attached. When the core buffer is full the data are transferred to a temporary file and then are written onto magnetic tape for permanent storage. To demonstrate the acquisition of data from the camera, a thyroid phantom was filled with ^{131}I and was scanned by the camera-computer (Fig. 44-A). The computer-prepared scan pictures (Fig. 44 B-C) are raw counts without any scan enhancement supplied by the computer. The well-defined points shown in Fig. 44-C indicate the need for increased resolution of the binary coordinates. The acquisition program is being modified to accept 10-bit coordinates and the resulting increase in significance should improve the presentation of our scan images, especially when computer

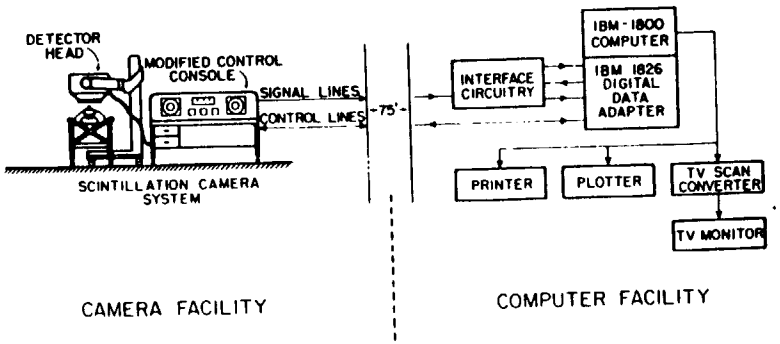


Fig. 43. Block diagram of the Pho/Gamma-IBM 1800 computer interface.

enlargements are made. The programming for the analysis and enhancement of these scan images is in progress; two programs are completed that produce isocount contours and three-dimensional prospective plots. Since the computer stores not only the X and Y coordinates of each event but also the time of each occurrence, later replays of preselected areas of the picture, or timed sequences during a study, are possible. Control of a study is provided by supplying the computer with the START, STOP, and RESET functions of the camera.



Fig. 44A. Pho Gamma image of an ^{131}I thyroid-phantom taken from the camera oscilloscope.

B. Computer-produced scan image of Fig. A. taken from the TEKTRONIX 4501 scan converter.

C. Computer enlargement of the thyroid image in Fig. B.

Computer-Interfaced Four-Probe Counter

Last year we reported technical progress toward developing an electronic interface from the four-probe counter to the IBM 1800 computer. In a joint effort between the instrumentation, clinical, and computer departments, we have recently completed the basic instrumentation for this project; the equipment is operational for dynamic-function studies (A. C. Morris, Jr., Francis A. Goswitz, Paulette Aaron, and A. E. McDow, Jr.). Figure 45 is a block diagram of the major signal paths between the counting site and the computer, 450 feet away. Equipment operating at the counting site includes four detector-probes, a four-channel spectrometer, a four-pen analog recorder, a switch-connected scaler, and an interface control of ORAU design. Our interface enables the counter operator to encode identification numbers of patients to the computer, call programs for the analysis of the counting data, and start or stop the data-acquisition process, all accomplished through use of computer-connected switches and indicator lamps on the control panel of the interface. Moreover, the interface contains circuits that receive pulses from the four spectrometers, and reproduces pulses of the correct polarity and length (−10 to +1 volt pulses, 5 μSec long) for transmission to the computer input. The schematic for one of these line drivers is illustrated in Fig. 46, and it operates in the following way. Output pulses from the spectrometer (−2 volts amplitude, 500 nsec long) are

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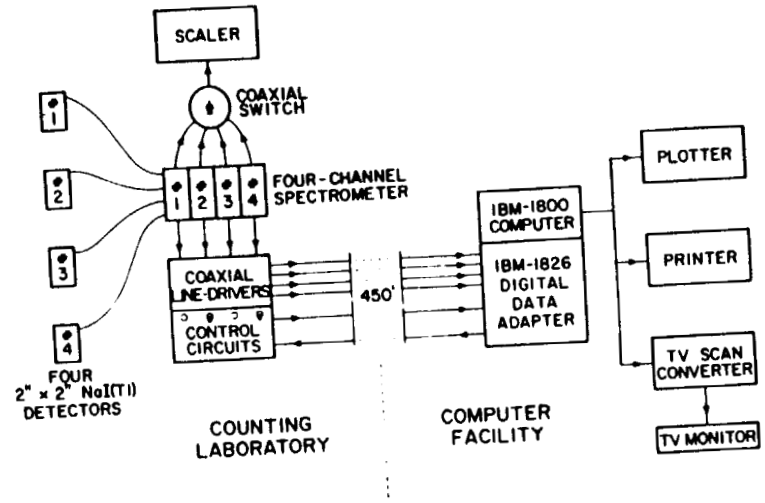


Fig. 45. Block diagram showing interface connection from four-probe counter to the computer.

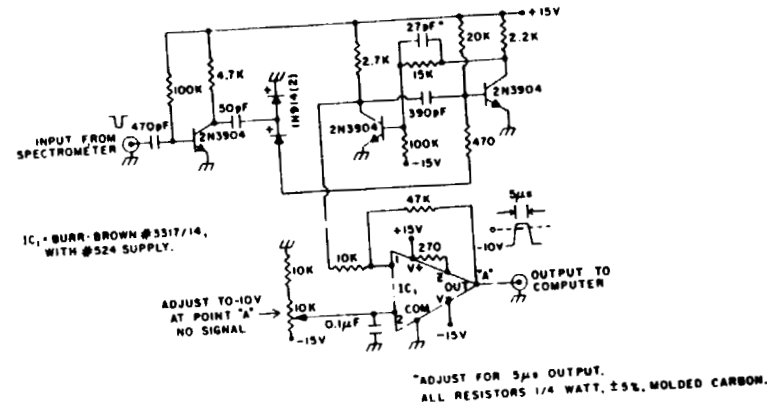


Fig. 46. Schematic of line-driving electronics for the four-probe counter.

capacitor-coupled to an inverter stage, the input 2N3904 transistor. We then differentiate the inverter output and apply a negative spike to trigger one transistor base of a 5-μsec monostable circuit. A negative voltage pulse from the monostable circuit is fed through a 10K resistor to the inverting side of an operational amplifier; the noninverting input to this amplifier is biased to −10 volts, the required quiescent voltage of the computer's pulse-counter input. Pulses from the monostable circuit cause the output voltage of the operational amplifier to rise from the quiescent −10 volts to +1 volt, hold this polarity for 5 μsec, and then return to −10 volts. These voltage changes are transmitted to the computer using four 450-ft RG-62/U cables, and each excursion is read as a detected count. Counting rates as high as 10^5/sec can be handled by the computer in this manner. Since the capacitance of each cable is nearly 0.01 μF, the line driver must have a very low impedance to obtain good, high-frequency transmission, a function readily fulfilled by the operational amplifier. By means of these circuits, counting pulses from the four detectors are simultaneously read into the computer during a dynamic radionuclide study. The computer acquisition and display programs used with this four-probe counter are now

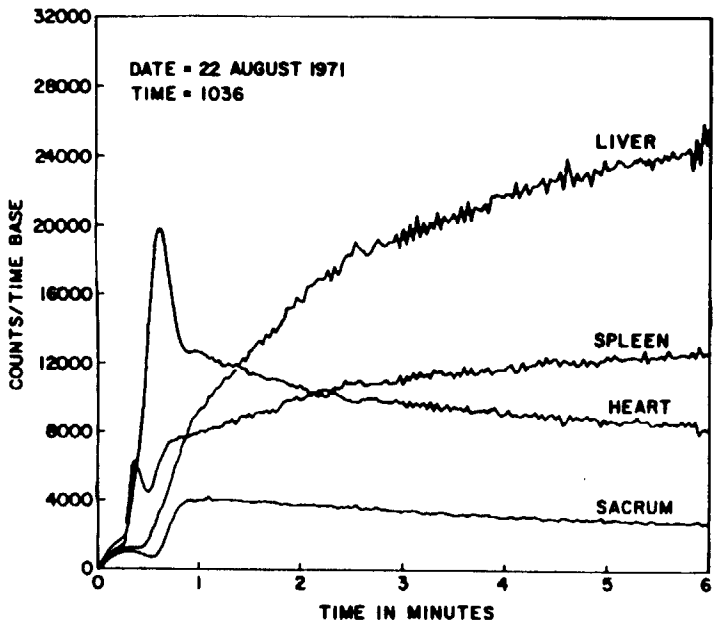


Fig. 47. Results from computer-interfaced four-probe counter used on patient injected with 350 μCi of ^{198}Au.

1028063

complete (Paulette Aaron). By actuating appropriate switches on the interface control, the computer acquires the identification information and count data from the probes. These data are then stored in a permanent tape-file and are plotted for analysis by the researcher.

Figure 47 illustrates the kind of performance we have obtained using this computer-interfaced counter. Normally the "experiment start" push button on the interface control is pressed simultaneously with a quick injection of radionuclide into a basilic vein; the accumulation of counts begins immediately on the switch closure. For the study shown in Fig. 47, we administered 350 μCi of ^{198}Au colloid. Incremental counts were started, spaced every 2 sec, and the heart curve is seen to make an immediate rise and fall. The liver curve shows a continuous rise to a high counting-rate level, with lesser concentrations indicated in the sacrum and spleen. Figure 47 represents the initial 6-min plot of a 27-min study of a patient; another graph covering the full 27-min-run, showing later trends, was also produced by the computer.

Studies on the Minimum Detectable Tumor Size with Gallium-67

A study was undertaken to systematically assess the factors influencing the minimum size of gallium-concentrating tumor that can be detected by scanning (Y. Tateno,* W. D. Gibbs, and C. L. Edwards). Assuming a given degree of biologic concentration, two approaches to the problem were used. One of these was theoretical, based upon a formula for contrast visibility used in radiology and expanded to take into account the statistical problems of radiation detection. The theoretical model was further expanded to include such factors as duration of scanning procedure, collimator performance, background, and quality of display systems. The other approach was to determine by experiments with phantoms the smallest lesions that could be seen under various scanning conditions. The values obtained from the theoretical model and the experimental results showed reasonably good agreement. The information was used to assess the most practical approaches to improving the scanning procedure. With current scanning methods and average tumor uptake of ^{67}Ga we estimate that we can see lesions about 1 cm in diameter (under conditions used in the phantom). With more skillful use of pulse height selection, a better display system and improved collimators, this might be reduced to about 0.5 cm.

*Chiba University Medical School, Chiba, Japan

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM

Most of these investigators are tied closely to other parts of the program. They represent important fundamental areas of study, some of which grew out of discoveries and unusual research progress that occurred at the Medical Division, and some of which were initiated specifically to give needed basic support to an ongoing program. These research projects take particular advantage of clinical resources in the Division and the special scientific community in Oak Ridge.

SUMMARY OF ACCOMPLISHMENTS

The biochemistry program continued a major emphasis on the biosynthesis and degradation of ether-linked lipids, especially in malignant tissues. Work was done on the mechanisms by which alkyldihydroxyacetone phosphate may be formed by way of an acyl exchange mechanism. Evidence indicates that O-*alk-1-enyl moieties of glycerolipids are formed from an* O-*alkyl moiety. New information suggests that the conversion of* O-*alkyl lipids to* O-*alk-1-enyl compounds involves a substitution on the* O-*alkyl moiety rather than simple dehydrogenation. Earlier work showed that in malignant cells fatty alcohols instead of being oxidized to fatty acids are incorporated into ether-linked lipids that are not rapidly broken down or removed. New studies are being done to explore this pathway as a mechanism for concentrating large amounts of tritium in cancers by administering 1-*^{3}*H-fatty alcohols. It was shown that human leukemic lymphocytes and granulocytes synthesize alkyl glycerolipids from long-chain fatty alcohols as do other nonhematologic types of malignant cells. Work on the endoplasmic reticulum of rat liver cells showed that the lipid composition and turnover of rough and smooth microsomal fractions were similar. It appears that endoplasmic reticulum may be a mosaic array of structural lipoproteins and enzymes in a dynamic state.*

In the marmoset project, in response to challenge with Salmonella typhosa *and sheep erythrocyte antigens, a particular pattern of antibody response was seen, different from that of other species. Earlier studies had shown wild-bred marmosets to be quite sensitive to total-body irradiation (TBI) and the role of parasites in mortality had been questioned. Recent studies in a small number of animals suggest that the parasite-free laboratory-bred marmoset is as sensitive as the wild-caught animal. Marmoset hemopoietic cells, previously cultured in liquid medium, are now being grown successfully as colonies of separate types on soft-agar medium. Recent data suggest that the megakaryocytic cells, as well as lymphocyte, neutrophil, and erythrocyte forms, may exhibit chimerism. Mixed lymphocyte cultures are being used successfully to elucidate the immunologic reactivity of marmoset cells to their co-twins, and to other animals of the same and different species. An interesting development has been the*

1028064

discovery and extensive investigation of four cases of cancer of the small intestine in marmosets; such lesions are either very rare or have been overlooked in most other primates.

In the ultrastructural anatomy program, further evidence was obtained that the subcellular structures that contain ^{67}Ga are lysosomes. When mice were given sublethal doses of TBI, there followed a distinct, transient decrease in the ability to retain ^{67}Ga in most tissues except for spleen and bone. In collaboration with ORNL it has been shown that when ^{67}Ga is given to pregnant mice it concentrates to a considerable degree in fetal tissues. Preliminary studies have compared gallium with ^{203}Hg, another nuclide sometimes recommended for tumor localization and one that concentrates in and may damage normal kidneys. In the kidney, mercury in contrast to gallium is localized in the mitochondria.

Cytogenetic studies included detailed sequential observations on a man accidentally exposed to external irradiation. Comparable studies done on the same patient at ORNL brought out the fact that small differences in cultural conditions can greatly affect the number of abnormal chromosomes. Collaborative studies with ORNL on the effects of irradiation on chromosomes in marmoset lymphocytes showed an aberration yield comparable to that for human beings, and identical results for cells irradiated in vivo and in vitro. The initial segment of the large study on the effects of the contraceptive pill on human chromosomes is nearing a conclusion; a modest but distinct increase in chromosome abnormalities was found in women taking these medications, and valuable data were obtained on normal variations in a population group followed over a period of months. An American girl with Burkitt's lymphoma was found to have a characteristic chromosome abnormality like that reported for some cases of this disease studied in Africa.

BIOLOGICAL CHEMISTRY*

Metabolic Pathways Involving Glycerolipids: Effects of Neoplasia and Irradiation

This portion of the biological chemistry research program has concerned itself with the exploration of the metabolic pathways responsible for the biosynthesis and degradation of ether-linked lipids and related compounds at the enzyme level. We are attempting to elucidate the chemistry and mechanism of each reaction step and the nature of the control mechanisms (M. L. Blank, R. H. Lumb, B. Malone, C. Piantadosi,† F. Snyder, and R. L. Wykle). Our research is providing answers on how the individual reaction steps are sequentially integrated as complete pathways. A primary goal is to evaluate the effects of

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1028065

metastatic growth and irradiation injury on the metabolic pathways responsible for the formation and turnover of membrane lipids at cell surfaces and in organelles.

Enzymic Synthesis of Alkyl Glycerolipids. Alkyldihydroxyacetone phosphate appears to form via an acyl exchange mechanism. The complete enzymic system for the synthesis of alkyldihydroxyacetone phosphate (alkyl-DHAP) and alkyldihydroxyacetone (alkyl-DHA) requires the following components: long-chain fatty alcohols, DHAP, CoA, ATP, Mg^{++}, and microsomes. The first detectable product in this reaction (alkyl-DHAP) can be dephosphorylated to alkyl-DHA.

The fact that the cofactors (CoA, ATP, Mg^{++}) are the same as those necessary for fatty acid activation and that alkyl-DHAP synthesis is completely inhibited when NADPH is present at the beginning of the reaction led Hajra (*Biochem. Biophys. Res. Commun.* 39, 1037, 1970) to suspect that acyl-DHAP might somehow be involved in the mechanism responsible for the biosynthesis of alkyl-DHAP (Fig. 48). We have also observed this exchange reaction in

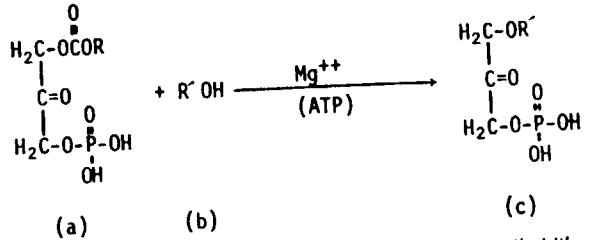


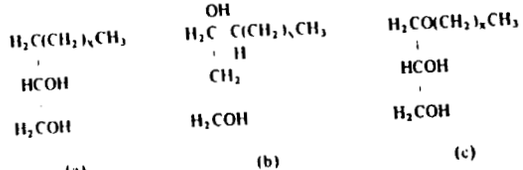
Fig. 48. Exchange reaction involved in the biosynthesis of alkyldihydroxyacetone phosphate (c) from long-chain fatty alcohols. (a = acyldihydroxyacetone phosphate and b = long-chain fatty alcohols.)

microsomes from Ehrlich ascites cells and preputial gland tumors. Magnesium ions appear to be required, but it is not well documented. Furthermore, Murooka and colleagues (*Biochem. Biophys. Res. Commun.* 41, 407, 1970) have obtained data on the alkylation of homoserine that supports the principle of the exchange mechanism. Although the polarity of the reactant molecules can account for such an exchange, the exact mechanism has not yet been elucidated.

Nucleotide specificity for reduction of the ketone group in alkyl glycerolipids is an important consideration. There are two ketone-containing alkyl glycerolipids that have been carefully characterized in the cell-free systems that synthesize alkyl glycerolipids: alkyl-DHAP and alkyl-DHA. In addition, both classes of compounds have been prepared by chemical synthesis (*J. Pharm. Sci.* 59, 1201, 1970). The alkyl-DHAP can be dephosphorylated by phosphatases in the microsomal and soluble fractions of cells. Microsomal reductases that utilize the two *O*-alkyl ketone substrates appear to differ in their specificity for the reduced nucleotide. Under certain conditions, we have found that either NADPH or NADH will serve as hydrogen donors for the reduction of alkyl-DHAP, whereas NADPH is much more effective than NADH for the

reduction of alkyl-DHA. Since the extent of phosphatase activity in the enzyme systems can mask the nucleotide specificity for alkyl-DHAP, one must use caution in assessing nucleotide specificities for these lipid classes.

Alkane diols potentially interfere in the analysis of alkylglycerols. When identifying ether-linked lipids in biochemical studies, it is important to make sure that alkane-1,2-diols (a) and alkane-1,3-diols (b) are not mistaken for alkylglycerols (c), since their structures are similar except for the ether linkage.



(a) (b) (c)

These compounds all have the same R_f in thin-layer chromatographic systems, but their isopropylidene derivatives can be resolved to some extent by thin-layer chromatography (TLC), and completely by gas-liquid chromatography (GLC). We have isolated alkane diols after incubating fatty acids in mitochondrial systems and reducing the extracted lipids with $LiAlH_4$. The alkane-1,3-diols produced under these conditions are derived from a β-hydroxy fatty acid ($RCHOHCH_2COSCoA$), an intermediate formed during β-oxidation of fatty acids by mitochondrial enzymes. We have described the methodology to distinguish a variety of alkane diols from corresponding alkylglycerols in a paper by Blank, Cress, Stephens, and Snyder[3].

Enzymic Synthesis of Ethanolamine Plasmalogens. Since 1957 when the *O*-alk-1-enyl structure in ethanolamine plasmalogens was elucidated, there has been much speculation on potential reactions responsible for the biosynthesis of the *O*-alk-1-enyl moiety (*J. Pharm. Sci.* 59, 283, 1970). Possibly more than one pathway for the biosynthesis of plasmalogens exists; however, at present only a cell-free system from tumor preparations has been isolated that forms plasmalogens. We have obtained data from this system strongly indicating that *O*-alk-1-enyl moieties of glycerolipids are formed from an *O*-alkyl moiety. The data suggest that this conversion involves a type of substitution (Fig. 49, reaction I) on the *O*-alkyl moiety rather than simple dehydrogenation by a hydrogen-accepting nucleotide (Fig. 49, reaction II).

Perhaps one of the most difficult problems in investigating plasmalogen biosynthesis is to prove unequivocally that the product formed in the system is indeed a plasmalogen and not an artifact. To circumvent this problem, one must isolate the ethanolamine phospholipid fraction (the main source of plasmalogens in most cells) and identify the plasmalogen structure on the basis of chemical derivatives that take into account all three positions of the glycerol moiety (Fig. 50). Using this approach, we demonstrated the enzymic synthesis of ethanolamine plasmalogens in a cell fraction containing microsomes and the soluble supernatant isolated from Ehrlich ascites cells and preputial gland tumors. These systems utilized the same substrates (fatty alcohols and DHAP)

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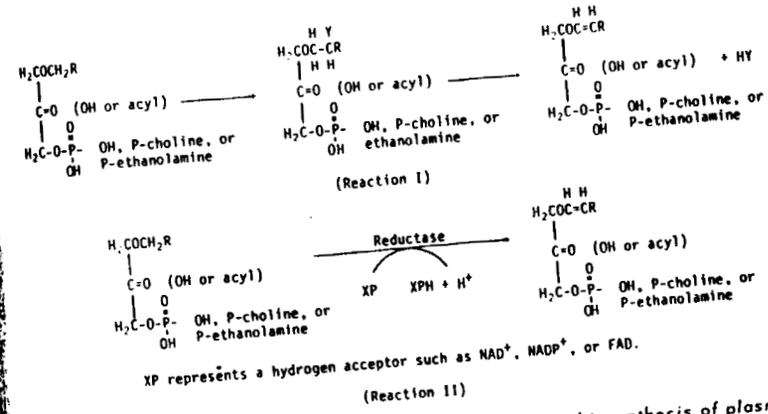


Fig. 49. Possible enzymic reactions responsible for the biosynthesis of plasmalogens from an alkyl glycerolipid.

and cofactors (CoA, ATP, and Mg^{++}) required for the biosynthesis of alkyl glycerolipids by microsomes, except for the additional requirement of $NADP^+$ or NAD^+ and the soluble supernatant fraction. Under these conditions, most of the radioactivity in plasmalogens from labeled fatty alcohols and DHAP is in the ethanolamine-containing fraction. Supplemented CDP-ethanolamine in the system increases the total quantity of plasmalogen synthesized. 1-^{14}C-palmitic acid could not substitute for the 1-^{14}C-hexadecanol, and added unlabeled hexadecanal had little effect on the incorporation of 1-^{14}C-hexadecanol into the ethanolamine plasmalogens. Washed microsomes as the enzyme source will not alone synthesize plasmalogens; they also require the addition of a soluble protein fraction from either the tumor or normal rat liver.

Carbon-14 was recovered in a number of derivatives formed from intact ethanolamine plasmalogens and isolated by TLC and GLC. These included (a) hexadecanal liberated by aqueous HCl, (b) dimethylacetals of hexadecanal formed by HCl-methanol treatment, (c) alk-1-enylglycerols liberated by reduction with $LiAlH_4$ or $NaAlH_2(OCH_2CH_2OCH_3)_2$, (d) alkylglycerols obtained by hydrogenation of the alk-1-enylglycerols, (e) isopropylidene derivatives of the hydrogenated alk-1-enylglycerols, (f) lysoethanolamine plasmalogens after phospholipase A treatment, and (g) dinitrobenzene derivatives of the intact ethanolamine plasmalogens.

The reaction(s) involved in the formation of alk-1-enyl glycerolipids and the roles of $NADP^+$ and NAD^+ and the soluble protein fraction in plasmalogen biosynthesis are not known. However, since no ketone compounds appear to be reduced unless NAD^+ or $NADP^+$ is added, even though the soluble fraction is present, it would seem that the oxidized forms of the nucleotides relate to the reduction-oxidation of alkyl-DHAP; in addition, they could also serve as an acceptor of hydrogens from the α,β carbon atoms of the *O*-alkyl moiety, or, they could be involved in some other mechanism.

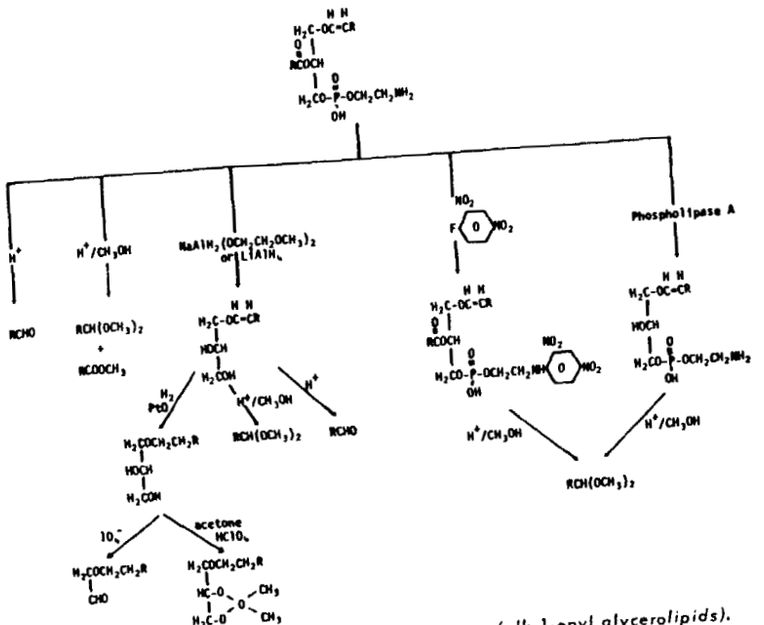


Fig. 50. Reactions used to identify plasmalogens (alk-1-enyl glycerolipids).

Although the substrate and cofactor requirements for the biosynthesis of plasmalogens are essentially identical to those for the biosynthesis of *O*-alkyl lipids, this by itself was insufficient evidence to conclude that an alkyl glycerolipid was converted to an alk-1-enyl glycerolipid. However, thin-layer radiochromatographic data obtained with uniformly ^{14}C-labeled DHAP and 9,10-^{3}H-hexadecanol demonstrated that the $^{3}H/^{14}C$ ratios were the same for the alkylglycerols and alk-1-enylglycerols isolated from the total lipids of the incubation mixture after $LiAlH_4$ reduction. Collection and radioassay of the isopropylidene derivatives of the hexadecylglycerols and hydrogenated hexadecl-enylglycerols from GLC confirmed the TLC results and strongly suggested that an *O*-alkyl moiety was converted to an *O*-alk-1-enyl moiety.

In subsequent experiments, we prepared alkylacylglycerol-P as a substrate containing ^{3}H in the 9,10-position of the *O*-alkyl moiety and U-^{14}C in the glycerol portion. This doubly labeled substrate was incorporated, in the absence of CoA, into alk-1-enylacylglycerylphosphorylethanolamine without any alteration in the $^{3}H/^{14}C$ ratio. These data represent the first enzymic evidence for a direct conversion of an *O*-alkyl group to an *O*-alk-1-enyl group on a glycerolipid molecule.

An in vivo Technique for Evaluating the Quantitative Contribution of Ether-Lipid Pathways. Glycerolipids containing esterified aliphatic moieties are synthesized from long-chain fatty acids, whereas those containing ether-linked

1028067

aliphatic moieties (*O*-alkyl and *O*-alk-1-enyl) are synthesized from long-chain fatty alcohols. We have demonstrated how 1-^{3}H-hexadecanol can be used in complex biological systems to assess the contribution of the pathway that produces the ether bond in glycerolipids. Since the ^{3}H on the first carbon atom is lost as $^{3}H_2O$ during oxidation to the fatty acid, no radioactivity can be incorporated into the acyl portion of glycerolipids, but when ether linkages are formed the ^{3}H is retained (Fig. 51). The 1-^{3}H-hexadecanol (3.66 millicuries/millimole) was synthesized by reducing palmitaldehyde* with ^{3}H-labeled sodium borohydride. The cells and tissues were selected on the basis of their relative differences in the composition of ether- and ester-linked aliphatic moieties in the glycerolipids. For example, fibroblasts grown as monolayers and normal rat livers contain only negligible quantities, whereas the tumors contain substantial quantities of glycerolipids with ether bonds.

The data in Fig. 52 demonstrate that fibroblasts oxidize more than 80% of the hexadecanol during a 22-hr incubation; the oxidized product, fatty acid, contained no ^{3}H. The residual ^{3}H associated with lipids was present as alkylglycerols and unreacted substrate. In contrast, the distribution of ^{14}C differed completely from that obtained for ^{3}H; of the ^{14}C activity recovered as lipid, 85% was as methyl esters derived from acyl groups, but only 3.7% was present as alkylglycerols and 7.0% as unreacted substrate.

In contrast with the fibroblast results, injections of 1-^{3}H-hexadecanol into rats bearing either Novikoff or Morris 7777 hepatomas, or mice bearing Ehrlich ascites cells, demonstrated that ^{3}H concentrated in the tissues containing significant amounts of ether-linked lipids. Most of the injected activity recovered was in the tumor cells since they synthesize more ether-linked glycerolipids than any of the other tissues (Table 43). Activity appearing in the liver is probably transient and reflects the role of this tissue in oxidizing the administered fatty alcohols. The recovery of significant quantities of tritiated hexadecyl glycerolipids from tumor cells 5 days after injection of 1-^{3}H-hexadecanol indicates that the ether-linked lipids are extremely stable cellular components.

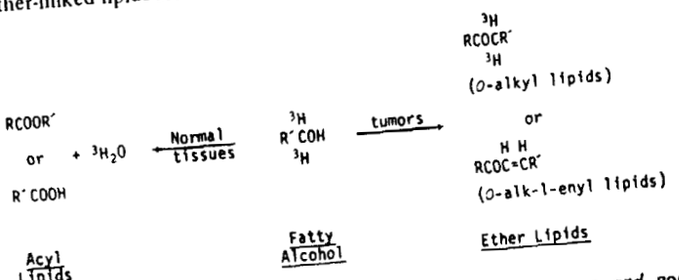


Fig. 51. The metabolism of long-chain fatty alcohols in tumors and normal tissues (R' = aliphatic chain and R = glycerol moiety).

*Applied Science Laboratories, Inc., State College, Pennsylvania

Table 43

^{3}H LOCALIZATION IN VARIOUS TISSUE LIPIDS FROM RATS BEARING NOVIKOFF OR MORRIS 7777 HEPATOMAS AND FROM MICE BEARING EHRLICH ASCITES TUMOR

Organ	Rats with Novikoff hepatoma*				Rats with Morris 7777 hepatoma†				Mice with Ehrlich ascites tumor‡			
	Disint./min per organ	Disint./min per g of fresh wt.	% of dose	% of recovered activity	Disint./min per organ	Disint./min per g of fresh wt.	% of dose	% of recovered activity	Disint./min per organ	Disint./min per g of fresh wt.	% of dose	% of recovered activity
Tumor	1,049,000	210,000	5.10	82.83	1,589,000	59,600	7.03	92.99	2,413,000	1,215,000	18.91	90.86
Liver	125,000	16,700	0.50	9.87	59,900	6,300	0.26	3.50	101,000	134,000	0.79	3.79
Heart	18,600	24,000	0.08	1.32	41,800	38,000	0.18	2.44	8,600	75,800	0.06	0.32
Kidney	11,700	7,200	0.05	0.92	14,800	6,800	0.06	0.86	31,200	47,000	0.24	1.17
Brain	62,000	34,800	0.30	4.89	3,200	2,000	0.01	0.18	107,000	260,000	0.83	4.02

*9.66 μCi (2.63 mmoles) of [1-^{3}H] hexadecanol injected intravenously
†10.3 μCi (2.81 mmoles) of [1-^{3}H] hexadecanol injected intravenously
‡5.8 μCi (1.60 mmoles) of [1-^{3}H] hexadecanol injected intraperitoneally

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Our data clearly demonstrate that the incorporation of 1-^{3}H-hexadecanol into glycerolipids can provide a rapid means of assessing the extent of biosynthesis and turnover of the ether-linked moieties of glycerolipids. However, in such studies some of the ^{3}H is also retained in the unreacted hexadecanol and it is theoretically possible for ^{3}H to be incorporated into wax esters and fatty aldehydes. The latter possibility is very unlikely under in vivo conditions, since we were unable to detect any ^{3}H in wax esters or fatty aldehydes in any of the tissues from rats and mice or in the fibroblasts grown as monolayers. The prominent route for the biosynthesis of ether bonds in tumor tissues, but not in normal cells, becomes obvious after inspection of the data in Table 43. Approximately 80-90% of the ^{3}H recovered appeared in the tumors. These data suggest that the ^{3}H from 1-^{3}H-labeled fatty alcohols is concentrated in neoplastic cells and could have a potential application in destroying radiosensitive cells. (We will discuss this point in the following paragraphs.) The selective incorporation of the 1-^{3}H label of fatty alcohols into ether moieties might also provide a physiological means of marking biomembranes, since it appears that lipids with ether bonds are important components at the peripheral surfaces of cells.

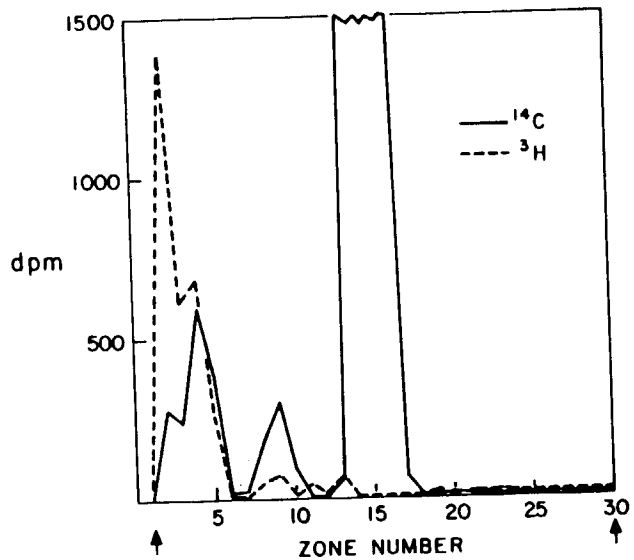


Fig. 52. 5-mm zonal scan of methanolysis products derived from total lipids of L-M cells grown as monolayers. Cells were incubated 22 hr with 1.1 μCi 1-^{14}C-hexadecanol and 2.0 μCi 1-^{3}H-hexadecanol. Peak 1, alkylglycerols (61.76% ^{3}H, 3.66% ^{14}C); Peak 2, unreacted fatty alcohol (28.65% ^{3}H, 7.05% ^{14}C); Peak 3, dimethylacetals derived from plasmalogens (3.48% ^{3}H, 4.16% ^{14}C); and Peak 4, methyl esters of fatty acids derived from acyl moieties of glycerolipids (0.00% ^{3}H, 84.56% ^{14}C).

A Biochemical Basis for Localizing Tritium in Tumor Cells

Fatty acids and fatty alcohols can be interconverted enzymically through an aldehyde intermediate; NAD^+ (NADH) and/or $NADP^+$ (NADPH) are required for these interconversions. In previous work we found that in normal tissues fatty alcohols are rapidly oxidized to fatty acids, whereas in neoplastic tissues they are primarily incorporated into ether-linked glycerolipids (Fig. 51). The results suggested to us (E. A. Cress, R. H. Lumb, and F. Snyder) that 1-^{3}H-fatty alcohols could be used to concentrate large quantities of radioactivity in neoplastic cells. The tritium could not build up in normal tissues because of the presence of an active *O*-alkyl lipid cleavage system and because the tritium from 1-^{3}H-fatty alcohol would be lost as ^3H_2O during the oxidation of the alcohol to the acid. Thus this approach permits selective localized irradiation of neoplastic cells with little or no exposure to normal tissues.

The principle of this idea was tested by administering 1-^{3}H-hexadecanol to mice bearing Ehrlich ascites cells. The highest concentration of tritium appeared in the tumor cells at all three time periods tested, at 2, 5, and 7 days; the lowest concentration appeared in the bone samples which included the marrow. Of the tritium dose, 74% was found in the tumor cells 2 days after injection and 26% 5

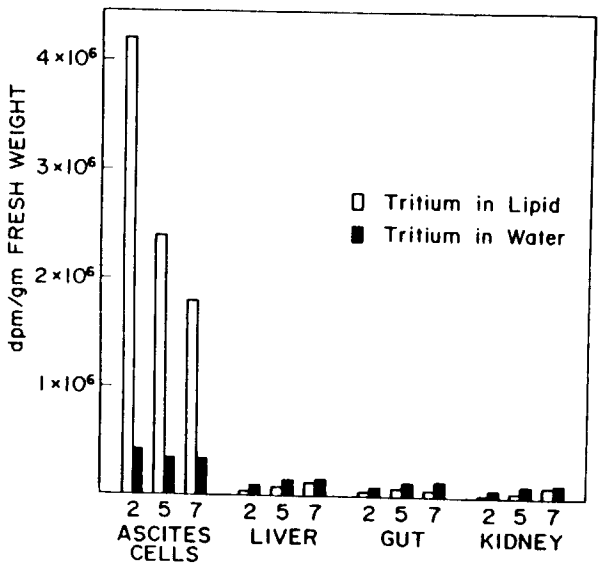


Fig. 53. Total amount of tritium found in tissues 2, 5, and 7 days after intraperitoneal injection of 10.1 μCi 1-^{3}H-hexadecanol expressed as disint./min per g fresh weight of each tissue. The ascites cells contained 74%, 26%, and 26% of the injected dose on the 2nd, 5th, and 7th day after injection, respectively. All other tissues contained less than 3% of the injected dose at all three time periods.

1028069

days after injection. Other organs contained less than 3% of the administered dose at all time periods. Two days after injection, 98% of the administered dose was recovered; recovery dropped to 72% (5th day) and 61% (7th day) at the later periods because the tritium was lost by excretory processes. All of the recovered tritium was found in either lipid or water (Fig. 53) in all tissues examined. Only the tumor cells contained major percentages of tritium incorporated as lipid, whereas in other tissues the tritium was mainly present in water. When high levels of tritiated water were injected intravenously into rats, essentially no tritium exchanged between water and other cellular components. These data indicate that 1-^{3}H-fatty alcohols might be useful for localizing tritium in radiosensitive tumor cells.

Biochemical Studies of Human Leukemic Cells

We have recently initiated a project on lipid metabolism in blood cells from patients with leukemia and erythrothrombocythemic disorders (M. L. Blank, M. Clevenger, F. A. Goswitz, B. Malone, and F. Snyder). The primary aim of this work is to determine whether human blood cells considered malignant have characteristic abnormalities of lipid metabolism that are seen in nonhematologic malignancies. The blood samples under investigation include intact cells, homogenates, and subcellular fractions from lymphocytes, granulocytes, erythrocytes, platelets, and whole blood.

Preliminary results from eleven different patients have demonstrated that alkyl glycerolipids are synthesized from long-chain fatty alcohols by intact leukemic lymphocytes and granulocytes and their cell-free homogenates. Typical data are depicted in Table 44. Maximal synthesis occurred after about 1 hr of incubation. The ether-linked aliphatic moieties of the glycerolipids synthesized by leukocytes from patients with chronic granulocytic and lymphocytic

Table 44
BIOSYNTHESIS OF ALKYL GLYCEROLIPIDS BY CELL-FREE HOMOGENATES OF LEUKEMIC CELLS*

Patient number	Picamoles per mg protein	Picamoles per million cells
	Granulocytes	
1	477	12.7
2	649	23.7
3	842	37.3
	Lymphocytes	
4	495	34.0

*Other cell fractions did not synthesize alkyl glycerolipids. Incubations contained dihydroxyacetone-P, 1-^{14}C-hexadecanol, CoA, ATP, Mg^{++}, and homogenate in phosphate buffer at pH 7.1. All values have been corrected for appropriate cofactor controls.

leukemias are similar to those found in nonhematologic neoplasms (mainly 16:0, 18:0, and 18:1) except that the leukemic cells also contain a significant quantity of longer carbon chains (Tables 45 and 46). Our data indicate that the leukemic cell is an excellent system for investigating the ether-lipid pathways and their regulation in human subjects with neoplasms. Continued work on this project is contemplated.

Table 45

CHRONIC LYMPHOCYTIC LEUKEMIA: COMPOSITION OF *O*-ALKYL AND *O*-ALK-1-ENYL MOIETIES OF GLYCEROLIPIDS* FROM LYMPHOCYTES AND ERYTHROCYTES

Chain length	Alkyl		Alk-1-enyl	
	Lymphocytes	Erythrocytes	Lymphocytes	Erythrocytes
16:0	28.8	28.0	27.4	25.6
17:0 + 16:1 + 17:1	1.9	2.0	5.[illegible]	2.7
18:0	15.4	29.5	29.2	44.8
18:1	32.0	25.6	28.9	21.4
18:2	tr	tr	1.2	1.0
20:0	1.6	1.2	1.3	1.1
20:1	2.9	1.3	1.9	1.0
20:2	1.2	tr	1.7	tr
22:0	tr	tr	tr	tr
22:1	16.2	12.4	tr	tr
A	tr	tr	3.3	2.4

*Derived from total lipids extract

Table 46

CHRONIC GRANULOCYTIC LEUKEMIA: COMPOSITION OF *O*-ALKYL AND *O*-ALK-1-ENYL MOIETIES OF GLYCEROLIPIDS FROM GRANULOCYTES AND ERYTHROCYTES

Chain length	Alkyl		Alk-1-enyl	
	Granulocytes	Erythrocytes	Granulocytes	Erythrocytes
16:0	22.8	19.3	26.0	23.2
17:0 + 16:1 + 17:1	2.8	Present	2.0	1.7
18:0	20.1	18.2	39.9	44.5
18:1	29.2	38.5	12.1	16.7
18:2	tr	tr	tr	tr
20:0	3.7	tr	5.5*	1.7
20:1	2.7	tr	2.9	2.6
20:2	2.4	tr	2.7	tr
22:0	1.7	tr	1.1	tr
22:1	12.2	24.0	3.5	tr
A	2.4	tr	4.3	9.6

1028070

Biomembranes: Composition, Metabolic Turnover, and Enzymic Properties of the Endoplasmic Reticulum

Liver Microsomes. The endoplasmic reticulum of rat hepatocytes can be divided into rough- and smooth-surfaced components. Other workers have shown that it is a dynamic structure with continually changing constituents and enzyme activities; the total proteins of both rough- and smooth-membrane fractions have identical turnover rates with a mean half-time of 2.0 days, whereas the relative rates of degradation of individual protein species are heterogeneous. The present study (T-c. Lee, A. Moehl, F. Snyder, and N. Stephens) was undertaken to evaluate the distribution and turnover of acyl moieties at the 1- and 2-positions of phosphatidylcholine and phosphatidylethanolamine, and the degradation of the major phosphoglycerides of the smooth and rough endoplasmic reticulum of normal rat liver, separated by zonal centrifugation. In addition, the distribution of several key enzymes in lipid metabolism (1-acylglycerol-3-phosphate acyltransferase, acyl CoA synthetase, acyl CoA hydrolase) and reactions with 1-^{14}C-oleic acid, an indicator of acyltransferase and phospholipase activities, were determined for the submicrosomal fractions. We found the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in the submicrosomal fractions to be very similar to that reported for whole rat livers. The 1-position is predominantly occupied by saturated fatty acids and the 2-position by unsaturated fatty acids. The relative rates of degradation for the phospholipid classes in the rough microsomes are the same as those in the smooth microsomes. However, the different phospholipid species are degraded differently within a given subfraction. Furthermore, acyl moieties esterified at the 1-position of phosphatidylcholine and phosphatidylethanolamine turned over faster than those esterified at the 2-position (Table 47). A delicate balance of synthetic and degradative enzymes apparently accounts for the similar gross features of lipid composition and turnover in the two microsomal subfractions derived from the endoplasmic reticulum. For example, the specific activities of 1-acylglycerol-3-phosphate acyltransferase, palmityl CoA hydrolase, and palmityl CoA synthetase are higher in the smooth-surfaced microsomes (Table 48). Yet when measuring in vitro exchange (indicative of phospholipase and total acyltransferase activities) with 1-^{14}C-oleic acid, the smooth microsomes incorporated more ^{14}C-oleic acid into phosphatidylethanolamine than the rough microsomes, but essentially no difference was observed for other lipid classes within the two microsomal subfractions. The results obtained in vivo and in vitro support the concept that the endoplasmic reticulum is a mosaic array of structural lipoproteins and enzymic proteins in a dynamic state of heterogeneous turnover.

Preputial Gland Tumor Microsomes. Cholesterol represents an important constituent of all mammalian membranes. Knowledge of its dynamic status during neoplastic growth could provide clues on the structural and functional role of sterols in the formation of newly synthesized membranes. Cholesterol: acylCoA acyltransferase activity, assayed in fractions from mouse preputial

gland tumors, we found to exist mainly in the 100,000 x g sediment (microsomal fraction) and to a lesser extent in the 15,000 x g sediment (mitochondrial fraction). CoA and ATP were necessary for esterifying cholesterol and palmitic acid, and the reaction was stimulated by Mg^{++} and bovine serum albumin. The pH optimum was close to 8. The microsomal enzyme esterified the membrane cholesterol as well as the cholesterol added to the incubation mixtures. Both cholesterol pools were esterified linearly with time, indicating that they equilibrate rapidly to form a single pool accessible to the enzyme (Fig. 54). The enzyme preferred palmitic acid or pentadecanoic acid as substrate over oleic and linoleic acids. Our data suggest that membrane-bound cholesterol is not solely a structural constituent of the endoplasmic reticulum in tumors (M. R. Grigor and F. Snyder).

Table 47

RELATIVE TURNOVER OF FATTY ACIDS ESTERIFIED AT 1- AND 2-POSITIONS OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDYLETHANOLAMINE IN MICROSOMAL SUBFRACTIONS OF RAT LIVERS AFTER THE INTRAPERITONEAL ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE*

Cell fraction	Ratio $^{3}H/^{14}C$			
	Experiment 1		Experiment 2	
	1-Position	2-Position	1-Position	2-Position
	Phosphatidylcholine			
Rough-surfaced microsomes	0.68	0.19	1.98	0.44
Smooth-surfaced microsomes	0.66	0.24	2.03	0.47
	Phosphatidylethanolamine			
Rough-surfaced microsomes	0.55	0.20	1.47	0.34
Smooth-surfaced microsomes	0.53	0.24	1.55	0.36

*Two rats weighing approximately 150 g were injected with 200 μCi of sodium acetate-2-^{14}C; 63 hr later, each rat was injected with 500 μCi (experiment 1) or 1 mCi (experiment 2) of sodium acetate-2-^{3}H. The animals were killed 4 hr later.

The Biochemistry of Developing Brain

The very high content of plasmalogens in nerve tissue (20-50% of the total phospholipids) indicates that they have an important biological function in these cells. Furthermore, the well-established correlation between myelination and plasmalogen formation emphasizes that the ether-linked lipids must play an important role in processes that cause demyelination. Several laboratories have suggested the ether bond is prominent in surface membrane lipids; the quantity

1028071

and their arrangement could be responsible for the relative stability of membranes from different cell types. Biological properties attributed to lipids that possess ether bonds, e.g., transient muscular relaxation and paralysis, also suggest that these compounds are capable of exerting potent pharmacological effects in the central nervous system.

Table 48

DISTRIBUTION OF 1-ACYLGLYCEROL-2-PHOSPHATE ACYLTRANSFERASE, PALMITYL CoA SYNTHETASE, AND PALMITYL CoA HYDROLASE IN SMOOTH- AND ROUGH-SURFACED MICROSOMES OF NORMAL RAT LIVER*

Fraction	nmoles/min per mg protein	nmoles/min per mg lipid	nmoles/min per μg lipid phosphorus
	1-Acylglycerol-3-phosphate acyltransferase		
Total microsomes (3)	9.8	23.0	0.83
Rough-surfaced microsomes (3)	12.4	27.4	1.05
Smooth-surfaced microsomes (3)	21.2	59.4	1.49
	Palmityl CoA synthetase		
Total microsomes (3)	5.5	12.5	0.53
Rough-surfaced microsomes (3)	5.7	12.2	0.46
Smooth-surfaced microsomes (3)	6.8	15.2	0.51
	Palmityl CoA hydrolase		
Total microsomes (2)	29.1	78.9	2.6
Rough-surfaced microsomes (2)	20.2	45.5	1.7
Smooth-surfaced microsomes (2)	33.5	99.7	2.5

*The numbers in parentheses refer to the number of experiments used to obtain average values indicated under each activity heading. The reaction products of 1-acylglycerol-3-phosphate acyltransferase were isolated on Silica Gel HR layers with chloroform-methanol-NH_4OH (60:35:8, v/v). The reaction products of palmityl CoA hydrolase were isolated on Silica Gel G with hexane-diethyl ether-acetic acid (50:50:1, v/v).

The aim of this on-going project is to determine the role of ether-linked lipids and the enzymic and hormonal factors controlling their biosynthesis and biocleavage in nerve tissue during myelination and demyelination processes (O. E. Bell, Jr.,* M. L. Blank, M. Hibbs, and F. Snyder). Neurogenic properties of the ether-linked glycerolipids and the physiological significance of these properties will also be assessed. Under this project the effect of irradiation, disease, and genetic mutations on lipid metabolism of nerve tissue is being evaluated.

*From the Department of Biochemistry, University of Mississippi Medical Center, Jackson

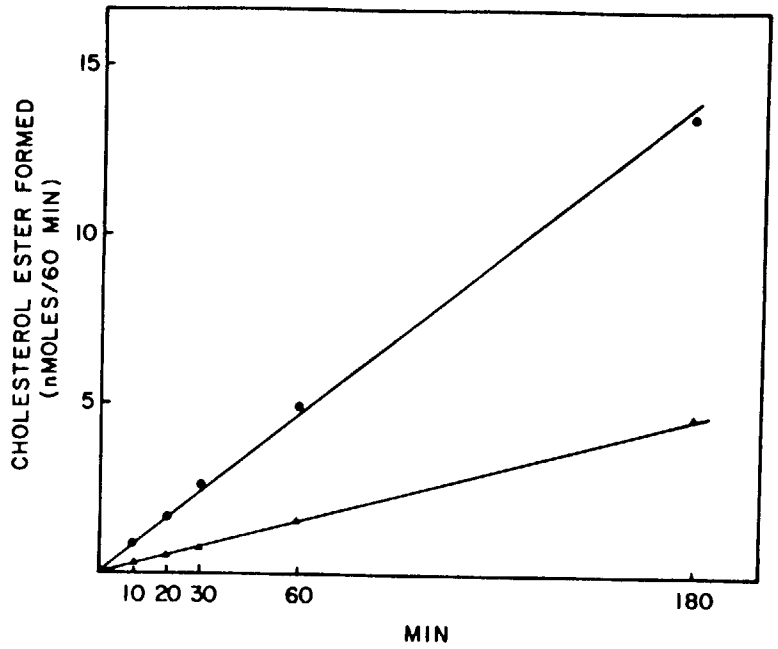


Fig. 54. Effect of time on cholesterol ester synthesis from membrane cholesterol, labeled with 7-^{3}H-cholesterol (●), and from added 4-^{14}C-cholesterol (50 nmoles) (▲). Each value represents the mean of duplicate incubations.

Enzymic Synthesis of Alkyl Glycerolipids in Brain and Liver of Rats During Fetal and Postnatal Development. Brain tissue is rich in plasmalogens, primarily as the ethanolamine type. On the other hand, nerve tissue contains only small amounts of alkyl glycerolipids, but they could have an important role as precursors of the plasmalogens. Figure 55 shows the relative activities of the *O*-alkyl-synthesizing enzymes in the microsomal fraction of brains and livers of rats at various stages of development. The same general trend in the enzymic activities is apparent for both brain and liver, although the latter is lower at most of the ages. The highest activity for liver appears 1 day before birth; activities then and 1 day after birth were about the same for brain, whereas they reached a maximum at 5 days. The activities for both tissues gradually declined and by 45 days reached a minimal level. However, even the maximum activities (1.0–1.5 nanomoles/mg protein per hr) for brain and liver in these experiments are extremely low when compared to the activities of the *O*-alkyl-synthesizing enzymes (8–20 nanomoles/mg protein per hr) in neoplastic cells.

The similar activities of the *O*-alkyl-synthesizing enzymes in brain and liver

1028072

microsomes suggest that the levels of ether-linked lipids in tissues can be controlled by enzymic systems that cleave the ether-linked moieties prior to their conversion to other compounds. For example, the *O*-alkyl cleavage enzyme (a hydroxylase requiring tetrahydropteridine) is very active in rat liver, whereas we have found that it is essentially lacking in neoplastic cells and other tissues that are rich in the ether-linked lipids. This same relationship is reflected in the present experiments with the 5-day-old animals. At this age, the alkylglycerol cleavage enzymic activities were 0.25 nanomole/10 min per mg protein for brain microsomes and 58 nanomoles/10 min per mg protein for liver microsomes.

Our data demonstrate that the *O*-alkyl-synthesizing enzymes in brain and liver are most active during the early stages of growth and development. The findings are compatible with the idea that the alkyl glycerolipids are precursors of plasmalogens in nerve tissue since the activity of *O*-alkyl-synthesizing enzymes is highest during the period of most active myelination, a time that is known to reflect maximum plasmalogen accumulation.

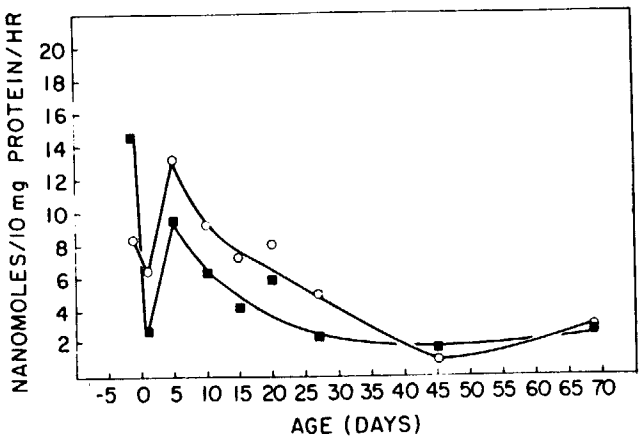


Fig. 55. The enzymic synthesis of alkyl glycerolipids in the 100,000 x g fraction of brains (○) and livers (■) of rats at various ages. The radioactivity in the ^{14}C-alkylglycerols was determined after $LiAlH_4$ reduction of the total lipids extracted from the incubation mixtures. The overall range of the experimental values for duplicate incubations was between 0.1 and 1.6 nmoles/10 mg protein. Gas-liquid chromatography of the isopropylidene derivatives of the alkylglycerols revealed that between 70 and 80% of the ^{14}C activity was associated with the O-hexadecyl derivative. The ratio of alkyl to alk-1-enyl glycerolipids in brain decreased from 0.17 in the 10-day-old rats to 0.10 in the 70-day-old rats; the content of ether-linked lipids was too low for quantitation in liver.

The Incorporation of ^{18}O and ^{14}C from Long-Chain Alcohols into the Alkyl and Alk-1-enyl Ethers of Phospholipids of Developing Rat Brain in vivo. In tissues other than nerve tissue, considerable in vivo data have been obtained that indicate an alkyl glycerolipid is the precursor of ethanolamine plasmalogens. As a result, we undertook the present study to clarify the role of alkyl glycerolipids as a precursor of alk-1-enyl glycerolipids in developing rat brains.

Data in Table 49 provide evidence that the 1-^{14}C-hexadecanol is incorporated into both the alkyl and alk-1-enyl moieties of the phospholipids. The declining ratio of ^{14}C-labeled alkylglycerols to ^{14}C-labeled alk-1-enylglycerols from 6.58 at 2 hr to 2.25 at 48 hr reflects the conversion of *O*-alkyl moieties to *O*-alk-1-enyl moieties in glycerolipids of rat brain. Our data show that in the total phospholipids the radioactivity in *O*-alkyl groups always exceeds that in *O*-alk-1-enyl groups over a 48-hr period. Between 2 and 48 hr the alkylglycerols lose 5.7×10^4 disint./min while the radioactivity in the alk-1-enylglycerols gains 4.8×10^4 disint./min, suggesting that alkyl ethers are being converted to alk-1-enyl ethers. Our experiments also show that the oxygen of ^{18}O-labeled

Table 49

INCORPORATION OF RADIOACTIVITY FROM 1-^{14}C-HEXADECANOL INTO ALKYLGLYCEROLS AND ALK-1-ENYLGLYCEROLS DERIVED FROM PHOSPHOLIPIDS AFTER $LiAlH_4$ REDUCTION

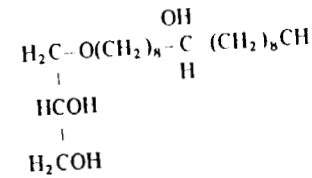
	2 hr	6 hr	24 hr	48 hr
Alkylglycerol (disint./min x 10^{-3})	251.50	302.00	216.25	194.50
Alk-1-enylglycerol (disint./min x 10^{-3})	38.25	79.50	84.25	86.50
Alkylglycerol/ alk-1-enylglycerol	6.58	3.80	2.57	2.25

hexadecanol was incorporated into both the *O*-alkyl and *O*-alk-1-enyl lipids of developing rat brain. The mass spectrum of the isopropylidene derivative of ^{18}O-hexadecylglycerol and hydrogenated ^{18}O-hexadec-1-enylglycerols isolated from brains after administering ^{18}O-hexadecanol shows that 11-12% of the alkyl oxygen contains the ^{18}O isotope and that 2-3% of the alk-1-enyl oxygen contains the ^{18}O isotope. Thus the oxygen from hexadecanol is incorporated into both the alkyl and alk-1-enylglycerol lipids of developing rat brain. The distribution compares favorably with the ^{14}C data in that after 24 hr 15.6% of the ^{14}C activity was in the alkylglycerols and 6.03% of the activity was in the alk-1-enylglycerols.

1028073

Newly Identified Lipid Classes

Hydroxy-Substituted Alkyl Glycerolipids. During the course of our investigation of glandular tissue lipids, we identified a new class of alkyl glycerolipids in the triglyceride fraction of lipid extracts of pink harderian glands of rabbits (M. L. Blank, K. Kasama,* W. T. Rainey, Jr.,† and F. Snyder). After $LiAlH_4$ reduction, the esterified lipid yields the following structure:



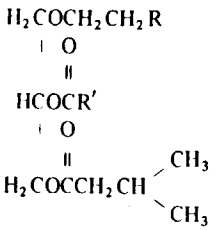
Identification was based on chromatography, infrared spectroscopy, and mass spectra of various derivatives and on comparisons to standards prepared chemically. The hydroxyl group on the *O*-alkyl moiety is attached to carbon atoms 11 or 12. All the hydroxyl groups are thought to be esterified with aliphatic moieties in the natural state and it appears that one of the acyl moieties contains less than ten carbon atoms.

An Alkyldiacylglycerol that Contains Isovaleric Acid Exclusively at the 3-Position of the Glycerol Moiety. *O*-Alkyl moieties in glycerolipids exist primarily as alkyldiacylglycerols and as ether analogs of phosphatidylcholine and phosphatidylethanolamine. In mammalian cells, the *O*-alkyl chains consist of 14, 16, 18, and 20 carbon atoms and the *O*-acyl moieties range from C_{12}–C_{24}. During the course of a biochemical investigation of rabbit harderian glands, a rich source of *O*-alkyl lipids, we detected a unique alkyldiacylglycerol fraction that contained a significant quantity of a short-chain acyl moiety, identifying it as 1-alkyl-2-acyl-3-isovalerylglycerol by chemical, enzymic, chromatographic, and physical procedures. More than 50% of the *O*-alkyl moieties consist of 16:0 and 18:0 carbon chains, whereas the other major *O*-alkyl moieties are 15:0 and 17:0 branched chains (≃30%). The acyl groups in the 2-position of the glycerol portion of the alkyldiacylglycerol subclass consist primarily of saturated fatty acids (60% 16:0 and 30% 18:0) and a small amount of branched chain fatty acids (≃5%), whereas the 3-position is exclusively occupied by isovaleric acid.

*From the Department of Public Health, Mie Prefectural University School of Medicine, 174, 2-chome Edobashi, Tsu, Japan

†From the Analytical Chemistry Division, Oak Ridge National Laboratory

The formula of this unique subclass of *O*-alkyl lipids is shown below:



(R = primarily 16:0, 18:0, 15:0 br, and 17:0 br)

(R′ = primarily 16:0 and 18:0)

Although isovaleric acid has previously been detected in triacylglycerols from the mandibular canal of a porpoise, our work is the first to describe its presence in a specific acyl position of alkyldiacylglycerols. Furthermore, the significant proportion of branched *O*-alkyl moieties in this lipid class indicates that some tissues containing isovaleric acid might also be selectively incorporated into long-chain fatty alcohols (precursor of the *O*-alkyl moiety).

MARMOSET RESEARCH PROJECT

The Medical Division maintains one of the few research colonies of marmosets in the country. These small South American primates are biologically unique because of their high frequency of fraternal twinning. To the transplantation immunologist this feature is further enhanced by the placental vascular anastomosis that occurs between the twins and ultimately leads to hemopoietic chimerism in the young. Our previous studies (ORAU-107, p 259; ORAU-110, p. 144; ORAU-113, p. 157) have characterized this chimerism extensively, i.e., frequency of occurrence, degree, hemopoietic elements involved, stability, and tolerance induction. Our more recent efforts have concentrated on defining immunologic capabilities of these animals, under both in vivo and in vitro conditions. Challenge of these animals to particulate antigens has revealed an essentially normal system, although their responses, when compared to other species inoculated with similar antigens, suggest a subnormal immune mechanism. Previous skin allograft studies suggested that the taxonomic classification schema devised for these animals by Hershkovitz (*Folia Primat.* 4: 381-395, 1966) has an immunogenetic basis; further analysis of the interrelationships of four subspecies (of *S. fuscicollis*) by in vitro mixed lymphocyte culture (MLC) tests has yielded analogous information. Thus, MLC (blood) tests between more distantly related animals have shown high activity (as measured by label incorporation), while MLC reactions between more closely related animals have yielded data which do not necessarily reflect histoantigenic

1028074

differences. One of the difficulties encountered in the use of blood lymphocytes for the MLC test has been the observation that hemic precursors can arise from these elements during in vitro culture, some persisting for over 50 days. We are continuing to investigate this peculiar property of these cells, particularly bone marrow, since there is a greater probability of obtaining long-term in vitro cultures with this tissue.

In a continuing program aimed at monitoring the various disease entities encountered among our marmosets, necropsies and histopathologic examinations are made of all animals dying in the colony. This has resulted in the surprising finding of a high incidence of spontaneous mucinous adenocarcinoma of the small intestine. Three cases were found in 21 necropsies of imported *S. oedipus* marmosets; a fourth was found in a young lab-bred marmoset, *Callimico goeldii*. In addition to detection of this intestinal cancer, our examinations of laboratory-bred animals dying in the colony suggest that the major cause of death is a bacterial gastrointestinal infection that is fatal to young animals, two to five months of age. Having shown this directly, we should now be able to devise specific preventive and therapeutic regimens to overcome these infections during this critical period of growth.

Antibody Formation in Marmosets to Sheep Erythrocytes and *Salmonella typhosa* Organisms

The immune competence of the marmosets was demonstrated in previous studies by their ability to reject allografts from unrelated animals in a manner suggesting a completely normal system. The present study (Nancy Basford* and N. Gengozian) was undertaken to further characterize the immunologic mechanism of the marmoset by measuring its humoral antibody response to multiple injections of heterologous particulate antigens.

Marmosets were injected intramuscularly with either *Salmonella typhosa* organisms or sheep erythrocytes (SRBC). For *S. typhosa*, groups of marmosets were subdivided to receive three different antigen doses, and for the SRBC antigen two different concentrations were used. We gave a series of four injections for each antigenic dose and bled the animals for serum at varying intervals after each injection. The serum samples were tested for agglutinating and/or hemolytic antibodies and the molecular species of the antibodies characterized by treatment of the serum with 2-mercaptoethanol (2-ME), sucrose-density gradient centrifugation, and Sephadex G-200 gel filtration. On the basis of their agglutinating properties, individual serum samples were selected for formation of antigen-antibody complexes in vitro which were then analyzed for their immunoglobulin components by specifically prepared reagents.

*Graduate student

Since the animals responded similarly to the three different doses of *S. typhosa*, we combined the data. As shown in Fig. 56, the response was characterized primarily by the formation of IgM antibodies as revealed by their sensitivity to 2-ME. They did not form significant amounts of IgG antibody (2-ME resistant) after the first injection; although a second and third injection induced the smaller molecular-weight antibody, this appeared in the serum for only a short time, and then dropped to undetectable levels. Sucrose-density gradients and gel filtration studies verified the IgM character of the antibodies suggested by the 2-ME sensitivity tests. In contrast, the response to SRBC antigens was predominated by the formation of IgG antibodies. As with the *S. typhosa*, the two different concentrations (10% and 50%) gave similar results. Although IgM antibodies were synthesized throughout the response, the IgG antibodies appeared 1 week after the primary injection and remained in high titers thereafter. In defining the in vitro requirements (complement source, dilution) for optimal detection of hemolytic antibodies, we found that "natural" hemolysins and both early immune and hyperimmune IgM hemolysins lost their hemolytic activity upon heating at 56°C for 30 min. The SRBC hemagglutinins and the SRBC IgG hemolysins and agglutinins retained their activity under the same conditions.

During the course of this study a prozone (zone agglutination inhibition) appeared in some of the hyperimmune antityphoid antisera. Antigen-antibody complexes were then prepared from sera in this zone of inhibition and injected into rabbits with Freund's adjuvant; the antisera were then tested by immunodiffusion and immunoelectrophoresis against whole marmoset serum. As shown in Fig. 57, the immune complexes stimulated the formation of antibody

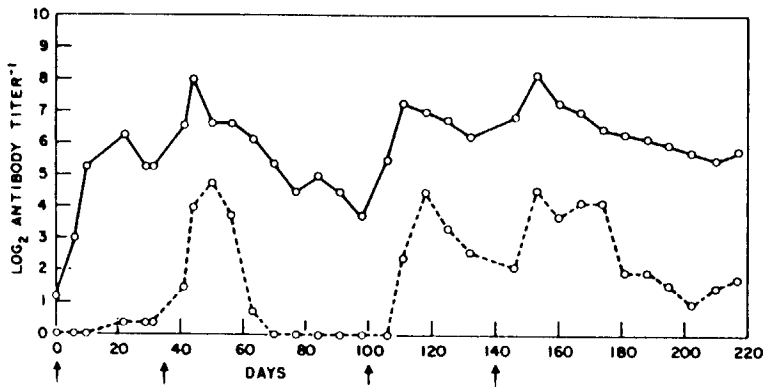


Fig. 56. Mean agglutinin titers of marmosets immunized with multiple injections of typhoid vaccine. Titration before (o—o) and after (o- -o) treatment of sera with 2-ME. ↑= day of injection.

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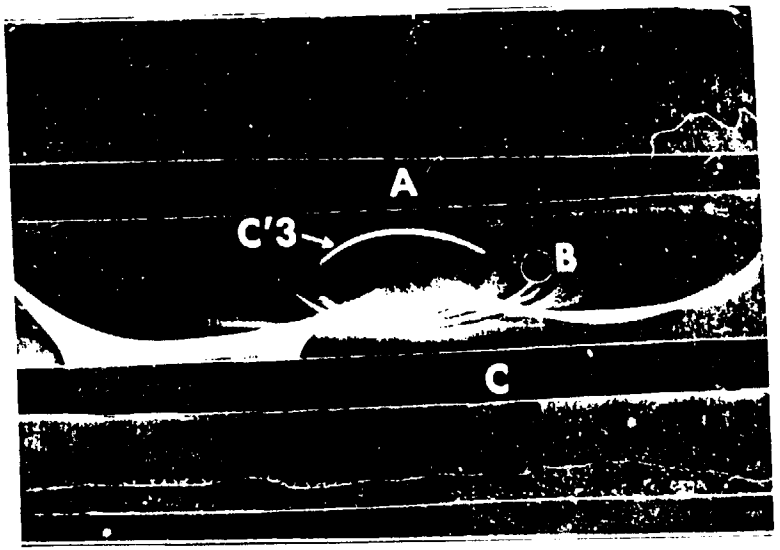


Fig. 57. Antiserum obtained from rabbit immunized with prozone (typhoid antigen-antibody system) immune complex has been placed in the upper trough (A) and reacted with gradient fractions of marmoset serum (B) following electrophoretic migration. Trough C contains a rabbit antiserum to normal marmoset serum. The primary protein identified by antiserum (A) is the third component of C'3.

against the third component of complement (C'3) and only trace activity to the IgG immunoglobulin was detectable. In contrast, immune complexes prepared from nonprozone antisera contained IgG, IgM, and C'3 as revealed by the antisera produced after their injection into rabbits (Fig. 58).

Although the marmosets responded to the two antigens, the titers of antibody were less than that observed for other species (e.g., mouse, rat) under similar conditions. Further, the characteristic sequential conversion from IgM synthesis to IgG synthesis was not seen with the *S. typhosa* challenge until the third injection. In the mouse and rat this occurs within the first 2 weeks after a single injection. The significance of this is yet to be defined. The finding of greater amounts of C'3 than either IgG or IgM immunoglobulins attached to the antigen (typhoid particle) in antisera demonstrating a prozone phenomenon is of considerable interest. The role of C'3 in prozone phenomena in this and other antigen-antibody systems is not known. However, binding of C'3, IgG, and IgM in nonprozone complexes has been observed in other systems.

Radiosensitivity of Marmosets to Total-Body Irradiation

Paralleling the in vivo studies on leukocyte chromosome aberrations (see p. 163), we obtained data on the radiosensitivity of the laboratory-bred marmo-

sets (N. Gengozian, J. G. Brewen,* and J. Batson). Previously we found the marmoset to be one of the most sensitive animals, having a 30-day LD_{50} of approximately 200 R. Although the time of death and various hematologic parameters indicated injury to the hemopoietic system was the primary cause of death, we had some reservations because those were parasitized, imported marmosets. All animals used in the present study were laboratory-bred and parasite free. Although the numbers irradiated are still too limited to make an unequivocal assessment, the mortality and hematologic response suggest that the laboratory-bred marmoset is as sensitive as the imported animal. Of four receiving 100 R, one died on day 31, and one of three receiving 200 R died on day 16. Two others receiving 300 R and 400 R died on days 12 and 10 respectively. The hematologic response as measured by red blood cells, white blood cells, and platelets paralleled the previous studies for each radiation dose with one exception, i.e., recovery of these elements appeared to occur earlier in the laboratory-bred animal.

We had previously speculated that hemopoietic chimerism may play a role in

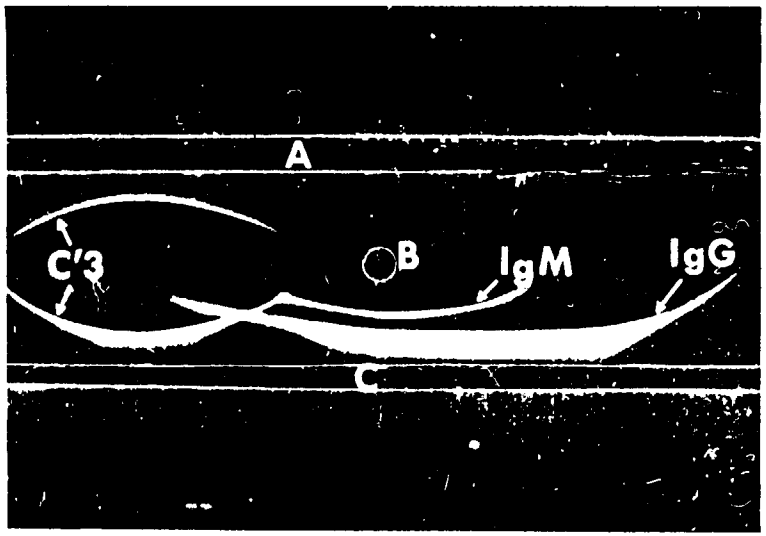


Fig. 58. Antiserum obtained from rabbit immunized with nonprozone (typhoid antigen-antibody system) immune complex has been placed in the lower trough (C) and reacted against whole marmoset serum (B) following electrophoretic migration. Trough (A) contains a goat antiserum to human C'3.

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1028076

this radiation sensitivity in that the immunologic tolerance that exists between the two genetically different systems may be disrupted by radiation. With this thesis, therefore, at varying intervals before and after radiation we drew blood for chimerism evaluation by sex chromosome analyses. Of the nine animals in this study, four died from radiation injury and only among this group were there any significant changes in the chimerism status. In two, there was a significant decrease at the time of death, and in a third a marked increase was noted immediately after radiation. Cells from this animal and another at time of death were not suitable for scoring. Whether the chimerism changes and mortality itself were related to any immunologic phenomenon is yet to be demonstrated; however, the observations dictate additional studies be made to identify this possibility.

Culture of Hemopoietic Marmoset Tissue

We have expanded the study of marmoset hemopoietic cultures to include the colonization of the various differentiating cell types in semisolid (soft-agar) medium to learn more about the transitions of the nonadherent populations of mixed cells (Raymond P. Porter and N. Gengozian). As with the liquid cultures, the discrete colonies in a soft-agar medium survived 7 or 8 weeks. Morphologic differences of colonies developing in the semisolid medium were seen with time. Diffuse colonies, made up of cells resembling macrophages morphologically and by their migrational and phagocytic properties, expanded over broad areas in the agar. Large colonies (up to 8 mm in diameter at 6 or 7 weeks) having compact centers and progressively more diffuse cell growth peripherally, contained cells of the myelocytic series. Other tightly compacted colonies with discrete perimeters were composed of more primitive, less differentiated cell types. We found megakaryocytes in the excess fluid medium under and over the agar layer but not as colonies. Adherent cell populations grew on the bottom of the petri dish under the agar. Many colonies containing only 20–30 cells terminated during the time that other colonies were undergoing cellular expansion. Few of the initial number of colonies increased extensively; these were the myelocytes and an occasional nondifferentiated cell colony, the cell types in liquid medium culture shown to be the most prevalent.

We have shown by various markers that marmoset blood lymphocytes, polymorphonuclear neutrophil leukocytes, and red blood cells can be a chimeric mixture but a marker for thrombocytes has not been reported. Evidence for chimerism is now suggested for the thromobocyte precursors by the finding that polyploid metaphases from marrow cultures containing free-floating cells believed to be megakaryocytes can be scored by a cytogenetic marker, a technique not easily possible with direct marrow preparations. We scored $n = 4$, $n = 8$, and $n = 16$ metaphases as male or female by either the presence or absence of multiple, small metacentric Y "male" chromosomes. One culture containing megakaryocytes yielded 18 well-spread polyploid metaphases. Four (22%) of these polyploid metaphases had male markers. When 100 diploid metaphases

from the same slides were analyzed, nearly the same percentage (18%) were scored male. Presumably the majority of the diploid metaphases from this 17-day-old marrow culture from a female are divisions of promyelocytes or more primitive cells since they were the prominent nonadherent cell type at that time.

The cell types of the adherent population of cells from liquid cultures of marmoset marrow or blood were also scrutinized more closely during the past year. With an inverted phase-optical system, we frequently saw numerous nests or clusters of round, adherent cells which increased in diameter with time. They were allowed to develop on a cover glass introduced into the culture vessel, and after 5 to 6 days the cover slips were recovered, the cells fixed in methanol, and stained with Wright's stain. Figure 59 shows the low power magnification of one of the larger clusters of these adherent cells. We always observed a large nucleus associated with these clusters. When a cover slip was recovered from a culture that had had clusters forming on it for only 3 days many areas were seen that had only beginning clusters (Fig. 60). The detail of the cytoarchitecture shown in this phase-microscope view was preserved in this preparation by fixing the cells with 2% gluteraldehyde in 0.1 M cacodylate buffer and Wright's stain. One 9 x 22 mm cover slip from this culture had approximately 50 clusters; some of the round cells were in division. The origin and identification of these large cells that seem to attract other cells remain obscure. Their precursors are passed with

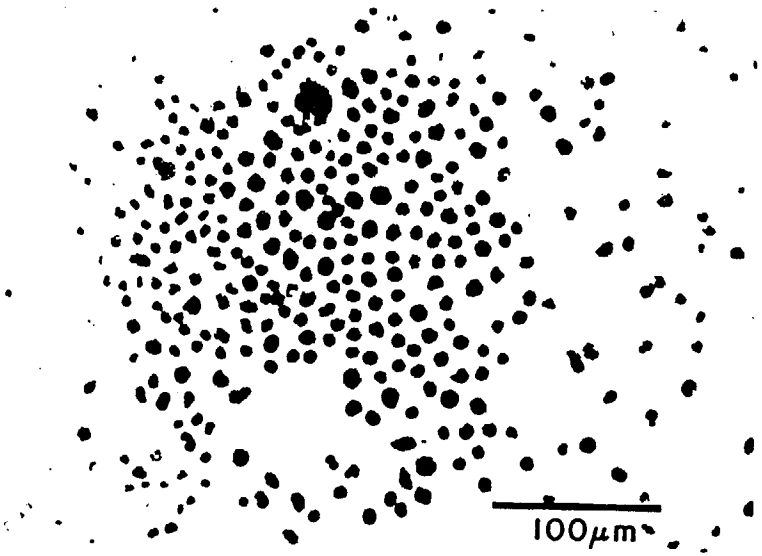


Fig. 59. Photomicrograph of a cluster of round cells growing among the mixed adherent population of cells in marmoset hemopoietic cultures. Methanol fixation, Wright's stain.

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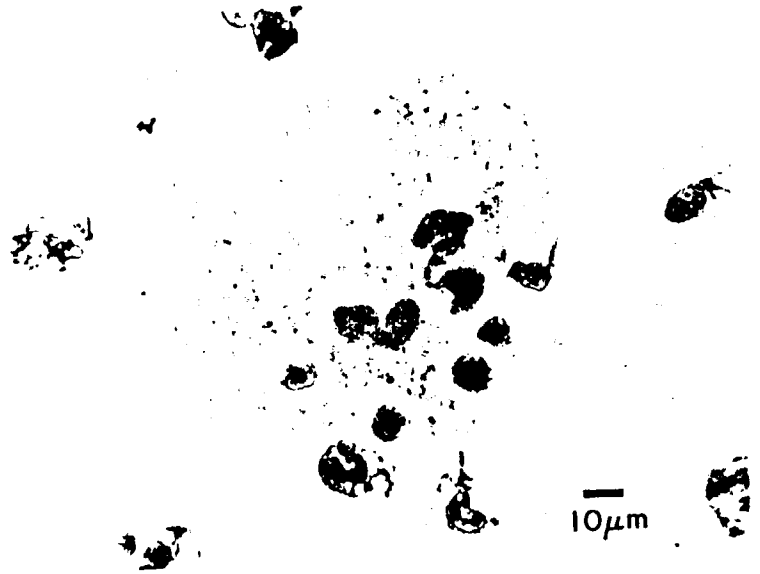


Fig. 60. Phase contrast photomicrograph of an early cluster-forming adherent cell. Round cells similar in appearance to some of the free-floating population appear to nest on the broad cytoplasmic extension of these unidentified cells Gluteraldehyde fixation, Wright's stain.

the nonadherent cell population and new clusters form in the next culture vessels. They appear only during the most proliferative stages of the cultures.

We have previously reported (ORAU-113, p. 155) on the use of the in vitro mixed lymphocyte culture (MLC) as an index of histocompatibility in the marmoset. As anticipated from our early skin-graft studies, no stimulation (measured by ^{14}C-thymidine incorporation) occurred in mixtures of lymphocytes from chimeric, fraternal co-twins. In MLC reactions involving cells from unrelated animals, lymph node lymphocytes of the white-lipped tamarin (W–L) were stimulated almost equally by allogeneic and xenogeneic cotton-top (C–T) cells.

The response of C–T lymphocytes in allogeneic MLC reactions was investigated this year. In contrast to what was observed with the W–L system, the MLC reactions within the C–T species showed little stimulation, i.e., there was little difference between the response to allogeneic cells in comparison to their own; no definite peak response occurred up to 8 days. The allogeneic MLC response for these two species of marmosets when compared to the in vivo data for skin-graft survival time (ORAU-110, p. 144) correlates well. For example, the mean survival time (MST) of intraspecies skin grafts in the C–T tamarin was greater than 70 days and C–T intraspecies MLC reactions showed weak

stimulation; with the W–L tamarin, the MST for intraspecies grafts was 38 days and the MLC tests showed a good response to intraspecies stimulation.

The MLC tests are now being extended to use blood lymphocytes instead of solid-tissue lymphoid cells because of the convenience of working with these easily obtainable cells. We reported last year that without exogenous histoantigens some marmoset peripheral blood cell cultures incorporated rather high values of ^{14}C-thymidine after 4–6 days of culture. We have aimed preliminary studies at lowering this high "nonspecific" background and have found that a decrease in the antibiotic level and the concentration of fetal bovine serum (FBS) from 20 to 2% in the culture medium reduced the counts considerably (Table 50). As controls, autologous serum or plasma was used also at these two concentrations; in addition to lowering the counts further, with 2% protein in the medium there was greater incorporation of the label than with 20%, opposite to the findings with FBS. Current studies are aimed at determining whether the low "background" activity provided by the different protein concentrations and the decrease in antibiotics will permit use of the peripheral blood cells for allogeneic and xenogeneic MLC reactions.

TABLE 50

VARIABLES AFFECTING INCORPORATION OF ^{14}C-THYMIDINE INTO BLOOD LYMPHOCYTES OF MARMOSETS DURING IN VITRO CULTURE

Supplements to culture medium (RPNI 1940)	Average counts/min of 1×10^6 peripheral blood lymphocytes labeled at day 5
None	700*
Antibiotic concentration A‡	1400*
Antibiotic concentration B§	600*
Antibiotic concentration B§ + 20% FBS	5200†
Antibiotic concentration B§ + 2% FBS	2560†
Antibiotic concentration B§ + 20% autoplasma	400†
Antibiotic concentration B§ + 2% autoplasma	1520†
Antibiotic concentration B§ + 20% Autoserum	340*
Antibiotic concentration B§ + 2% Autoserum	670*

*Average of three experiments.

†Average of six experiments.

‡Antibiotic concentration A = 100 units penicillin, 100 μg; streptomycin, 2.5 μg Fungizone.

§Antibiotic concentration B = 10 units penicillin, 10 μg streptomycin.

1028078

Spontaneous Mucinous Adenocarcinoma of the Upper Small Intestine of Two Species of Marmosets (*Saguinus oedipus oedipus* and *Callimico goeldii*)

Four instances of spontaneous mucinous adenocarcinoma were found infiltrating and metastasizing from the upper small intestinal (duodenal and jejunal) muscosa of four adult marmosets among 285 necropsied routinely (Gretchen Humason, N. Gengozian, Raymond P. Porter, and C. C. Lushbaugh). Three occurred in imported cotton-top marmosets (*Saguinus oedipus oedipus*) and one in a colony-bred Goeldi's marmoset (*Callimico goeldii*). All had metastasized to peripancreatic, mesenteric lymph nodes.

The rarity of recorded spontaneous neoplasms in nonhuman primates is well known. Although it has led some to believe that these species have a susceptibility lower than that of man, this low incidence seems to be related to the young age at which most captive primate species die or are killed for experimental purposes. Human cancer experience suggests that as more of these animals live to natural old age their true cancer incidence will be revealed particularly as deaths from infections and parasitic infestations are prevented.

The observations of Adamson et al. (*J. Nat. Cancer Inst.* 45, 555, 1970) suggest that marmosets have as high a sensitivity to carcinogens as primitive primates and are not as resistant as higher species of monkeys and apes.

Previously, in a study of the diseases found in marmosets imported into the ORAU marmoset research colony up to 1965, only one neoplasm, a malignant lymphoma, was found in 506 necropsies of young adult, hairy-faced tamarin marmosets (largely *Saguinus fuscicollis* subspecies). In the ensuing 5 years we have studied 285 adult marmoset necropsies among which were 28 young colony-bred animals who died when greater than 1 year of age. The numbers of animals of the various species and subspecies whose necropsy findings are included in the present study are shown in Table 51, along with the four instances we found among them of metastasizing mucinous adenocarcinoma of the small intestine. These four intestinal carcinomas differed from each other only in the characteristics of their local invasion and distant metastases; three occurred in imported cotton-top marmosets (*S.o. oedipus*) of unknown age and one in a relatively young, laboratory-bred animal (*Callimico goeldii*). Although this type of cancer is rare in human beings, its high incidence in this animal along with its high degree of malignancy makes understanding of these spontaneous intestinal neoplasms important to the human cancer problem. Several benign thyroidal adenomas were the only other true tumors found in this set of necropsies.

Case 1. A 45-month-old Goeldi marmoset that was born to a pair of imported animals developed rickets as an infant. It recovered after ultraviolet-ray therapy and was well until several months before death when it began to eat poorly, losing weight progressively. At death the animal had lost 25% of its estimated best weight (~100 g).

At necropsy, the distal duodenum or first port of the jejunum was encircled by a firm, irregularly nodular fibrous mass that decreased the lumen to less than half diameter for about 1 cm. Mesenteric lymph nodes in the region of the middle colic artery seemed to be adherent to this mass and led us to believe that this was some kind of parasitic granuloma. Within the mass the intestinal surface was irregularly ulcerated but not hemorrhagic or tumorous in appearance.

TABLE 51

SPECIES AND NUMBERS OF MARMOSETS NECROPSIED (1965 THROUGH 1971)

Species	Number of Necropsies	Tumors found	
		Small intestinal	Other
1. Bare-faced tamarins			
S. oedipus oedipus	21	3	0
S. leucopus	2	0	0
2. Hairy-faced tamarins			
S.mystax mystax	18	0	0
S. nigricollis	4	0	0
S. fuscicollis ssp.*	86	0	0
S.f. fuscicollis	2	0	0
S.f. nigrifrons	22	0	0
S.f. illigeri	62	0	1
S.f. leucogenys	13	0	0
S.f. lagonotus	20	0	0
S.f. (sp.) colony-bred young adults	27	0	0
3. Pygmy marmoset *Cebuella pygmaea*	2	0	0
4. Goeldi's marmoset *Callimico goeldii* (one colony-bred)	4	1	0
Totals	285	4	1

*Not identified as to subspecies.

Histologically, the walls of the ulcerated small intestine were distorted by dense, sclerotic connective tissue and smooth-muscle hyperplasia in the submucosa, muscle coats, and subperitoneum. The ulcers were located in a mucosa composed of tall, thin villi covered entirely by mucin-secreting cells. A few dark-staining, proliferative crypts remained in their immediate borders. Mucin-rich cells in the necrotic debris and in the ulcer floor had the typical signet-ring appearance of human mucinous adenocarcinoma. These cells were present singly, or as cords of disorganized cells, in submucosal connective tissue, lymphatic vessels, smooth muscle, subperitoneal connective tissue, and scattered in large mucin-filled spaces in adjacent lymph nodes.

1028079

The parents of this animal died of infectious disease 1 and 3 years after it was born. Neither had any tumorous disease.

Case 2. This *S.o. oedipus*, male, was imported as an adult animal of unknown age 6 years before the onset of an emaciating disease that was accompanied by intermittent diarrhea for about 6 months before death. It was used in the laboratory only as a donor source of *S.o. oedipus* serum proteins for immunologic studies and received no injections of proteins or medicines other than oral vermifuge once 5 years before its death.

At necropsy the small intestine, about 5 cm proximal to the cecum, was involved in what grossly appeared to be a gangrenous intussusception. Fecal peritonitis was present. A malignancy was not suspected grossly. The other organs appeared normal.

Histologically, we found a typical, widely infiltrating, mucinous adenocarcinoma of the upper small intestine. This malignancy was histologically identical to that of Case 1 but necrosis and postmortem changes in the intestinal mucosa made identification of the precise location of the initial primary lesion impossible. Although the histology of much of the mucosa was obscured by postmortem autolysis, we found foci of typical adenocarcinoma cells from which cells have ramified through the muscularis mucosae into submucosal connective tissue and elicited an intense desmoplastic reaction as in Case 1.

This carcinoma differed from that in Case 1 in the voluminous nature of its distant metastases in the intestinal muscle layers, subperitoneal connective tissues, adjacent lymph nodes, mesenteric nerves (Fig. 61) and lymphatics of the ureters. These metastases consisted chiefly of mucus-filled lakes in which adenocarcinoma cells were scattered sparsely. The mucus in this and the other cases was identified as being diastase resistant with periodic acid Schiff reaction on sections after incubation of sections with saliva. Alcian blue (at pH 2.5 and 1.0), toludine blue *O* metachromatic, and acridine orange fluorescent stains were also used and confirmed the presence of mucin.

Case 3. This imported cotton-top marmoset (*S.o. oedipus*), male, had lived uneventfully in our colony for 24 months until it developed a progressively debilitating disease that terminated fatally 6 months later. Weight loss was estimated to be about 25% of peak body weight.

At necropsy the large intestine contained red blood. Emaciation was the only other gross notation made as all organs appeared normal. No tumors or ulcerative process of the intestinal tract was found grossly to explain the intestinal hemorrhage. Sections were taken as usual, however, of stomach, proximal and distal duodenum, jejunum, ileum, cecum, and rectum in addition to sections of the other organs.

Histologically, in sections of the midportion of the duodenum, we were surprised to find again a mucinous adenocarcinoma identical in appearance to the previous two carcinomas. As in the other two animals, the cancer arose from a grossly inconspicuous lesion obscured by advanced postmortem changes in a mucosa composed largely of simplified mucin-secreting cells. In this instance the

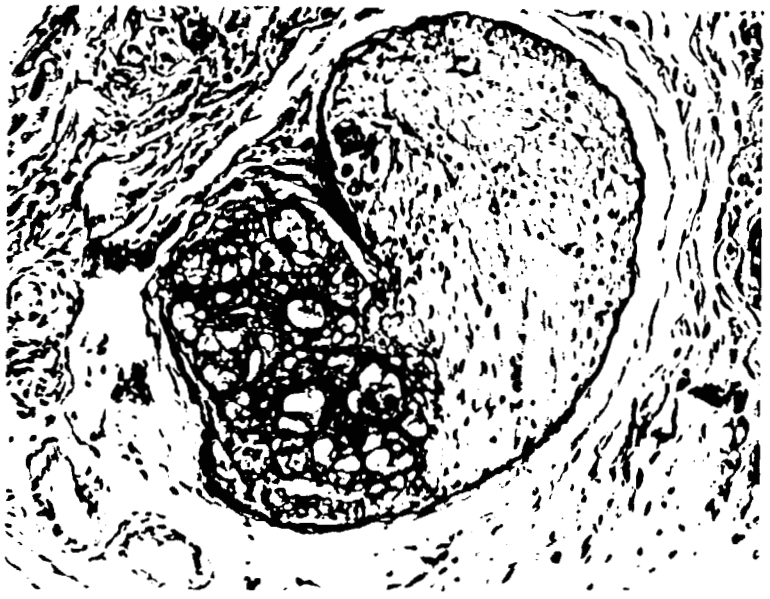


Fig. 61. Mucin-rich metastasis in a branch of the celiac nerve; Case 1. Hematolylin and PAS. 300 X.

cells looked more malignant because they contained less mucin and had greater nuclear and cytoplasmic hyperchromatism and pleomorphism. Unlike the other two cases, the malignant cells were often found lining the walls of dilated lymphatic spaces. Mitoses were readily detected, whereas in the mucin-rich metastases of the other two animals mitoses were difficult to find. Both kinds of cells (mucin-secreting and poorly differentiated) were present as cellular infiltrates in the intestinal muscular and connective tissue coats of the duodenum. One small metastasis (consisting of an estimated 1000 cells) was found in the peripheral sinus of a single, peripancreatic lymph node. Fourteen other peripancreatic, mesenteric nodes did not contain metastases.

Case 4. This cotton-top marmoset (*S.o. oedipus*), female, began losing weight 1 year after being imported and about 1 year before its death. At first weight loss was slow. Four months before death diarrhea began to occur intermittently and weight loss became progressively more rapid. Antibiotic therapy was not helpful. Its terminal weight of 250 g resulted from a total weight loss of 210 g (46% of maximum weight).

At necropsy gross findings were minimal. No intestinal lesion was evident. Feces were mucinous and scant. The diameter of the common bile duct, however, was about twice normal size and the enlarged gallbladder seemed

1028080

greatly distended. The liver was normal in appearance. An immediate cause of death was not grossly evident.

Histologically, there was an acute endometritis, suppurative salpingitis, and local peritonitis, anemia, hyperplastic marrow, and extramedullary erythro- and myelopoiesis. Five lymph nodes from the mesentery and peripancreatic connective tissues contained numerous small carcinoma metastases in their peripheral and radial sinuses. A prolonged microscopic search of multiple sections from the distal duodenal limb finally uncovered a minute primary mucosal neoplastic lesion. It was partially ulcerated, poorly staining, and extremely disorganized. Adjacent mucosal glands were simplified but not as mucinous as in the other cases. Therefore, this case differed from the others by having the most minute primary site in the duodenum-jejunum mucosa, no local fibrosis, but numerous lymph node metastases.

The four cases of primary mucinous adenocarcinoma of the small intestine, reported here, are unusual in many respects but, in particular, because they are indicative of a previously unreported high incidence of a kind of neoplasm that is infrequent in man and seen in rodents only after carcinogen ingestion. High incidence is difficult to explain in view of the experience of others except on such nonscientific bases as serendipity and the known vagaries of coincidence of a small series of cases. Unlike many reported instances of "adenocarcinoma" in nonhuman primate species, the malignancy of these small-intestinal neoplasms is proven for us in each instance by lymph node metastases and in one instance by lymphatic spread to the ureters and nerves.

The discovery of these four tumors resulted chiefly from our routine use of the PAS stain [instead of hematoxylin and eosin (H & E)] and of a meticulous necropsy protocol in which many specimens are taken routinely for later histologic examination even when grossly discernible pathologic processes are absent and postmortem autolysis is advanced. Since these four cases would have probably been overlooked permanently by us if only a few sections had been taken and stained with H & E, it seems quite possible that the occurrence of such minute intestinal tumors may be going undiscovered in other colonies of these species.

Alimentary-tract parasitism is common in these animals and might be a factor in the production of the lesions described in this report. The intestinal granulomatous disease of primates caused by the Acanthocephalus, *Prosthenorchis elegans*, has a high incidence in newly imported marmosets and is chiefly responsible for a majority of their deaths in our colony. It is common in this chronic granulomatous infection for hyperplastic glands of the cecum and terminal ileum to penetrate into the submucosa and proliferate there, occasionally forming mucus-filled cystic spaces. These pseudoneoplasms, however, like the gastric papillomata caused by *Nochtia nochti* never have been known to metastasize and are reversible if the parasites and the bacterial infections that cause them are treated successfully. Such parasite-induced hyperplastic epithelial lesions occur in the terminal ileum and cecum and not in upper jejunum and duodenum. Parasitism of the biliary system by *Athesmia foxi* was present in the

fourth animal reported here, but, as far as is known, this parasite does not produce an epithelial reaction. One animal (Case 1) with this cancer was colony-bred and raised under strict conditions to control any intermediate insect vectors. The absence of any exposure to intestinal parasites would seem to rule out a parasitic etiology for the tumor at least in this case.

We have no clue to what might be unique in the environment of our colony that would cause this cancer to develop.

The greater than 1% incidence (4 out of 285 necropsies) of small intestinal adenocarcinoma in our colony is unusual not only in comparison with the less than 0.1% incidence of all cancers of *all* types reported from nonhuman primate colonies (only one colonic carcinoma has been reported in a monkey) but also with human cancer statistics. In man, the incidence of cancer of the small intestine is less than 1 : 20 cancer of the colon (<1% of all cancer deaths). It originates in the second portion of the duodenum and first 2 feet of the jejunum. A primary ileal site for it is extremely rare as is the first portion of the duodenum where ulcerative disease is common and Brunner's glands are abundant. Although it is thought that most duodenal malignancies originate in the region of ampulla or in the paraduodenal portion of the common bile and pancreatic ducts, such precise primary foci frequently cannot be identified at human necropsy because of regional invasion and tissue destruction. In our animals, precise location of the site of origin was made difficult grossly by the small size of the mucosal lesions, and histologically by the lack of differentiating histologic features of the ascending duodenum jejuno-ileum in the marmoset. The close proximity of pancreas in sections of lymph nodes and intestine containing mucosal and submucosal carcinoma cells was the best single localizing criterion especially since, in addition, the normal, small-intestinal mucosal architecture seems to have been obliterated by a preexisting mucosal change in which an entirely mucin-secreting epithelium develops and the usual proliferative crypts are destroyed. This simplified mucin-secreting mucosa may be the precancerous mucosal condition. The close resemblance of the more benign-appearing, mucin-filled cancer cells to the cells of Brunner's glands, submandibular salivary glands, and periductal choledochal glands is provocative; it suggests that the target cell in marmoset intestinal carcinogenesis may be derived during an epithelial conversion to mucus production induced by some chronic irritant or inflammation. We are now making a more thorough review of our necropsy material with this possible correlation in mind.

Causes of Postnatal and Prepubertal Deaths in our Marmoset Colony

In addition to studying in great detail the newly discovered intestinal cancer among the cotton-top marmosets in the ORAU colony, we (Gretchen Humason and C. C. Lushbaugh) made a survey of the necropsies of the colony-bred animals that died during calendar year 1971. This was done to see if a common cause of death could be determined so appropriate corrective measures might be taken to reduce the seemingly increasing large yearly loss among these animals:

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1965, 2; 1966, 12; 1967, 13; 1968, 22; 1969, 29; 1970, 45; and 1971, 62 necropsied colony-bred animals. Reevaluation of the colony status by age, however, showed that this yearly increase in number of deaths reflected chiefly the increase in numbers of colony-bred animals surviving the neonatal period each year and being at risk. This finding suggested that the diseases responsible for this toll were similar to human childhood diseases, most likely infectious in nature. In Table 52, the 1971 monthly incidence of (necropsied) deaths are shown for animals less than 1 year old ("young") and greater than 2 years of age ("adult"). These data demonstrate that there were two periods of high death rate (February to May and August through November) and that the young animals were most susceptible.

TABLE 52

INCIDENCE OF DEATH AMONG COLONY-BRED MARMOSETS DURING CY 1971

Age (yr)	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Total
1	1	7	3	3	1	0	2	4	6	5	4	0	36
1–2	0	1	5	1	0	0	1	0	1	1	2	0	12
2	0	1	1	2	3	2	1	1	2	2	0	0	14
Total	1	9	9	6	4	2	4	5	9	8	6	0	62

The histopathologic study of these necropsies has been completed for the 36 young animals. Thirty-two of them had histologic evidence of acute or subacute and chronic enteritis. In addition they had mesenteric lymphadenitis and a phlebitis associated with small hepatic abscesses, suppurative pericarditis, pneumonitis, and focal suppurative adrenal cortical necrosis. One animal died from a wound infection, another from toxic hepatitis, and another from pneumonia. One animal had a disease (autoimmune?) we have not seen previously; an "allergic" glomerular sclerosis that involved only the outer cortical glomeruli that are still undergoing differentiation and development at time of birth. While 10 animals had various degrees of thyroid hyperplasia, this is a much smaller incidence of this condition than we have seen in previous years. We are inclined to attribute this decrease in active thyroid disease to the fact that we have added iodized salt blocks to the cages. However, we have recently seen four large colloid goiters and these suggest that the thyroid problem is not yet completely solved.

These histopathologic findings suggest that the major cause of death in these young animals is a bacterial gastrointestinal infection that is fatal if the animal does not rapidly develop an adequate, specific immune response. This conjecture is supported by the occurrence of the highest incidence of this disease between 2 and 5 months of age: Forty-six percent were 3 or 4 months old. Only 12% of the deaths were in animals older than 6 months. Bacterial studies (Raymond P.

Porter) of blood and fecal cultures are incomplete but support these observations since they indicate the presence of such pathogens as *Pseudomonas* and *Clostridia* as well as some instances of *Escherichia coli* bacteremia.

In retrospect, it may not be surprising that this study shows that the 2- to 6-month-old marmoset, like most other mammals, is highly susceptible to gastrointestinal infections. Nevertheless, having shown this directly, we should now be able to devise specific preventive and therapeutic regimens to overcome these infections during this critical period of growth.

ULTRASTRUCTURAL STUDIES OF TUMORS

Localizing Radionuclides in Animal Tissues

Research efforts in ultrastructural anatomy have been concerned with autoradiographic localization of injected radionuclides in various experimental animal tissues. To study ^{67}Ga localization we have used both light and electron microscopic autoradiography and the combination of electron microscopic autoradiography with a cytochemical test for acid phosphatase. We reported the predominant localization of ^{67}Ga in lysosomes or lysosomal-like granules of different cell types, notably in macrophages of leukemic and nonleukemic murine tissues[1]. In preliminary work we (D. C. Swartzendruber and Gretchen Humason) determined by the modified Gomori histochemical technique for acid phosphatase that ^{67}Ga was concentrated in granules that contained the enzyme reaction product (Fig. 62 and 63). The presence of acid phosphatase plus a single limiting membrane is evidence for the designation of these ^{67}Ga-labeled granules as lysosomes. These in vivo observations support the findings obtained by subcellular fractionation methods of Dr. David Brown of the Medical Division staff.

As a continuation of collaborative research with staff members of the Biology Division of the Oak Ridge National Laboratory on gallium in Rauscher virus-induced hematologic disorders in BALB/C mice, we studied the degree and specificity of ^{67}Ga localization during embryogenesis (J. A. Otten,* R. L. Tyndall,* P. C. Estes,* and D. C. Swartzendruber). This study was prompted by analogies between fetal and malignant tissues. Gallium-67 uptake in embryos was determined 24 hr after i.v. injection of pregnant mice with 1 μCi of ^{67}Ga. Autoradiograms were prepared from embryos and placentas of mice inoculated i.v. with 0.2–2 mCi of either ^{67}Ga or ^{3}H-thymidine. Total ^{67}Ga counts in 6–8 day embryos were comparable to those previously seen in normal adult mouse tissues, whereas uptake of ^{67}Ga in 9–11 day embryos was equivalent to or surpassed that noted in leukemic tissues of adult mice. By day 13 ^{67}Ga counts were diminished (Table 53). Autoradiograms revealed that ^{67}Ga localized in fetal membranes, limb buds, and vertebrae of the embryos and in the decidua. Conversely, embryos and decidua labeled with ^{3}H-thymidine indicated no such selective deposition. Therefore, ^{67}Ga incorporation is neither selected for nor a

*Biology Division, Oak Ridge National Laboratory

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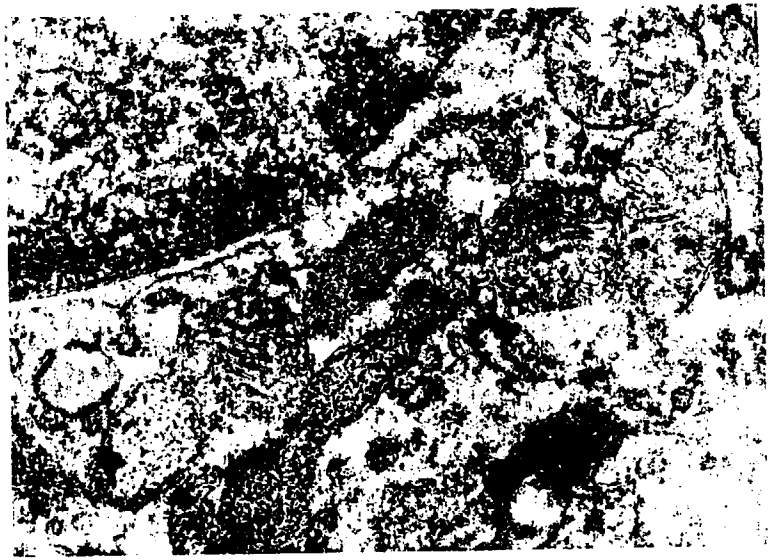


Fig. 62. Electron micrograph of portion of rat spleen cell with single membrane limited granules (lysosomes) containing electron dense acid phosphatase reaction product. This is a histochemical technique (Gomori) modified for electron microscopy.

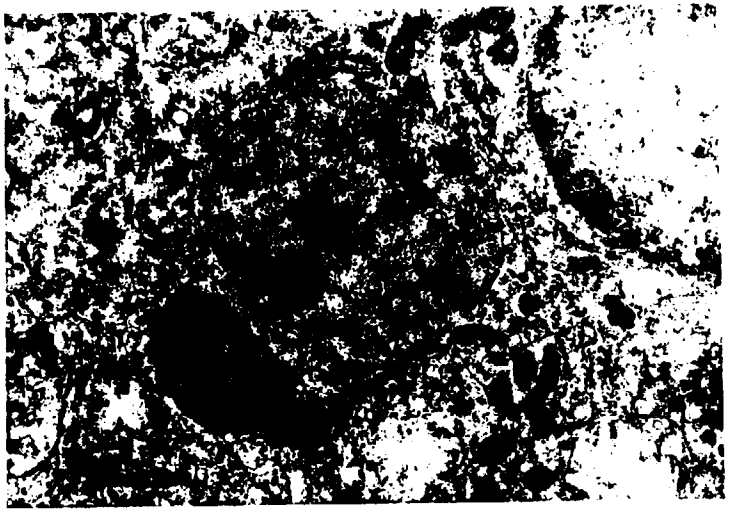


Fig. 63. Electron micrograph of portion of rat spleen cell showing single membrane limited granules (lysosomes) containing electron dense acid phosphatase reaction product with silver grains resulting from ^{67}Ga. This represents a combined histochemical and autoradiographic technique.

reflection of cell turnover as judged by its comparison with ^{3}H-thymidine uptake.

Another interesting point in this study was the observation that the ^{67}Ga concentration in the thymus of the pregnant mouse declined steadily to the end of gestation, whereas that of the liver and spleen remained nearly constant throughout this period (Table 54). Further work on this problem is required to assess the meaning and possible significance of these findings.

TABLE 53

CONCENTRATIONS OF ^{67}Ga IN WHOLE EMBRYOS AT DIFFERENT AGES

Age of embryo in days	Number animals/point	% injected dose/g*
4	8	8.29(0.43)†
5	13	7.48(1.03)
6	13	7.15(0.57)
8	18	10.45(0.16)
9	9	13.59(0.90)
10	14	11.45(0.44)
12	4	5.38(0.16)
13	8	5.86(0.30)
14	4	6.31(0.19)
15	6	6.07(0.35)
16	8	5.77(0.28)
17	4	3.22(0.11)
18	4	4.35(0.38)

*Average values.
†Standard errors.

TABLE 54

CONCENTRATIONS OF ^{67}Ga IN THE LIVER, SPLEEN, AND THYMUS OF VIRGIN AND PREGNANT MICE

Day of gestation	Number/time point	% injected dose/g*		
		Liver	Spleen	Thymus
Virgin	6	5.85(9.23)†	5.67(0.39)	4.06(0.61)
4–7	11	5.21(0.31)	4.28(0.39)	3.29(0.33)
8–11	11	4.49(0.38)	4.55(0.63)	2.35(0.28)
12–16	13	4.26(0.28)	5.68(0.35)	1.15(0.14)
17–19	7	4.43(0.28)	4.42(0.35)	0.43(0.04)

*Average values.
†Standard errors.

1028083

Changes in ^{67}Ga Distribution after Total-Body Irradiation

Because of our findings that ^{67}Ga localized notably in macrophages of various experimental animal tissues, we initiated experiments to investigate the role of macrophages in ^{67}Ga uptake. X irradiation is believed to be lymphocytolytic with little effect on macrophages. We therefore studied the effect of acute whole-body X irradiation on ^{67}Ga distribution in lymphoreticular tissues, bone, kidney, and liver of C3BF$_1$ mice (D. C. Swartzendruber and Karl F. Hübner). Irradiation doses of 25, 50, 100, 450, and 950 R were given in single exposures at approximately 100 R/min. Mice were injected intraperitoneally with a single dose of 2μCi ^{67}Ga in citrate (7 mg/kg of body weight) immediately after or at daily intervals after irradiation. Animals were killed 24 hr after the injections, and tissues were removed and assayed in a well-scintillation counter. In general, X irradiation diminished ^{67}Ga localization in all tissues except bone. However, ^{67}Ga concentration was significantly increased in spleen 1 day after 950 R. Essentially the ^{67}Ga concentration in all tissues except bone was near maximally diminished by doses of 100 R at day 2. Recovery rates of ^{67}Ga uptake were dose dependent (Table 55). Our data would indicate that the localization of this element in lymphoreticular tissues is not simply a function of macrophage engulfment and sequestration into lysosomes as has been shown for several colloidal metals. Sublethal doses of X irradiation are reported not to impair phagocytosis although the synthetic processes of intracellular degradation may be affected. Further, these data demonstrate that the reduction in gallium retention must represent an impairment of some other metabolic function and may be potentially useful as a tool to measure cell damage and recovery after irradiation.

Mercury-203 Localization at Subcellular Level

We have also investigated the localization of another radionuclide, ^{203}Hg, given as $^{203}HgCl_2$, in kidney cells of mice. This study was undertaken for three reasons. (1) To provide another method to precisely localize ^{203}Hg in proximal convoluted tubule cells of the kidney for comparison with data obtained from X-ray analysis of the same tissue in the scanning electron microscope, (2) to assess the resolution of ^{203}Hg attainable in electron microscopic autoradiography for comparisons with ^{67}Ga, and (3) to correlate the localization of ^{203}Hg with damaged renal cells on the ultrastructural level after the intravenous injection of $^{203}HgCl_2$.

The first step was to undertake a resolution study of ^{203}Hg (D. C. Swartzendruber, Gary Wolfe, and Roger J. Cloutier). We used a thin gold foil (530 Å thick) which had been exposed to $^{203}HgCl_2$ and was subsequently embedded in Epon 812. Sections were cut at right angles to the embedded foil, picked up on copper grids, and coated with Ilford L-4 emulsion. This method resulted in a line source of radioactivity from which measurements could be made on exposed autoradiographs to indicate how far the silver grains were away from the points of the radiations that caused them. This method is a

TABLE 55

EFFECT OF ACUTE X-IRRADIATION EXPOSURE ON ^{67}Ga CONCENTRATIONS IN FOUR TISSUES OF C3BF$_1$ MICE

	% Administered dose/g*			
	Liver	Spleen	Thymus	Blood
Control	6.9 ± 0.4†	4.5 ± 0.5	2.5 ± 0.2	2.2 ± 0.1
Day 1‡				
50 R	4.3 ± 1.1	3.2 ± 1.1	2.2 ± 0.4	2.3 ± 1.1
100 R	3.3 ± 1.0	3.7 ± 1.6	1.8 ± 0.8	0.9 ± 0.6
450 R	4.8 ± 0.6	6.5 ± 1.1	3.3 ± 1.0	0.4 ± 0.2
950 R	4.6 ± 1.0	8.0 ± 2.6	3.1 ± 0.9	0.8 ± 0.4
Day 2				
50 R	3.2 ± 1.3	1.9 ± 0.8	1.9 ± 0.6	2.5 ± 0.8
100 R	1.0 ± 0.5	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.3
450 R	0.9 ± 0.2	0.6 ± 0.2	1.7 ± 0.7	0.1 ± 0.1
950 R	0.8 ± 0.1	0.6 ± 0.1	0.9 ± 0.2	0.1 ± 0.0
Day 3				
50 R	6.3 ± 0.8	4.3 ± 0.6	4.1 ± 0.9	4.0 ± 0.6
100 R	5.1 ± 0.4	2.9 ± 0.4	2.7 ± 0.3	2.5 ± 0.2
450 R	1.0 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	0.1 ± 0.01
950 R	1.0 ± 0.4	1.1 ± 0.7	0.3 ± 0.1	0.1 ± 0.03
Day 5				
50 R	5.0 ± 0.6	3.7 ± 0.3	3.2 ± 0.8	3.6 ± 0.8
100 R	5.0 ± 0.6	3.7 ± 0.2	2.7 ± 0.6	2.3 ± 0.4
450 R	2.6 ± 0.2	1.6 ± 0.1	1.5 ± 0.6	0.7 ± 0.3
950 R	1.0 ± 0.5	0.8 ± 0.4	0.5 ± 0.2	0.2 ± 0.1

*Average values, 5 mice each dose.
†Standard error.
‡Day refers to time of sacrifice after X irradiation; ^{67}Ga always administered 24 hr before animals were killed.

modification of the line source method used by Salpeter et al. (*J. Cell Biol.* **41**: 1, 1969) to determine experimentally the resolution of ^{3}H in electron microscopic autoradiography (EMARG). A representative photograph of the results obtained with the gold foil containing ^{203}Hg as a line source is shown in Fig. 64. Our data indicate that the resolution of ^{203}Hg is about 0.5 μm under the conditions that we routinely employ in EMARG. The experimentally determined resolution values for ^{3}H reported by Salpeter et al. was 0.1 μm and 0.2 μm for ^{14}C and ^{35}S (Salpeter and Salpeter, *J. Cell Biol.* **50**: 324, 1971).

In contrast to our findings with ^{67}Ga, ^{203}Hg was not localized in lysosomes of renal cells (Fig. 65). Our preliminary data indicate that more than 50% of the grains were localized over swollen mitochondria in the damaged tubule cells. It

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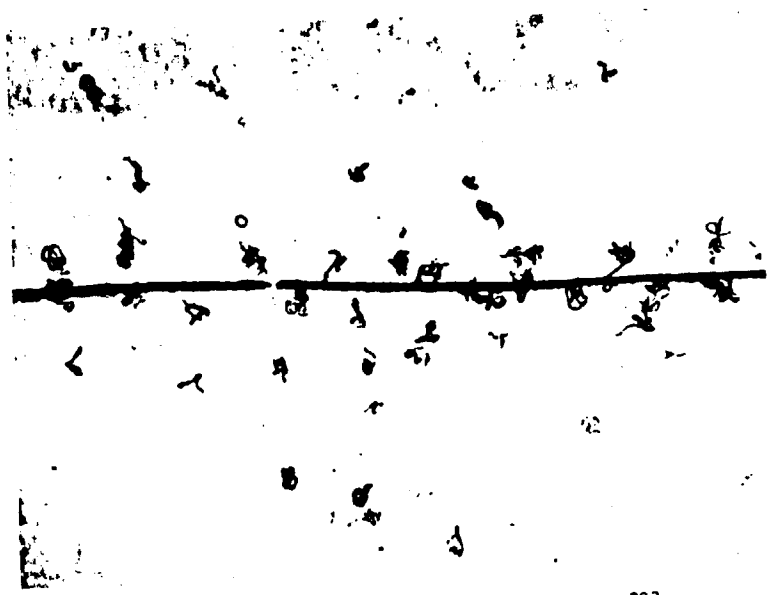


Fig. 64. Electron micrograph of the developed autoradiograph of ^{203}Hg absorbed to a thin film (gold, foil, 530 Å thick) as a radioactive line source. The distance that silver grains are located away from the line source is an indication of the resolution attainable with ^{203}Hg in electron microscopic autoradiography.

appears that the localization of ^{203}Hg can be correlated with ultrastructural evidence of damage to the cells, i.e., swollen mitochondria and disrupted membranes.

Elemental Analysis of Biologic Tissues

In collaboration with staff members from the Metals and Ceramics Division of the Oak Ridge National Laboratory (Helen Mateer and Ralph Gehlbach), we have initiated experiments to perform elemental analyses on relatively thick sections (~2000 Å) of biologic tissues. We are using energy dispersion X-ray analysis techniques with the scanning electron microscope. It is reportedly now possible with these techniques to detect the elements heavier than Na in concentrations as low as 10^{-16} g and to localize them with a spatial resolution of about 100 Å. Although our preliminary results are fragmentary, we are

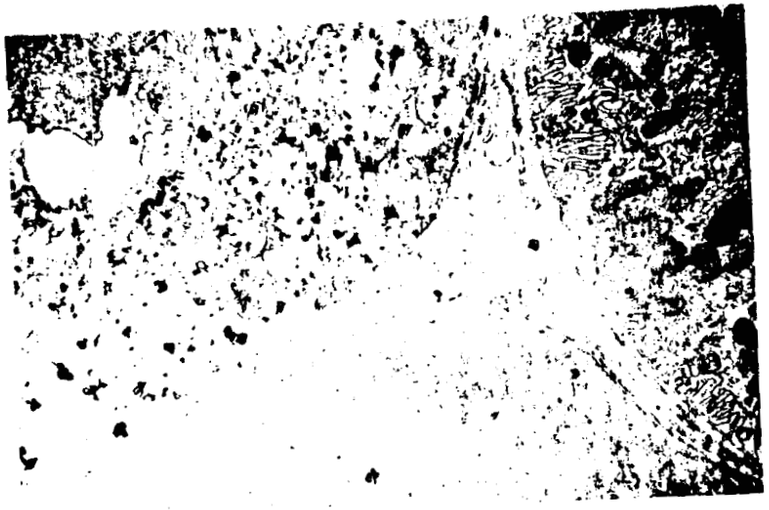


Fig. 65. Electron microscopic autoradiograph of ^{203}Hg localization in mouse kidney proximal convoluted tubule 6 hr after intravenous injection of 0.5 mCi $^{203}HgCl_2$. Note the numerous silver grains located over the damaged cytoplasm.

encouraged enough to pursue the development of this approach as a method of the future that will support or possibly supplant currently used autoradiographic and histochemical methods.

1028085

INDUCED CYTOGENETIC ABNORMALITIES

Cytogenetic Studies of a Man Accidentally Exposed to Total-Body Gamma Irradiation: Effects of Time-in-Culture on Chromosomal Breakage Frequencies

In February 1971 a 32-year-old man employed in one of the Oak Ridge laboratories was accidentally exposed to cobalt-60 gamma radiation. A thermoluminescent dosimeter on his left front waist received a dose of 260 R.

We obtained blood samples for evaluating radiation-induced chromosomal aberrations within a few hours after the exposure (L. Gayle Littlefield). Although there are numerous reports of the cytogenetic consequences of human exposure to ionizing radiation, this accident was unique in that (1) the radiation was of one quality, (2) the dose was distributed fairly homogeneously over the anterior surface of the body, and (3) blood samples were obtained within 3 hr. To ensure obtaining high quality preparations, duplicate samples were drawn for processing by two different laboratories (ORAU, ORNL). At the beginning we decided to harvest cultures at two early fixation times, with one group culturing samples for 52 hr, the other for 56 hr. We initiated, incubated, and harvested the cultures according to methods routinely employed by the respective laboratories.

Two blood samples were obtained within 3–6 hr and subsequently at weekly intervals for 8 weeks, and twice monthly thereafter. Personnel at each laboratory scored their own preparations for radiation-induced lesions, generally examining 300 metaphases at each culture time. The frequency of centric rings and dicentric chromosomes, and the percentages that had accompanying fragments were recorded.

Early it became apparent that the frequency of ring and dicentric chromosomes in the cultures incubated for 56 hr was considerably lower than in the cultures harvested after 52 hr (Fig. 66-A), the difference persisting over 90 days. In the samples incubated for 56 hr, a high percentage of the metaphases with centric rings or dicentric chromosomes did not have accompanying fragments, suggesting that this population of cells was contaminated with second division metaphases (Fig. 66-B). To determine whether more than one division had occurred in vitro in the 56-hr preparations, we pulse-labeled two cultures with tritiated thymidine (1 μCi/ml of culture medium) 23–24 hr after their initiation. Cultures were harvested at 56 hr, and several slides dipped in Kodak NTB liquid nuclear track emulsion. The resulting autoradiograms were scored for unlabeled versus labeled mitoses, and the labeled metaphases analyzed for label segregation between the sister chromatids. We scored 697 metaphases, of which 22.8% had incorporated tritiated thymidine (Table 56). Of the labeled cells, 93.9% could be positively identified as second division metaphases by their labeling patterns (Fig. 67).

The striking differences in aberration yields between 52-hr and the 54- to 56-hr cultures cannot be explained by the small differences in absolute culture time, and demonstrates the variability in the length of the cell cycle in

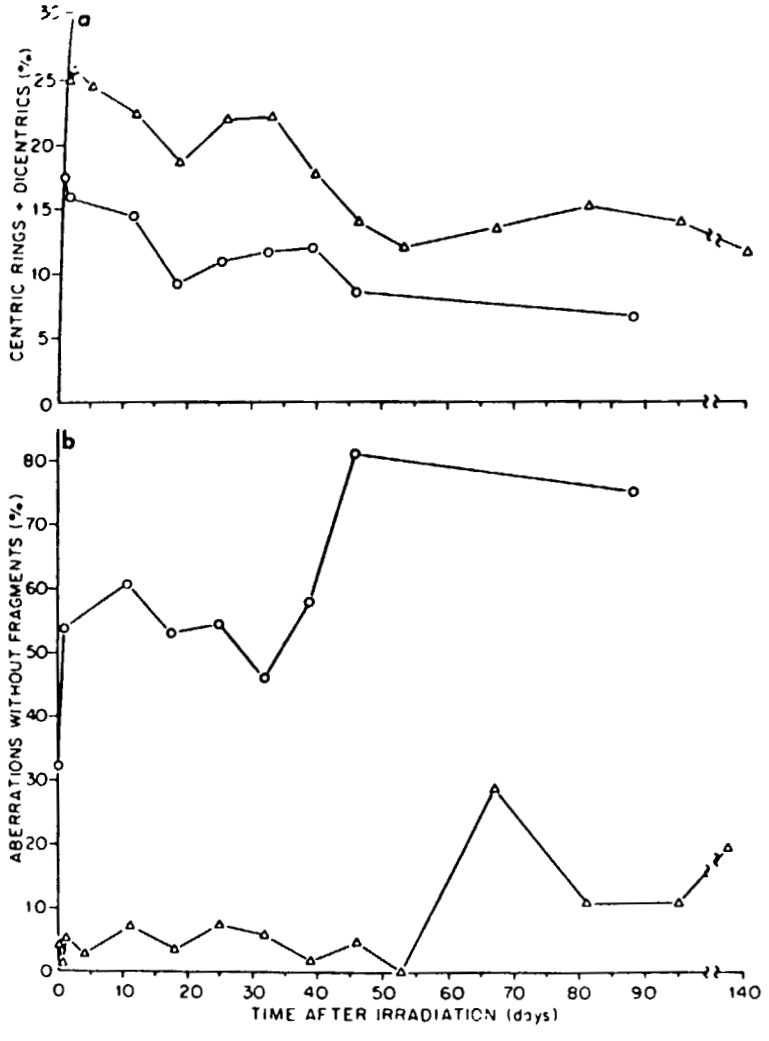


Fig. 66A. Comparison of the yields of asymmetrical exchanges observed under two culture conditions at various intervals after an accidental whole-body exposure to ^{60}Co γ rays.

B. Frequency of asymmetrical exchanges without accompanying fragments under the two culture conditions.

Δ—Δ 52-hr culture (data obtained by J. G. Brewen and R. J. Preston, ORNL)

O—O 56-hr cultures (data obtained by Littlefield, ORAU)

1028086

lymphocytes cultured under different conditions. In the 52-hr cultures, buffy coats were inoculated into 10 ml medium consisting of 70% RPMI-1640, 27% heat inactivated heterologous AB+ human serum, and 3% phytohemagglutinin (PHA). The cultures were incubated in 5% CO_2 in air at 35 ± 1°C for a total period of 52 hr. In the 56-hr cultures 0.5 cc cell-rich plasma was introduced into 7 cc TC 199 culture medium which contained 100 units penicillin G, 100 μg

TABLE 56
FREQUENCY OF LABELED AND SECOND-DIVISION METAPHASE FIGURES IN A 56-HR LEUKOCYTE CULTURE

No. of cells	% Unlabeled	% Labeled	% 2nd division*
697	77.2	22.8	93.9

*Percent of those labeled cells that could be analyzed for label segregation.

streptomycin, and 0.01 cc reconstituted Difco PHA/cc. The cultures were supplemented with 2.5 cc autologous plasma, and incubated at 37 ± 1°C for 56 hr. Obviously the lymphocytes cultured with autologous plasma at 37°C for 56 hr had a much faster cell cycle than duplicate samples incubated with heterologous plasma at 35°C.

To obtain accurate quantitative estimates of radiation-induced aberrations, it is essential that cells that are evaluated be in their first in vitro division. Early fixation times ranging from 48 to 60 hr have been advocated by various groups as the best times to obtain first-division metaphases in human lymphocyte cultures. Here two early fixation times were employed (52 vs 54 to 56 hr) but the aberration yields were quite different in the two series of cultures.

The lower frequency of aberrations in the 56-hr cultures becomes quite important in estimates of biological dose. The mean yields of rings and dicentrics observed in cultures obtained during the first 32 days postexposure in the 52-hr preparations was 22.8% compared to 13.3% in comparable cultures harvested at 54 to 56 hr. If these yields are used to estimate dose using the quadratic model $Y = a + bD + cD^2$, with coefficients $b = 0.393 \pm 0.106) \times 10^{-3}$ and $c = (8.16 \pm 0.43) \times 10^{-6}$ for centric rings and dicentrics (coefficients obtained by J. G. Brewen and R. J. Preston, ORNL), a dose estimate of 144 rads is obtained from the 52-hr cultures compared to 102 rads in the 56-hr cultures. Dose measurements in a phantom used in the reconstruction of the accidental exposure gave a mean midline dose of 127 rads and a marrow dose of 118 rads. The hematological and clinical findings suggested that the patient received a somewhat higher dose (~150 rads). The estimated dose of 104 rads obtained from the 56-hr cultures was low, and obviously the result of the contamination of these cultures with second division metaphases.



Fig. 67. Autoradiogram of a metaphase from a 56-hr culture labeled with tritiated thymidine 23 hr after initiating culture. The segregation of the label between the sister chromatid indicates that the metaphase was in its second in vitro division at the time of harvest.

Other investigators have cautioned against using predetermined physical time for harvesting lymphocyte cultures from irradiated persons. Our findings dramatically demonstrate the influence of culture conditions on the rate of cell division, and emphasize the importance of initially determining the cell-cycle characteristics of the person being studied.

1028087

Radiation-Induced Chromosome Aberrations of Marmoset Leukocytes

Many reports describe aberrations in human leukocytes after accidental, therapeutic, or diagnostic exposure to ionizing radiation. These data are difficult to interpret, however, due to inhomogeneities of the distribution of dose over the body and of the quality of radiation. The objective of the present experiments (J. G. Brewen* and N. Gengozian) was to compare extensively the in vivo and in vitro production of chromosome aberrations by low-linear energy transfer (LET) radiation in the circulating lymphocytes of a primate. The marmoset was chosen because of the similarity of its karyotype (2n = 46) to the human karyotype, and also because its small size would eliminate most dosimetry problems that result from tissue absorbance of the low-LET radiations.

Whole blood cultures from humans and from *Saguinus fuscicollis* were irradiated with various doses of 250 kV X rays. The resulting centric ring plus dicentric aberration yields were fitted to the three models, $Y = a + bD$, $Y = a + bD + cD^2$, and $Y = a + cD^2$, by least squares regression. In both instances the best fit was to the model $Y = a + bD + cD^2$, with coefficients of the one- and two-tract components for human and marmoset being, $b = (0.78 \pm 0.09) \times 10^{-3}$ $c = (5.92 \pm 0.31) \times 10^{-6}$, and $b = (1.11 \pm 0.36) \times 10^{-3}$, $c = (7.1 \pm 1.7) \times 10^{-6}$, respectively.

We irradiated several marmosets with ^{60}Co gamma rays at a dose rate of 3.7 R/min. Blood samples were drawn immediately and 24 hr after the irradiation, and the aberration yields in the circulating leukocytes were measured. The yields at both sampling times were identical. Concurrently, freshly drawn whole blood was irradiated in the same manner to compare in vivo to in vitro radiosensitivity; the in vitro irradiation produced aberration yields identical to those from in vivo irradiation.

The fact that the yields of centric rings plus dicentrics in the human and marmoset dose-response curves gave a "best" fit to the quadratic model is not surprising. The results of the human in vitro irradiation agree well with those published recently, where whole blood was irradiated. The similarities in yields and dose kinetics when the human and marmoset data are compared are also not surprising (Fig. 68). We expect such similarities in radiosensitivity on the basis of target theory, in that the two species have identical chromosome numbers and deoxyribonucleic acid (DNA) contents.

Our experiments clearly indicate that there is no difference in aberration yields produced in circulating leukocytes by low-LET radiation in vivo and in vitro. Thus in vitro experiments truly measure the expected effect of in vivo exposure of somatic cells if the exposure is of a homogeneous whole-body type. Of course, in estimating the effect expected from a whole-body exposure to man the factors for tissue absorbance and inverse-square dose relations must be accounted for in estimating average midline dose. Because of the small size of the marmoset, these factors did not contribute significantly in the present study.

*Biology Division, Oak Ridge National Laboratory.

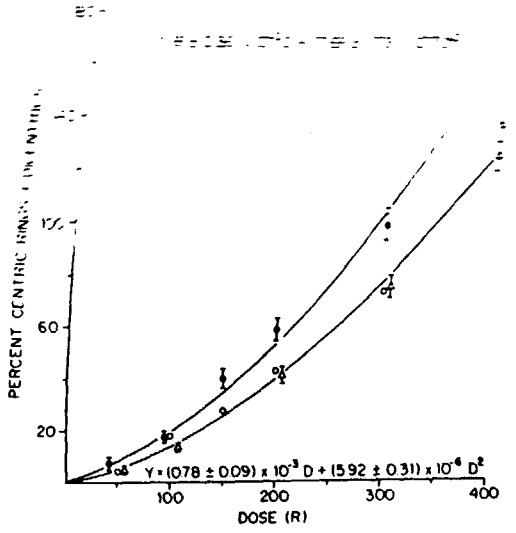


Fig. 68. The observed centric ring plus dicentric yields obtained when whole-blood cultures of human (Δ——Δ) and marmoset (●——●) were irradiated, plotted against the "best-fit" regression curves. The earlier data from irradiation of human whole-blood samples are also given (O). These points are offset from the actual dose levels for the purpose of clarity.

Frequency of Chromosome Breakage in Lymphocyte Cultures from Control Men and Women

In conjunction with an ongoing study of the effects of oral contraceptives on chromosome breakages in lymphocytes, we have accumulated data on the frequency of abnormal chromosomes in over 11,900 metaphases from 122 cultures of 10 control men, and over 17,795 metaphases from 185 cultures of 21 control women (L. Gayle Littlefield, Mary Beth Klepper, Eugene Joiner, and Lydia Corrill) (Table 57). Cultures have been obtained from various individuals in these two groups during the last 3 yr. Here we describe the types, frequencies, and variability in frequencies of abnormal chromosomes in cultures from them.

Ten men and 21 women participated in the study; most of them are employed in Oak Ridge. All range in age from 20 to 40 yr, have no phenotypic abnormalities or chronic disease and are not taking routine medications. At the outset all participants completed clinical histories describing immunizations, childhood and adult illnesses, and pertinent family history. At the time of every venapuncture, each person filled out a health form describing any illnesses or medications taken during the period preceding the sampling. Blood was initially obtained from each person at intervals of 1 month and later at 6 to 7 weeks.

Three-day blood lymphocyte cultures were established from 10 cc heparinized blood. Whenever possible, 100 metaphases were analyzed from each culture.

1028088

To avoid bias in selection of metaphases, each scorer scanned the preparations for well-stained and well-spread metaphases using the 10x objective. All suitable metaphases were then examined at 1000x. First the chromosomes of each metaphase were counted, and then systematically analyzed by pairing chromosomes 1, 2, 3, and 16, and observing each member of the B, D, E, F, and G group chromosomes. Thus we hoped to detect those more subtle aberrations that might be overlooked in routine scoring methods.

TABLE 57
ABNORMAL CELLS WITH BREAKS IN PERIPHERAL BLOOD LYMPHOCYTES FROM CONTROL MEN AND CONTROL WOMEN*

	$\bar{x}$	$s_{\bar{x}}$	d.f.	t	P†	
Men	5.58	0.37	121	303	1.598	<0.2
Women	6.48	0.38	182			

*Data adjusted for number metaphases analyzed per culture.
†2 tail "t" test for unequal N.

We have considered as abnormal any chromosome with a break involving both sister chromatids as observed at metaphase. These include isolocus breaks, chromosome breaks (deletions either with or without fragments), and all intra- and interchromosomal exchanges. All metaphases having one or more of these lesions were scored as abnormal. We also noted other unusual chromosome configurations such as presumptive somatic crossovers, partial endoreduplication figures, and pulverized metaphases or portions of metaphases.

In cultures from the men, 5.6% of the total metaphases had at least one chromosomal break. In the women 6.5% of the metaphases had similar aberrations. In both groups 92% of the abnormal cells had only one lesion; the majority had single break aberrations (isolocus breaks or deletions). Of the two-break lesions scored, translocations were identified most often. We found no ring chromosomes in any of the metaphases. Three somatic crossover figures were identified in cultures from the women, and none in cultures from the men. We found only two partial endoreduplication configurations, both in cultures from the women.

Since the incidence of metaphases with chromosomal breaks was approximately 1.0% greater in cultures from the women compared to the men, we analyzed the group means for statistical differences in breakage frequencies (Table 58). The difference in the means was not significant when tested at the 0.05 level of probability.

To determine whether any aberration type occurred more frequently in cultures from the men versus women, we calculated the percentage of the total aberrations scored which were isolocus breaks, deletions, etc. (Table 59). In the men 62.9% of the lesions were isolocus breaks, compared to 67.4% in the

TABLE 59
FREQUENCY OF OCCURRENCE OF SPECIFIC TYPES OF ABERRATIONS IN CULTURES FROM CONTROL MEN AND CONTROL WOMEN

	Men	Women
Total aberrations scored	707	1262
Isolocus breaks	445	851
	(62.9)*	(67.4)
Deletions	195	319
	(27.6)	(25.3)
Dicentrics	22	13
	(3.1)	(1.0)
Translocations	33	60
	(4.7)	(4.8)
Inversions	10	14
	(1.4)	(1.1)
Somatic Crossover	—	3
		(0.2)
Partial endoreduplication	—	2
		(0.15)
Pulverizations	15	20
	(2.1)	(1.6)

*Percent total aberrations.

women. With the exception of dicentric chromosomes, the frequency of the various types of lesions was similar in the two groups. Over 3.0% of the aberrations in men were dicentric chromosomes, whereas only 1.0% of the lesions in women were asymmetrical interchanges. At the time the culture data were decoded, we discovered that 8 of the 22 dicentric chromosomes in cultures from the men were found in metaphases from one man (MC7), and that 5 of the 13 dicentrics scored in cells from the women were found in metaphases from

1028089

(FC… The man had an estimated accumulated total body exposure of … radiation over the last 6 yr; whereas the woman had had multiple chest, …der, and spinal X rays following a riding accident 8 months before she had …d our study.

The number of metaphases with chromosomal breaks varied from 0–34% in individual cultures from both men and women. Sixty-one percent of the cultures from the men had between 0–5% abnormal cells, and 35% had 6–10% abnormal cells. Fifty-four percent of the cultures from the women had 0–5% abnormal cells, and 29% had 6–10% breaks. A detailed graph of the percentage of the total having 1–25% breaks is shown in Fig. 69. The individual culture means in samples from the control men cluster closely about the overall group mean, whereas more culture variability is evident in the women. Only 5 of the 122 cultures from the men had greater than 10% abnormal cells whereas 26 of the 183 cultures from the women had in excess of 10% abnormal cells. The three cultures with the highest percentage of abnormal cells (26, 30, and 34%) in cultures from the women were obtained over a 6-month period from one person (FC21). Her health histories revealed no illnesses or unusual medications during the months preceding her cultures. She had recorded dates of trips to the dentist, episodes of "flu," and specific drugs taken, which suggests that she was conscientious in completing health forms. She was 37 years old at the beginning of the study and the mother of four normal children.

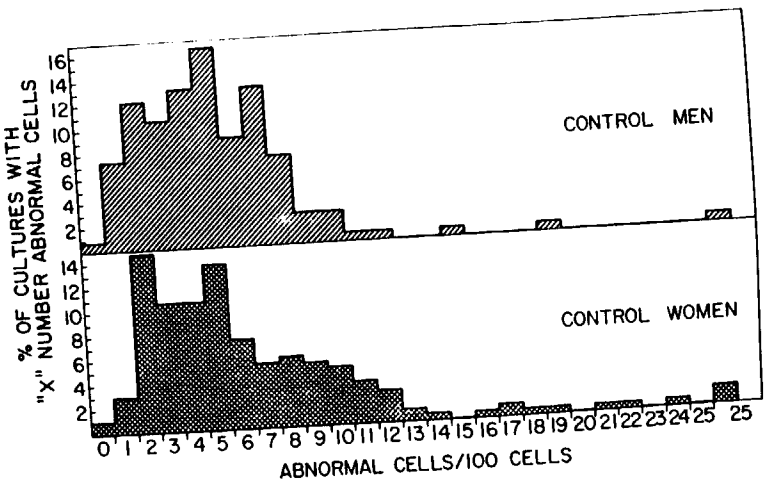


Fig. 69. Distribution of cultures according to the number of abnormal cells/100 cells analyzed. There is considerably more variability in breakages among cultures from the women than from the men.

TABLE 60
TYPES AND FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN INDIVIDUAL CONTROL MEN

Code	No. cultures	No. cells	Average for all cultures*							
			Abn. cells	Abn. chrom.	Isolocus breaks	Deletions	Dicent.	Trans.	Inver.	Somatic crossover
MC1	15	1500	3.8	4.1	2.1	1.8	—	0.13	—	
MC2	17	1669	4.7	4.9	3.4	0.6	0.1	0.4	0.1	
MC3	8	800	4.8	5.0	3.8	0.87	0.12	—	0.12	
MC4	17	1700	4.9	5.3	3.2	1.5	0.2	0.1	0.1	
MC5	7	503	5.5	5.7	3.9	1.3	0.19	0.19	—	
MC6	21	2044	5.7	6.6	4.3	1.8	0.04	0.39	0.4	
MC7	17	1700	6.4	7.1	3.7	2.2	0.4†	0.5	0.05	
MC8	7	700	6.5	7.1	4.8	1.8	0.28	0.14	—	
MC9	7	620	6.6	7.0	4.1	2.9	—	—	—	
MC10	8	722	6.6	6.7	4.8	1.2	0.27	0.13	0.27	

*Mean value (abnormal/100 cells).
†Received 2.7 R exposure to radiation 1963–present.

TABLE 61
TYPES AND FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN INDIVIDUAL CONTROL WOMEN

Code	No. cultures	No. cells	Average for all cultures*								
			Abn. cells	Abn. chrom.	Isolocus breaks	Deletions	Dicent.	Trans.	Inver.	Part. endo.	Somatic crossover
FC1	16	1600	4.0	4.2	2.8	0.9	—	0.38	0.13	—	—
FC2	17	1700	4.3	4.6	2.8	1.5	0.06	0.29	0.06	—	—
FC3	6	600	4.3	4.3	2.8	1.5	—	—	—	—	—
FC4	8	800	4.4	4.6	4.0	0.8	—	0.13	0.25	—	—
FC5	5	500	4.6	5.0	2.0	3.0	—	—	—	—	—
FC6	15	1500	4.9	5.1	3.2	1.3	0.13	0.33	0.13	—	—
FC7	16	1600	5.1	5.7	2.8	2.1	0.06	0.63	0.06	0.06	—
FC8	6	579	6.0	7.1	4.5	2.4	—	0.17	—	—	—
FC9	8	690	6.1	6.8	5.4	1.2	—	0.29	—	—	0.14
FC10	8	723	6.1	6.2	3.3	2.5	—	0.41	—	—	—
FC11	7	700	6.4	6.9	3.4	1.9	0.29	1.00	0.29	—	—
FC12	19	1900	6.4	6.8	4.7	1.7	0.05	0.21	0.11	—	0.5
FC13	3	250	6.8	9.6	7.2	1.6	—	0.80	—	—	—
FC14	7	650	7.1	7.2	6.0	1.1	—	0.15	—	—	—
FC15	5	500	7.2	8.6	4.2	3.2	1.00	0.20	—	—	—
FC16	3	300	8.3	10.0	8.7	1.3	—	—	—	—	—
FC17	7	700	8.4	9.0	6.9	2.0	—	0.14	—	—	—
FC18	5	450	8.7	8.7	4.2	3.6	0.22	0.67	—	—	—
FC19	7	700	8.9	9.6	7.0	2.4	—	—	0.41	—	—
FC20	7	567	11.5	12.2	8.8	2.5	—	0.71	0.18	—	—
FC21	8	750	18.0	21.8	18.8	2.5	—	0.53	—	0.13	0.13

*Mean values (abnormal/100 cells).

As an index of the variability in breakage frequencies in different people, we tabulated the average incidence of breakages in all cultures obtained from each participant (Tables 60 and 61). The average breakages in cultures from the 10

1028090

men varied only slightly, from 3.8 to 6.6% abnormal cells. A considerably wider range in variability was seen in the women, the lowest breakage being 4.0% and the highest 18.0%.

It became apparent during the study that some groups of cultures had higher breakages than others. Upon decoding we found higher breakages in cultures obtained during the late spring and early summer months, particularly in cultures from the women. We grouped all the cultures according to month and year and tabulated the mean and standard error in breakages for all cultures in each month (Figs. 70 and 71). Since blood samples were obtained from some subjects at intervals of 6 to 7 weeks, consecutive months do not always include consecutive cultures from the same person. The increase in average breakages for the cultures obtained during April, May, June, and July is particularly striking in cultures obtained during 1970. To test for statistically significant differences in breakages at different seasons, we arbitrarily grouped the 4 months of highest breakages, and tested the mean for this period against the means for the flanking 4-month periods (Table 62). The increase in breakages seen during April–August was highly significant in both the men and the women.

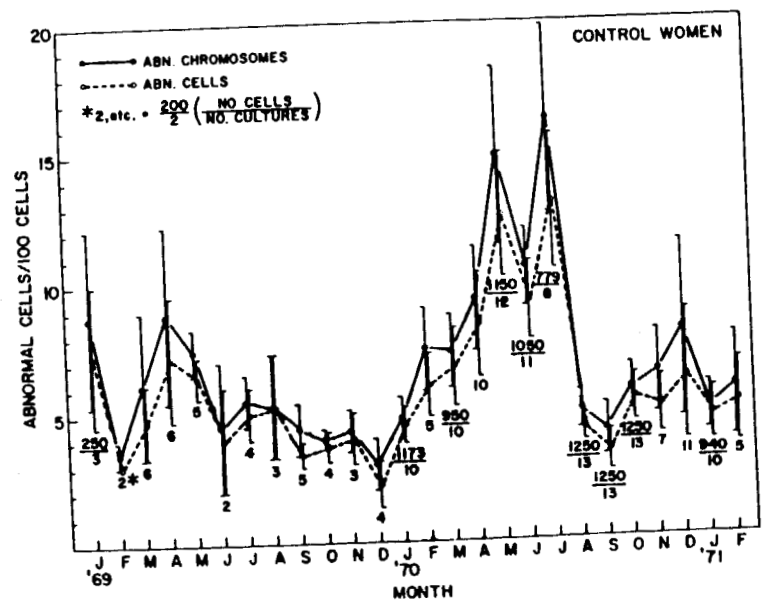


Fig. 70. Average breakages per month (±SE) in cultures from control women. The increase in breakages obtained during April-July significantly exceeded the breakages during August-November or December-March.

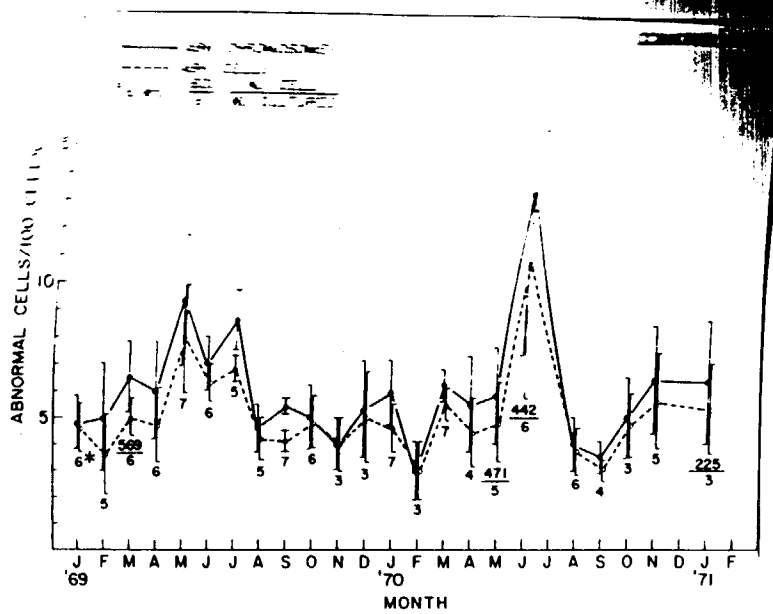


Fig. 71. Average breakages per month in cultures from control men. The increases in breakages obtained during April-July was significantly higher than the breakages during either December-March or August-November.

Whenever possible we identified the specific chromosome or chromosome group in which isolocus breaks or deletions occurred. (In some instances, it was impossible to determine the derivation of small acentric fragments.) We were able to identify 551 breaks in cultures from the men and 1047 breaks in cultures from the women. Using the standard mean percent chromatin per chromosome or per chromosome group (Chicago conference, Standardization in Human Genetics, 1966), we derived the expected number of breaks for both male and female diploid karyotypes. Chi square comparison of the observed versus expected breaks is shown in Table 63. In cultures from the men, we observed more breaks than expected in chromosome 3 and in the G + Y chromosome group. Considerably fewer breaks were observed in the F chromosomes than expected. Much more variability occurred in cultures from the women; we observed very exaggerated increases in breaks in the 3, 16, and G group

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[illegible] chromosomes, [illegible] fewer breaks than expected in the [illegible] chromosome groups.

Our results [illegible] chromosome breakages in grossly normal men and women demonstrate a marked variability in frequencies both among cultures from different persons, and among consecutive cultures from the same individual. Our data indicate that the lymphocyte is susceptible to breakages induced by many extraneous factors.

Investigators who are attempting to determine the effects of specific drugs on induced cytogenetic aberrations should be cognizant of the innate variability of chromosome breakage frequencies in lymphocytes from untreated subjects.

TABLE 62
STATISTICAL COMPARISON OF MEANS (ABNORMAL CELLS/100 CELLS) OF CULTURES OBTAINED DURING DECEMBER–MARCH, APRIL–JULY, AND AUGUST–NOVEMBER

Time period*	Control Men $\bar{x}$	Control Men $s_{\bar{x}}$	df	t	P<	Control Women $\bar{x}$	Control Women $s_{\bar{x}}$	df	t	P<
Dec.–Mar.	4.85	0.388	78	−2.33	0.050	5.46	0.550	125	−4.04	0.001
Apr.–Jul.	7.32	0.980	76	−2.91	0.010	9.43	0.844	111	−5.22	0.001
Aug.–Nov.	4.21	0.348				4.53	0.113			

*Included in each time period are data from all cultures obtained during the respective months in 1968, 1969, 1970, and 1971.

TABLE 63
X^2 DISTRIBUTION OF TOTAL CHROMOSOME BREAKS

Chromosome No.	Control men Obs.	Exp.	X^2	P	Control women Obs	Exp.	X^2	P
1	43	49	0.73	0.40	81	90	0.90	0.35
2	50	47	0.19	0.65	78	84	0.42	0.55
3	55	38	7.60	0.01†	111	70	24.01	>0.0005†
B	63	68	0.36	0.55	103	126	4.19	0.05*
C	176	187	0.64	0.45	352	400	5.76	0.025
D	54	57	0.15	0.70	77	104	7.00	0.01†
16	23	17	2.11	0.15	104	32	162.00	>0.0005†
E	36	32	0.50	0.50	60	58	0.06	0.80
F	10	26	9.84	0.005†	16	48	21.33	>0.0005†
G	41	30	4.03	0.05*	65	35	25.71	>0.0005†

*$P \leq 0.05$ significant.
†$P \leq 0.01$ highly significant.

[illegible]

We described in an earlier paragraph our culture methods and criteria for scoring lesions. We have completed analyses of 19,974 metaphases from 205 cultures from 26 women taking oral contraceptives, finding 9.24% of the metaphases with at least one abnormal chromosome. Breakages differed significantly from the control men and control women ($P \leq 0.001$) (Table 64).

TABLE 64
ABNORMAL CELLS WITH BREAKS IN BLOOD LEUKOCYTES*

	$\bar{x}$	$s_{\bar{x}}$	d.f.		t†	P
Control men	5.58	0.37	121	325	5.859	< 0.001
Experimental women	9.24	0.42	204			
Control women	6.48	0.38	182	386	4.745	< 0.001
Experimental women	9.24	0.42	204			

*Data adjusted for number metaphases analyzed per culture.
†2 tail "t" test for unequal N.

Ten different kinds of oral contraceptives were used by the 26 women in the study group, including eight combination and two sequential types of medications. The estrogen and progestogen components of these various compounds are shown in Table 65.

To determine whether there were any differences in breakages related to the different types of oral contraceptives, we tabulated the average number of abnormal metaphases in all cultures from each of the 26 women (Table 66). As in cultures from the control women, breakages varied markedly in cultures from the individual oral contraceptive users (4.1–21.16% abnormal cells). Some showed no apparent increase in the number of abnormal metaphases; whereas other women have a very high incidence of breakages. Of the six with the highest breakages, four (OC21, OC23, OC25, and OC26) were taking sequential medications.

To further examine the effects of specific contraceptive agents on breakage frequencies, we calculated the average number of abnormal cells and standard error of the mean for all cultures obtained from those taking the same kind of contraceptive (Fig. 72). Nine women were taking Ortho-Novum (2 mg progestogen), and the incidence of abnormal cells in 79 cultures from these women was 7.5%. In general, similar breakages appeared in cultures from all 22 women taking combination-type agents. On the other hand, the breakages from

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[illegible]

TABLE 65
TYPES OF ORAL CONTRACEPTIVES TAKEN BY WOMEN IN STUDY GROUP

Brand name	Estrogen	Progestogen	Number taking
Combined*			
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
Ovulen (OV)	Mestranol 0.1 mg	Ethynodiol diacetate 1.0 mg	4
Norinyl (NOR)	Mestranol 0.05 mg	Norethindrone 1.0 mg	1
Norlestrin 1 (NO1)	Ethinyl estradiol 0.05 mg	Norethindrone acetate 1.0 mg	1
Norlestrin 2 (NO2)	Ethinyl estradiol 0.05 mg	Norethindrone acetate 2.0 mg	1
Sequentials†			
C-Quens	Mestranol 0.08 mg	Chlormadinone acetate 2.0 mg	2
Oracon	Ethinyl estradiol 0.08 mg	Dimethisterone 25.0 mg	2

*Estrogen and progestogen combined in one dose, average 20 consecutive pills per month.
†Estrogen alone taken for 15–16 days, followed by 5 days progestogen plus estrogen.

TABLE 66
TYPES AND FREQUENCIES OF CHROMOSOMAL ABERRATIONS IN INDIVIDUAL WOMEN TAKING ORAL CONTRACEPTIVES

			Average for all cultures*								
I.D. code	Treatment	No. cultures	No. cells	Abn. cells	Total lesions	Isolocus breaks	Deletions	Dicent.	Trans.	Inver.	Rings
OC1	ON2	8	695	4.1	4.3	2.6	1.6	—	—	0.16	—
OC2	ON2	8	717	4.6	4.7	2.8	2.3	—	0.14	0.23	—
OC3	ON1/ ON1-50	5	500	4.8	5.2	2.2	1.6	0.4	0.8	—	—
OC4	ON2.5	10	973	4.9	5.7	3.6	1.8	—	0.2	—	—
OC5	ON	8	800	5.87	6.4	4.0	1.7	0.25	0.25	0.18	—
OC6	ON1-80	7	650	6.5	6.8	5.4	1.2	—	0.15	—	—
OC7	ON2	6	570	6.5	7.0	5.1	1.2	0.35	0.35	—	—
OC8	ON2	6	600	7.2	7.8	6.2	1.3	0.16	—	—	—
OC9	NO	8	800	7.8	8.3	5.5	2.1	0.13	0.5	—	—
OC10	ON1-50	4	400	8.0	8.8	6.5	1.8	0.3	0.3	—	—
OC11	NO1	12	1200	8.1	8.1	7.9	1.7	—	0.25	—	—
OC12	NOR	7	631	8.2	8.9	6.5	1.4	0.3	0.6	—	—
OC13	ON1-80	5	500	8.4	8.6	6.4	2.0	—	0.2	—	—
OC14	OV	8	718	8.5	8.5	6.0	2.0	0.42	0.3	0.14	—
OC15	OV21	6	520	8.9	9.6	6.9	1.5	—	0.7	—	—
OC16	ON2	19	1900	8.9	10.9	8.1	2.3	0.05	0.3	—	—
OC17	ON1-50	3	300	9.0	9.0	6.6	2.3	—	—	—	—
OC18	ON2	16	1600	9.3	11.1	6.7	2.9	0.12	1.0	—	—
OC19	OV	12	1200	9.6	10.8	6.5	3.2	0.16	0.9	—	—
OC20	OV21	7	700	10.6	10.6	8.0	2.0	0.14	0.14	0.28	—
OC21	OR	5	500	12.4	14.4	11.0	3.2	—	0.2	—	—
OC22	ON2	5	500	12.8	14.4	10.6	3.4	—	0.2	0.2	—
OC23	CO	17	1700	13.8	15.9	11.1	3.7	0.47	0.29	0.17	—
OC24	ON2	3	300	16.0	18.3	13.3	4.0	—	0.6	—	0.3
OC25	OR	7	700	18.0	22.7	18.8	3.6	0.4	0.14	—	—
OC26	CO OV	3	300	21.6	24.0	21.6	2.0	—	0.3	—	—

*Mean value.

the four women taking either C-Quens or Oracon was significantly higher ($P \leq 0.001$) than the breakages in cultures from women on combination oral contraceptives.

Since the regimen for the sequential medications consists of 15 or 16 days of estrogens, followed by 5 days of estrogen-progestogens, we attempted to determine whether the number of breaks in specific cultures could be correlated with the type hormone taken. We ascertained the time in the oral contraceptive cycle of 30 of the sequential cultures. The average incidence of breakages in 18 cultures obtained during the estrogenic phase was 13.05 ± 1.4%; whereas, in 12 cultures obtained during the progestogen phase the average breakage was 19.8 ± 2.79% (Table 67). The difference in these means is significant ($P \leq 0.05$). The specific synthetic estrogens of the sequentials (mestranol, ethinyl estradiol) are also components of several combination medications; whereas, the progestogens (chlormadinone acetate and dimethisterone) are not used in the combination contraceptives.

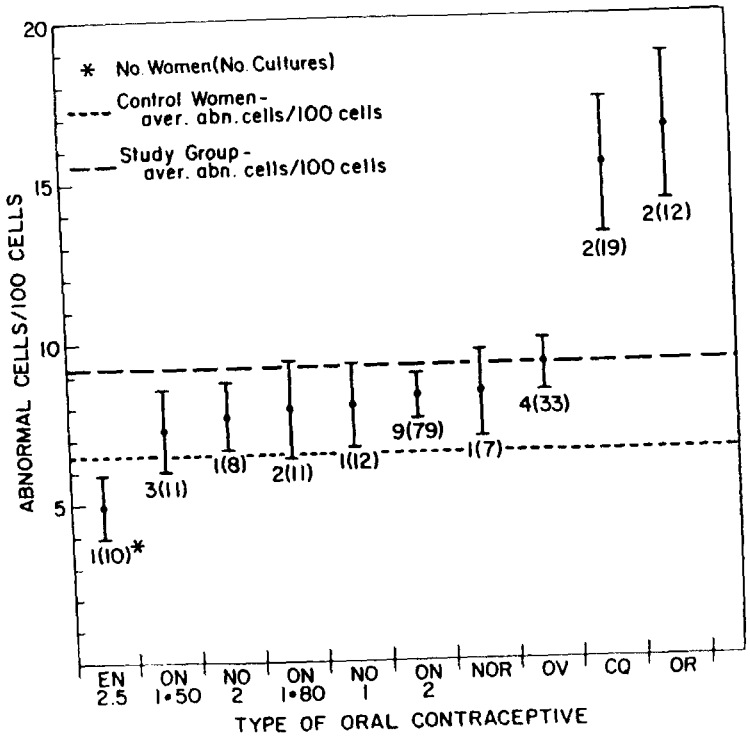


Fig. 72. Average incidence of chromosome breakages (±SE) from women taking different kinds of oral contraceptives. Note the high frequency of abnormal cells in cultures from four women taking sequential medications.

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TABLE 67
CHROMOSOMAL BREAKAGE FREQUENCY IN WOMEN TAKING SEQUENTIAL ORAL CONTRACEPTIVES: DATA OBTAINED DURING ESTROGENIC AND PROGESTOGENIC STAGES OF ORAL CONTRACEPTIVES CYCLE

		Estrogenic			Progestogenic		
No. women	Drug	No. cultures	No. cells	Abnormal cells	No. cultures	No. cells	Abnormal cells
2	C-Quens	18*	1800	235	12†	1200	238
2	Oracon						
Mean ± SE		13.05 ± 1.40‡			19.83 ± 2.79		

*Includes data from all cultures obtained during days 1–15 (estrogen only).
†Includes data from all cultures obtained during or immediately after (24 hr) days 16–20 (progestogen plus estrogen).
‡$t = 2.37$ (2-tail test); d.f. = 28; $P \leq 0.05$.

It became apparent that the high breakages in the 31 cultures from four women taking sequential medications were disproportionately raising the overall average breakages of the oral contraceptive group, so we retabulated our data excluding the sequential cultures. The average breakages in the women taking combination medications were 8.0% compared to 6.5% in cultures from the control women. The difference in these means was still highly significant ($P \leq 0.01$).

The high breakages in cultures from women taking sequentials cannot be explained at this time. Since the progestogen components differ from those used in the combination oral contraceptives, it is possible that these specific compounds are responsible for the increased breakages in cultures from the four women studied. On the other hand, the sudden shift from pure estrogen to combined estrogen-progestogen medication possibly could affect other metabolic systems which in turn result in increased chromosomal aberrations.

Sale of two sequentials (Provest and C-Quens) has already been discontinued because of undesirable side effects. Our data suggest that sequential oral contraceptives have a more pronounced effect on chromosomal breakages than combination-type medications.

Absence of Effect of Excess Vitamin A on Chromosome Breakage in Human Diploid Cells in Vitro

Numerous chemical, physical, and biological agents cause chromosome breakage in human diploid cells in vivo and in vitro. Some directly damage chromatin, for example by physical disruption of protein or DNA bonds (ionizing radiation) or by interference with protein or DNA synthesis (anti-

biotics, base analogues). The specific mechanism by which other clastogens (chromosome-breaking agents) induce breakage is not known. Obviously, chromosome damage is but one index of cell damage and agents causing chromosome breakage may, or may not, cause disruption at other cellular sites, and vice versa. Thus, it has been hypothesized that some substances causing chromosomal breakages may primarily affect other cellular organelles, resulting in secondary damage to genetic material. For example, agents that labilize lysosomal membranes might cause the release of hydrolytic enzymes into the cytoplasm; if acid nucleases or proteases, or both, gain access to nuclear contents, chromosomal damage could be induced indirectly. Increases in breakages appear in fibroblasts treated with photosensitizing dyes and exposed to high frequency light waves to disrupt lysosomal membranes.

We have seen a higher frequency of breakages in lymphocytes from women taking oral contraceptives. Other laboratories have reported that adding synthetic estrogens or progestogens to lymphocyte cultures does not cause increases in chromosome damage. Possibly these medications are inducing damage secondarily.

It has recently been reported that women taking oral contraceptives have an increase in plasma levels of vitamin A. We considered this to be significant in relation to our study for the following reasons. (1) Vitamin A is a potent teratogen when administered in excess quantities to pregnant rats; teratogenicity may or may not involve chromosomal breakages. (2) Vitamin A is known to primarily affect lysosomal membranes, resulting in labilization. Thus the possibility that the increased breakages in lymphocytes from women taking oral contraceptives might be related to the increased serum concentrations of the lysosomal labilizer, vitamin A, was suggested.

To test this hypothesis, we conducted a preliminary experiment (Lydia Corrill, Eugene Joiner, and L. Gayle Littlefield). Human diploid cells were incubated with an excess amount of vitamin A in vitro and checked for chromosomal breakages. Two human cell types were used: (1) lymphocytes and (2) skin fibroblasts. Lymphocytes from a normal male subject were cultured by standard techniques in autologous serum. Vitamin A [48 μg in 0.1 ml 5% ethanol (ETOH)] was added to 10 ml cultures at zero or 68 hr. Ethanol alone (0.1 ml, 5%) was added to one culture at hour zero. One culture was incubated without having been treated in any way. Cells were harvested at 72 hr.

We used sixteenth subcultured fibroblasts derived from a primary explant from a normal female subject. Vitamin A (48 μg in 0.1 ml 5% ETOH) was added to 10-ml cultures at 6 and 28 hr after subculturing. Ethanol alone was added to one culture at 6 hr. One normal untreated control culture was set up. Cells were harvested at 34 hr.

All cultures were coded and scored blindly. We analyzed 825 metaphases for gross chromosomal abnormalities including chromosome and isolocus breaks as well as single chromatid breaks. Results are presented in Tables 68 and 69. Although values show lowest breakage in untreated control cultures in the experiments using lymphocytes, the difference between these and values for

1028094

treated cultures cannot be considered significant. Chromosome and isolocus breaks occurred randomly in all fibroblast cultures, that is, no differently in control and treated cultures. Chromatid breaks, however, did appear to occur more frequently in treated than untreated cultures, but the number of cells available for analysis was not large enough to substantiate this observation.

These results using vitamin A as a possible chromosomal breaking agent were negative. Since the concentration of the vitamin (4.8 μg/ml culture media) was considerably higher than that reported in the serum of women taking oral contraceptives (1–2 μg/cc), we doubt that this substance is responsible for the increased breakages in the pill users.

We believe that despite these negative results our experimental design is basically sound, but that further investigation of this problem should include the following: (1) Means of checking the biological activity of our vitamin A

TABLE 68
CHROMOSOME AND ISOLOCUS BREAKS IN CONTROL AND VITAMIN A CULTURES (%)

	Control	ETOH(S)	Vitamin A*	Vitamin A†
Lymphocyte				
Expt. 1	6	4‡	4§	3§
Expt. 2	0	4	5	4
Fibroblast	4	8	0	8

*Added to lymphocyte cultures at hr 0; fibroblast, hr 6.
†Added to lymphocyte cultures at hr 68; fibroblast, hr 28.
‡0.1 ml 50% ETOH/10 ml culture.
§12 crystalline vitamin A dissolved in 0.1 ml 50% ETOH/ml.

TABLE 69
SINGLE CHROMATID BREAKS IN CONTROL AND VITAMIN A CULTURES (%)

	Control	ETOH(S)	Vitamin A*	Vitamin A†
Lymphocyte				
Expt. 1	0	4‡	6§	4§
Expt. 2	0	1	0	2
Fibroblast	8	4	12	12

*Added to lymphocyte cultures at hr 0; fibroblast, hr 6.
†Added to lymphocyte cultures at hr 68; fibroblast hr 28.
‡0.1 ml 50% ETOH/10 ml culture.
§12 μg crystalline vitamin A dissolved in 0.1 ml 50% ETOH/10 ml culture.

solution. Vitamin A is heat and light labile and precautions taken to protect the material may have been insufficient. (2) Means of checking exposure of lysosomes to vitamin A, that is, whether the vitamin penetrated the cell membrane. Use of radioactively labeled vitamin A would be an ideal way of checking this aspect. (3) Since lymphocytes are "lysosome poor" the use of fibroblasts, or myeloblasts, in future experiments would be advisable.

Cytogenetic Studies in an American Girl with Burkitt's Lymphoma

Cytogenetic studies of tumor cells from patients with Burkitt's Lymphoma (BL) have revealed aberrant stem-lines in most preparations. In general, the chromosomal changes do not appear to be specific for this neoplasm, but two different "recurring" marker chromosomes have appeared in malignant cells from several persons with this disease. A large acrocentric marker the size of the long arm of a number 2 chromosome has occurred in primary tumor tissue from seven patients with BL. In metaphases from 6 of the 7 patients, one number 2 chromosome was missing, indicating that the abnormal marker was derived from the missing group A chromosome. A second distinct marker has been seen in four African children with classical Burkitt's tumor, and in tumor cells from one American woman with poorly differentiated lymphocytic lymphoma morphologically resembling BL. This chromosome is an abnormal submetacentric, larger than any other chromosome in the human complement. It apparently replaced a number 1 chromosome in metaphases from three patients, and a number 3 chromosome in one patient. A morphologically identical marker replacing a number 1 chromosome has also been observed sporadically in the Jiyoye, Ogun, SL, and EB cell lines derived from Burkitt's tumors. Because of its distinctive morphology, it is readily identified in cytogenetic preparations, and it has been suggested that this chromosome be referred to as the recurring marker number 1 (RM-1) chromosome. A large submetacentric chromosome in lieu of a number 1 has also been found in tumor preparations from two patients with reticulum-cell sarcoma, in one patient with adenocarcinoma, and in a cell line derived from a patient with myeloma.

We have recently studied the chromosomes of marrow and blood preparations from a 15-year-old American girl with BL (L. Gayle Littlefield and Margo Steinman). Samples were obtained over a 6-week period, before chemotherapy, during treatment with Cytoxan, and at autopsy.

Five direct marrow samples were analyzed (Table 70). In the pretreatment marrow 87 of 100 metaphases had identical karyotypic abnormalities (Fig. 73). All aberrant metaphases were pseudodiploid, missing one number 1 and three group C chromosomes, replaced with four marker chromosomes. One of the markers was a large submetacentric chromosome apparently derived from the missing number 1, morphologically identical to the previously described RM-1 chromosome. In addition to the RM-1, metaphases from the marrow had three other consistent markers: an acrocentric similar to the D chromosomes, a metacentric slightly larger than the E 16, and a small metacentric slightly larger than the F chromosomes. The findings agreed with hematologic studies, which

1028095

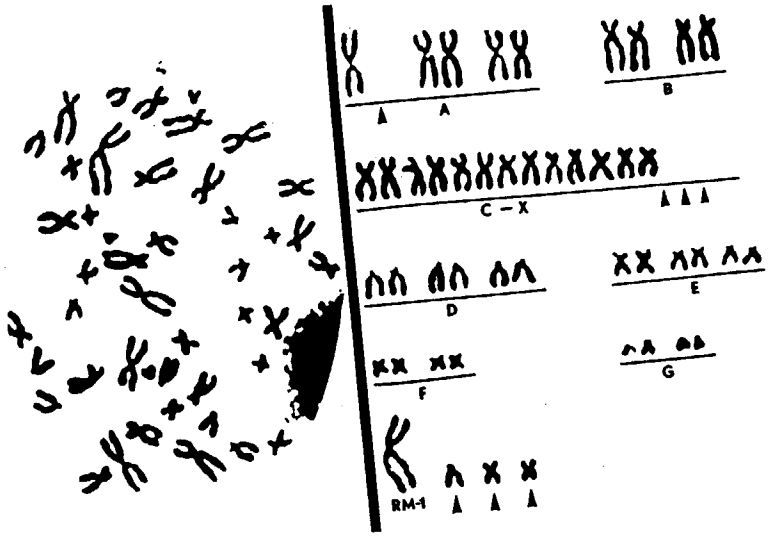


Fig. 73. Pseudodiploid metaphase from a direct marrow preparation showing four marker chromosomes. The large submetacentric chromosome is morphologically identical to the RM-1 marker seen in metaphases from at least five other persons with BL.

showed extensive infiltration of tumor cells into the marrow.

The abnormal stem-line persisted in the marrow throughout the course of Cytoxan treatment, but the percent of the marker cells varied in different preparations. During the course of chemotherapy the patient received two leukocyte transfusions from a woman with Ph^1-positive chronic granulocytic leukemia. Marrow aspirates obtained within a few days following the transfusions were contaminated with a significant number of Ph^1-positive metaphases (Fig. 74). The absolute ratio of RM-1 stem cells to nonmalignant marrow elements was difficult to assess in these preparations.

Phytohemagglutin-stimulated blood cultures were initiated on two occasions during the course of Cytoxan treatment. We analyzed 108 metaphases from the first sample and 200 metaphases from the second (Table 71). Five from the first culture (4.6%) and five from the second culture (2.5%) were hypodiploid, with a specific chromosomal rearrangement. Each of these cells was missing a G and group C chromosome and had an extra submetacentric the size of a group B chromosome but with longer short arms (Fig. 74). This abnormal chromosome probably represented an unbalanced C/G translocation. In one other study of biopsies of bilateral mandibular tumors in an African child with BL karyotypically different stem-lines were found in the left and right side tumors.

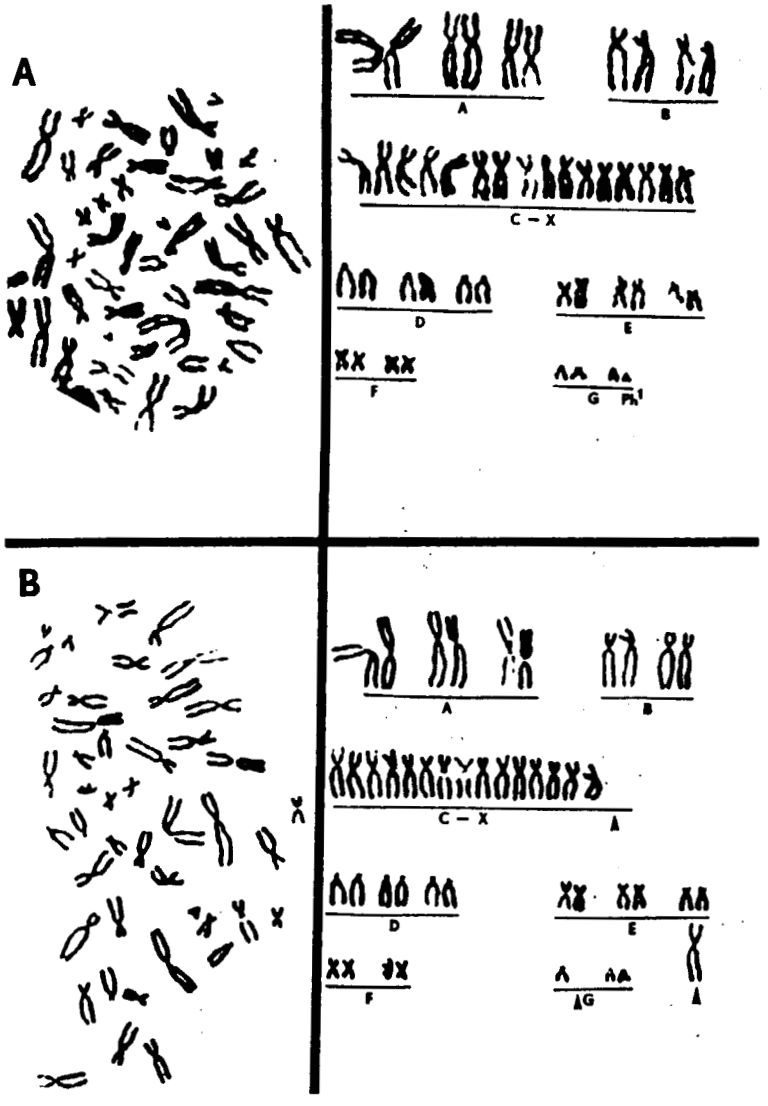


Fig. 74A. Ph^1-positive metaphase from a direct marrow preparation 3 days after a leukocyte transfusion from a female patient with CGL.

B. A hypodiploid metaphase from a PHA-stimulated blood culture showing a presumably C-G translocation marker chromosome. Ten out of 308 metaphases have this abnormal karyotype.

1028096

TABLE 70

PERCENT OF RM-1, Ph^1-POSITIVE, AND NORMAL DIPLOID METAPHASES IN DIRECT MARROW SAMPLES

Date	5-21*†	5-28	6-11*	6-23†	7-6‡
Metaphases analyzed	100	50	100	100	14
Karyotype					
Lymphoma stem-line 46,XX,$-A^1$,-C,-C,-C + 4 markers	87%	12%	82%	97%	10%
CGL marker 46,XX,Ph^{1+}	0	40%	0	0	90%
Normal 46,XX	13%	48%	18%	3%	0

*Cytoxan, 1.5 g, IV, 5-22 and 6-12.
†WBC transfusion from CGL donor 5-26 and 7-1.
‡Autopsy.

TABLE 71

PERCENT OF C/G TRANSLOCATION MARKER AND NORMAL DIPLOID METAPHASES IN PHA-STIMULATED BLOOD CULTURES

Date	5-25*	6-7†
Metaphases analyzed	108	200
Karyotype		
Lymphoma stem-line 45,-C,-G + marker	4.6%	2.5%
Normal 46,XX	95.4%	97.5%

*Cytoxan, 1.5 g, IV, 5-22.
†WBC transfusion from CGL donor 5-26.

The minor stem-line in the blood of our patient may have been derived from a different tumor site than the cells of the primary stem-line found in the marrow.

Abnormalities in chromosome number and/or morphology are common in neoplastic tissues, and may indicate malignancy or herald the transformation of one phase of the disease into another. Except for the Philadelphia chromosome

of CGL, disease specific aberrations have not been observed. In our search of the literature, we found cytogenetic studies of primary tumors from only 17 patients with BL. If we include our data, 6 of the 18 patients had what appears to be the same chromosomal marker in some or all of the metaphases examined. The high frequency of occurrence of this RM-1 marker in tumor cells suggests that it may be highly specific for BL.

1028097

PUBLICATIONS

From the Medical Division, Oak Ridge Associated Universities, 1971

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JOURNAL ARTICLES

Published

1. Andrews, G. A. and Haywood, F. F., *Clinical and Biological Consequences of Nuclear Explosions*, Practitioner, 207, 331-342, 1971.
2. Bell, O. E., Jr., Blank, M. L., and Snyder, F., *The Incorporation of ^{18}O and ^{14}C from Long-Chain Alcohols into the Alkyl and Alk-1-enyl Ethers of Phospholipids of Developing Rat Brain*, Biochim. Biophys. Acta, 231, 579-583, 1971.
3. Blank, M. L., Cress, E. A., Stephens, N., and Snyder, F., *On the Analysis of Long-Chain Alkane Diols and Glycerol Ethers in Biochemical Studies*, J. Lipid Res., 12, 638-640, 1971.
4. Blank, M. L., Wykle, R. L., and Snyder, F., *Enzymic Synthesis of Ethanolamine Plasmalogens from an O-Alkyl Glycerolipid*, FEBS Letters 18, 92-94, 1971.
5. Brewen, J. G., and Gengozian, N., *Radiation-Induced Human Chromosome Aberrations. II. Human in vitro Irradiation Compared to in vitro and in vivo Irradiation of Marmoset Leukocytes*, Mutat. Res., 13, 383-391, 1971.
6. Carlson, D. E. and Gengozian, N., *The Effect of Acute Radiation Exposure Rates on Formation of Hemagglutinating Antibody in Mice*, J. Immunol., 106, 1353-1362, 1971.
7. Carlton, J. E. and Hayes, R. L., *Rapid Separation of Generator-Produced Gallium-68 from EDTA, Eluate*, Int. J. Appl. Radiat, Isotop., 22, 44-45, 1971.
8. Gengozian, N., Congdon, C. C., Allen, E. A., and Toya, R. E., *Immune Status of Allogeneic Radiation Chimeras*, Transplant. Proc., 3, 434-436, 1971.
9. Gengozian, N. and Hübner, K., *"In situ" Visualization of a Graft-vs-Host Reaction*, J. Immunol., 106, 1159-1165, 1971.
10. Goh, K. O., *Chloramphenicol, Acute Leukemia and Chromosomal Vacuolizations*, S. Med. J., 64, 815-819, 1971.
11. Goh, K. O., *Total Body Irradiation and Human Chromosomes. II. Cytogenetic Studies of the Cultured Bone Marrow Cells Seven Years After Total Body Irradiation*, Amer. J. Med. Sci, 262, 43-49, 1971.

12. Grigor, M. R., Blank, M. L., and Snyder, F., *Structural Relationships Between Glycerides of Pig Serum and Adipose Tissue*, Lipids, 6, 965-968, 1971.
13. Lumb, R. H. and Snyder, F., *A Rapid Isotopic Method for Assessing the Biosynthesis of Ether Linkages in Glycerolipids of Complex Systems*, Biochim. Biophys. Acta, 244, 217-221, 1971.
14. Mardon, D. N., Hurst, S. K., and Balish, E., *Germ-Tube Production by Candida Albicans in Minimal Liquid Culture Media*, Can. J. Microbiol., 17, 851-856, 1971.
15. Morris, A. C., Jr., Barclay, T. R., Tanida, R., and Nemcek, J. V., *A Miniaturized Probe for Detecting Radioactivity at Thyroid Surgery*, Phys. Med. Biol., 16, 397-404, 1971.
16. Piantadosi, C., Ishaq, K. S., Wykle, R. L., and Snyder, F., *Synthesis and Characterization of 1-O-Alkyldihydroxy-Acetone Phosphates and Derivatives*, Biochemistry, 10, 1417-1421, 1971.
17. Savage, N. and Balish, E., *Morphology, Physiology, and Virulence of Some Mutants of Candida Albicans*, Infect. Immun., 3, 141-148, 1971.
18. Snyder, F., Blank, M. L., and Wykle, R. L., *The Enzymic Synthesis of Ethanolamine Plasmalogens*, J. Biol. Chem., 246, 3639-3645, 1971.
19. Snyder, F., Hibbs, M., and Malone, B., *Enzymic Synthesis of O-Alkyl Glycerolipids in Brain and Liver of Rats During Fetal and Postnatal Development*, Biochim. Biophys. Acta, 231, 409-411, 1971.
20. Swartzendruber, D. C. Nelson, B., and Hayes, R. L., *Gallium-67 Localization in Lysosomal-Like Granules of Leukemic and Nonleukemic Murine Tissues*, J. Nat. Cancer Inst., 46, 941-952, 1971.

In Press

21. Blank, M. L., Kasama, K., and Snyder, F., *Isolation and Identification of an Alkyldiacylglycerol Containing Isovaleric Acid*, J. Lipid Res.
22. Brewen, J. G., Preston, R. J., and Littlefield, L. G., *Radiation-Induced Human Chromosome Aberrations. III. Aberration Yields Following an Accidental Whole-Body Exposure to ^{60}Co γ-Rays*, Radiat. Res.
23. Cloutier, R. J., Watson, E. J., Hayes, R. L., Nelson, B., and Smith, E. M., *Summary of Current Radiation Dose Estimates to Humans from: ^{66}Ga, ^{67}Ga, ^{68}Ga and ^{72}Ga Citrate*, J. Nucl. Med.
24. Grigor, M. R., Pratt, R. D., and Snyder, F., *Acylation of Cholesterol by Microsomal Enzymes from Mouse Preputial Gland Tumors*, Arch. Biochem. Biophys.
25. Kniseley, R. M., *Marrow Studies with Radiocolloids*, Seminars Nucl. Med.
26. Littlefield, L. G., *An Abnormal Cell-Line in a Patient with Acute Granulocytic Leukemia: Cytogenetic Studies Before and After an Allogeneic Marrow Transplant*, Cancer.
27. Nelson, B., Hayes, R. L., Edwards, C. L., Kniseley, R. M., and Andrews, G. A., *The Distribution of Gallium in Human Tissues After Intravenous Administration*, J. Nucl. Med.

1028098

28. Piantadosi, C., Ishaq, K. S., Blank, M. L., and Snyder, F., *Synthesis of 1-O-Alkyl-3-O-Phosphorylethanolamine-2-Propanone and Related Derivatives*, J. Pharm. Sci.
29. Porter, R. P. and Gengozian, N., *In vitro Proliferation and Differentiation of Hemic Precursor Cells from Marrow and Blood of Naturally Chimeric Marmosets*, J. Cell. Physiol.
30. Vodopick, H., Rupp, E. M., Edwards, C. L., Goswitz, F. A., and Beauchamp, J. J., *Spontaneous Cyclic Leukocytosis and Thrombocytosis in Chronic Granulocytic Leukemia*, New Engl. J. Med.
31. Wykle, R. L., Piantadosi, C., and Snyder, F., *The Role of Acyldihydroxyacetone Phosphate, NADH, and NADPH in the Biosynthesis of O-Alkyl Glycerolipids by Microsomal Enzymes of Ehrlich Ascites Tumor*, J. Biol. Chem.

CHAPTERS IN BOOKS AND SYMPOSIUM PROCEEDINGS

Published

32. Gengozian, N., *Male and Female Cell Populations in the Chimeric Marmoset*, In: Medical Primatology, 1970; Selected Papers from the 2nd Conference on Experimental Medicine and Surgery in Primates, New York 1969; E. I. Goldsmith and J. Moor-Jankowski, eds., Basel, S. Karger, 1971, pp. 926-938.
33. Gengozian, N. and Porter, R. P., *Transplantation Immunology in the Marmoset*, In: Medical Primatology, 1970; Selected Papers from the 2nd Conference on Experimental Medicine and Surgery in Primates, New York, 1969; E. I. Goldsmith and J. Moor-Jankowski, eds., Basel, S. Karger, 1971, pp. 165-175.
34. Goh, K. O. and Heusinkveld, R. S., *Contributions of Cytogenetics to Hematology*, In: Hematology for Internists; R. I. Weed, ed., Boston, Little, Brown, 1971, pp. 275-285.
35. Hagen, P. O., *The Effect of Low Temperatures on Microorganisms: Conditions Under Which Cold Becomes Lethal*, In: Inhibition and Destruction of the Microbial Cell, W. B. Hugo, ed., New York, Academic Press, 1971, pp. 39-74.
36. McDow, A. E., Jr., and Ricks, R. C., *Uses of a Time-Shared Computer in Quantitative Nuclear Medicine*, In: Proceedings of Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Oak Ridge, 1971. USAEC Report CONF-710425, 1971, pp. 155-167.
37. Snyder, F., *The Chemistry, Physical Properties, and Chromatography of Lipids Containing Ether Bonds*. In: Progress in Thin-Layer Chromatography and Related Methods, Vol. II, A. Niederwieser and G. Pataki, eds., Ann Arbor Science Publishers, 1971, pp. 105-141.

38. Snyder, F., *Glycerolipids in the Neoplastic Cell: Methodology, Metabolism, and Composition.* In: Methods in Cancer Research, Volume VI. Harris Busch, ed., New York, Academic Press, 1971, pp. 399-436.
39. Snyder, F. and Moehl, A., *Mass Measurements in a Liquid Scintillation Spectrometer: Quantitation of Sulfhydryl Moieties by Color Quenching.* In: Organic Scintillators and Liquid Scintillation Counting, Proceedings of the International Conference on Organic Scintillators and Liquid Scintillation Counting, San Francisco, July 1970, D. L. Harrocks and C-T. Peng, editors, New York, Academic, 1971, pp. 419-424.
40. Snyder, W. S., Cloutier, R. J., and Smith, S., *Dose to a Developing Fetus from a Gamma Emitter in the Bladder.* In: Health Physics Division Annual Progress Report for Period Ending July 31, 1971. USAEC Report ORNL 4720, Oak Ridge National Laboratory, 1971, pp. 119-120.

In Press

41. Beck, W. L., Stokes, T. R., and Lushbaugh, C. C. *Dosimetry for Radiobiological Studies of the Human Hematopoietic System.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971.
42. Duggan, J. L. and Beck, W. L., *Charged Particle Induced X-Ray Fluorescence.* In: Proceedings of the 20th Annual Denver Conference on Application of X-Ray Analysis, 1971, New York, Plenum.
43. Lushbaugh, C. C., *Predicted Levels of Human Radiation Tolerance Extrapolated from Clinical Studies of Radiation Effects.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971.
44. Lushbaugh, C. C. and Ricks, R. C., *Some Cytokinetic and Histopathologic Considerations of Irradiated Male and Female Gonadal Tissues.* In: Sixth Annual San Francisco Cancer Symposium, October 1970. Proceedings.
45. Ricks, R. C., Lushbaugh, C. C. and McDow, E., *Pulmonary-Impedance Power Spectral Analysis: A Facile Means of Detecting Radiation-Induced Gastrointestinal Distress and Performance Decrement in Man.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971.
46. Snyder, F., *The Enzymic Pathways of Ether-Linked Lipids and Their Precursors.* In: Ether Lipids: Chemistry and Biology. F. Snyder, ed., New York, Academic Press.
47. Snyder, F., *Enzymic Systems that Synthesize and Degrade Glycerolipids Possessing Ether Bonds.* In: Advances in Lipid Research, Vol. 10, New York, Academic Press.
48. Snyder, F., *Ether-Linked Lipids and Fatty Alcohol Precursors in Neoplasms.* In: Ether Lipids: Chemistry and Biology. F. Snyder, ed., New York, Academic Press.

1028099

49. Yuhas, J. M., Stokes, T. R., and Lushbaugh, C. C., *Multifactorial Analysis of Human Blood Cell Responses to Clinical Total-Body Irradiation.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971.

GOVERNMENT REPORT

Published

50. Oak Ridge Associated Universities, Medical Division, Research Report, 1970, for the Year Ending December 31, 1970. USAEC Report ORAU 113, 1971.

MISCELLANEOUS PUBLICATIONS

51. Beck, William L., Jr., *K-Shell Ionization Cross Section for AG and L-Shell Ionization Cross Sections for Au, Bi, and U for Protons from 1.0 to 5.0 MeV.* Masters Thesis, University of Tennessee, 1971.
52. Cloutier, R. J., Book Review: *Protection in the Management of Patients Who Have Received Therapeutic Amounts of Radionuclides.* NCRP Report No. 37. NCRP Publications, Washington, 1970. Health Phys., 21, 343-344, 1971.

ABSTRACTS

Published

53. Beck, W. L., Cloutier, R. J., and Watson, E. E., *High-Dose Monitoring of Personnel with Film and Thermoluminescent Dosimetry,* Health Phys., 21, No. 6, 58 (abstracts section) December 1971. Abstract P/114.
54. Beck, W. L., Stokes, T. R., Cloutier, R. J., and Lushbaugh, C. C., *Dosimetry for Clinical Total-Body Irradiation,* Phys. Med. Biol., 16, 165, 1971, Abstract.
55. Blank, M. L., Cress, E. A., and Snyder, F., *The Metabolism of Fatty Acids and Fatty Alcohols in Fibroblasts (L-M Cells) Grown as Monolayers,* J. Amer. Oil Chem. Soc., 48, 100A, February 1971. Abstract 135.
56. Chaskes, S. and Kingdon, C., *Immunoglobulins (IgG, IgA and IgM) in Irradiated Humans,* Bacteriol. Proc., 71, 73, 1971. Abstract M 51.
57. Cloutier, R. J. and Berger, J. D., *Health Physics Practices in Laboratories Using ^{3}H- and ^{14}C-labeled Tracers,* J. Amer. Oil Chem. Soc., 48, 330A, 1971. Abstract 80.
58. Cloutier, R. J., Watson, E. E., Hayes, R. L., and Nelson, B., *Radiation Doses from Isotopes of Gallium,* J. Nucl. Med., 12, 348, 1971. Abstract.

59. Edwards, C. L. and Hayes, R. L., *^{67}Ga for Scanning and Staging Tumors,* J. Nucl. Med., 12, 355, 1971. Abstract.
60. Edwards, C. L., Hayes, R. L., Ominsky, S., Byrd, B. L., and Rafter, J. J. *Intralymphatic Injection of ^{67}Ga for Visualizing Abdominal Lymph Nodes,* J. Nucl. Med., 12, 431, 1971, Abstract.
61. Goswitz, F., Nelson, B., Vodopick, H., and Lushbaugh, C. *Human Marrow Changes After Treatment with Low-Dose-Rate Total-Body Irradiation,* J. Nucl. Med., 12, 359, 1971. Abstract.
62. Hayes, R. L., Byrd, B. L., Carlton, J. E., and Rafter, J. J., *Effect of Scandium on the Distribution of ^{67}Ga in Tumor-Bearing Animals,* J. Nucl. Med., 12, 437-438, 1971. Abstract.
63. Hayes, R. L., Nelson, B., Swartzendruber, D. C., Brown, D. H., Carlton, J. E., and Byrd, B. L., *Studies of the Intracellular Deposition of ^{67}Ga,* J. Nucl. Med., 12, 364, 1971. Abstract.
64. Kraus, L. M., Rasad, A., and Lushbaugh, C. C., *Fast Moving Hemoglobin (Hb) in C57BL/Cum Mice: Induced by Continuous Low Level Irradiation,* Amer. Chem. Soc., National Meeting, 162nd. Abstracts of Papers. Abstract 309, 1971.
65. Lee, T-c. and Snyder, F., *The Measurement of Lipid Turnover in Membranes by a Double Isotope Technique,* J. Amer. Oil Chem. Soc., 48, 330A, 1971. Abstract 82.
66. Littlefield, L. G., Goh, K-O, Klepper, M. B., and Joiner, E. E. *Chromosome Breakages in Women Taking Oral Contraceptives: A Two-Year Study.* In: Fourth International Congress of Human Genetics, Paris, 1971, Abstracts of Papers Presented, Amsterdam, Excerpta Medica, 1971. (International Congress Series, 233.) p. 113. Abstract 405.
67. Lushbaugh, C. C., Stokes, T. R., and Corrill, L. S., *Dose-Rate Dependence of Mouse Endogenous Splenic Colony Formation and Proliferation During Continuous and Fractionated Daily Irradiation at Low Exposure Rates,* Radiat, Res., 47, 344, 1971. Abstract Hc-2.
68. Nelson, B., Hayes, R. L., Edwards, C. L., Kniseley, R. M., and Andrews, G. A., *Distribution of ^{67}Ga in Human Tissues at Autopsy,* J. Nucl. Med., 12, 452, 1971. Abstract.
69. Otten, J. A., Tyndall, R. L., Estes, P. C., and Swartzendruber, D. C., *Localization of Gallium-67 Citrate During Embryogenesis,* Proc. Amer. Assoc. Cancer Res., 12, 41, 1971. Abstract 163.
70. Snyder, F. and Malone, B., *The Biosynthesis of Wax Esters: Enzymic Interconversions of Fatty Acids and Fatty Alcohols,* J. Amer. Oil Chem. Soc., 48, 85A, February 1971.
71. Swartzendruber, D. C., and Hübner, K. F., *Effect of Irradiation on ^{67}Ga Distribution in Mouse Tissues.* In: American Society for Cell Biology. Eleventh Annual Meeting, New Orleans, 1971. Abstracts of Papers, p. 296, 1971. Abstract 583.
72. Wykle, R. L., Blank, M. L., and Snyder, F., *The Enzymic Synthesis of Ethanolamine Plasmalogens,* Fed. Proc., 30, 1243, 1971. Abstract 1114.

1028100

MEDICAL DIVISION CONFERENCES–1971

January 5 *Some Concepts in the Radiation Therapy of Hodgkin's Disease.* Frank Comas, University of Tennessee Memorial Research Center Hospital.

January 12 *Lymphangiography—Worth the Time and Expense?* Edward Buonocore, University of Tennessee Memorial Research Center Hospital.

January 19 *Chemotherapy of Hodgkin's Disease and Other Lymphomas.* Stephen Krauss, University of Tennessee Memorial Research Center Hospital.

January 26 *Ultrastructural Studies in Mouse Leukemia and Lymphoma.* Donald Swartzendruber, Division staff.

February 2 *Management of Serious Infections in Hematologic Disorders.* Verne E. Gilbert, Oak Ridge.

February 7 *Histoproliferative Responses to Murine Leukemia Virus.* Michael G. Hanna, Jr., ORNL Biology Division.

February 16 *Clinical Importance of the Immunoglobulins in Lymphohistiocytic Disorders.* Joseph Hathaway, St. Mary's Hospital, Knoxville.

February 23 *In Vitro Studies of Lymphocyte Survival.* Francis A. Goswitz, Division staff.

March 2 *Medical Instrumentation Group Developments in Gamma-Ray Scanning.* P. R. Bell, ORNL.

March 23 *Medical Radiation Dosimetry by Thermoluminescence Techniques.* W. L. Beck, Division staff.

April 6 *Applications of Microanalytical Techniques to Biological Investigations.* Helen Mateer and Ralph Gehlbach, ORNL.

April 13 *Physiologic Monitoring and Total-Body Irradiation.* Robert Ricks, Division staff.

April 20 *The Selective Metabolism of Fatty Alcohols in Neoplastic Cells.* Fred Snyder, Division staff.

April 27 *Clinical Isotopic Studies in Germany.* G. Hoffmann, Nuclear Medicine Department, Medizinischen Universitäts-Klinik, Freiburg, Germany.

May 4 *The Ratio of Thyroxine/Triiodothyronine as a Possible Regulating Principle for Peripheral Hormone Supply.* Dieter Emrich, Göttingen University, Göttingen, Germany.

May 11 *Host-mediated Assay Systems for Environmental Mutagenesis.* J. G. Brewen, ORNL Biology Division.

May 18 *Antigenic Alterations of Neoplastic Cells.* Raymond W. Tennant, ORNL Biology Division.

May 25 *Radiobiology Simplified for Clinicians and Chemists.* A. H. Haber, ORNL Biology Division.

June 1 *Gallium Dosimetry.* Roger J. Cloutier, Division staff.

June 15 *Studies of the Distribution of Gallium in Animal Tissues.* Raymond L. Hayes, Division staff.

June 24 *Radioisotopic Studies in Congenital Anemias, Especially Thalassemia.* Basile Malamos, University of Athens, Greece.

Radioimmunoassay of Human Chorionic Somatomammotropin. C. Constantinides, University of Athens, Greece.

June 29 *Phagocytosis of Aged Mice.* Eugene H. Perkins, ORNL Biology Division.

July 13 *Highlights of the 18th Annual Meeting of the Society of Nuclear Medicine (Los Angeles, June 26–July 2).* G. A. Andrews, Division staff.

July 20 *Biomedical Computer Systems.* A. Earl McDow, Jr., Division staff.

July 27 *Sequential Testing.* Marvin Kastenbaum, Washington, D. C.

August 3 *In vitro Diagnostic Tests.* Clara Heise, Bowman Gray School of Medicine, Winston-Salem, North Carolina.

August 17 *Management of Lymphomas with Irradiation.* Frank Comas, University of Tennessee Memorial Research Center Hospital.

August 24 *Technical Experts in the Control of Technology.* Claire Nader, ORNL.

August 31 *The Effects of Partial Irradiation on Intestinal Epithelium.* Samuel W. Lesher, Allegheny General Hospital, Pittsburgh, Pennsylvania.

September 7 *Management: Its Role in Research and Development.* John T. Crockett, Jr., Division staff.

September 21 *Future Prospects of ORAU.* William G. Pollard, ORAU.

September 28 *Trace Element Analysis by Charged Particle Induced X-Ray Fluorescence.* Jerome Duggan, ORAU Special Training Division.

October 12 *The Calculation of Radiation Doses to Survivors of the Nuclear Bombing of Japan.* Harry H. Hubbell, Jr., ORNL Health Physics Division.

October 17 *Radiation and Chemical Safety.* James D. Berger, ORAU Radiation Safety.

October 24 *Some Applications of Physics in Medicine.* Fearghus T. O'Foghludha, Duke University.

November 23 *Gallium in Japan.* Raymond L. Hayes, Division staff.

December 7 *Spontaneous Intestinal Carcinoma in Animals.* C. C. Lushbaugh.

LIPID SEMINARS–1971

January 21 *A Time Study of the Microsomal Reaction Between Dihydroxyacetone Phosphate and Fatty Acyl-CoA and Fatty Alcohol.* Murray Grigor, visiting scientist from Medical Research Council of New Zealand.

February 25 *Characterization and Metabolism of a New Ether-Containing Glycerolipid from Harderian Glands of the Rabbit.* Kazuo Kasama, visiting scientist from Mie Prefectural School of Medicine, Mie Prefecture, Tsu, Japan.

April 8 *The Process of Membrane Assembly in Tetrahymena Pyriformis.* Guy A. Thompson, Jr., Department of Botany, University of Texas at Austin.

May 27 *Regulation of Protein Turnover.* Kai-lin Lee, ORNL Biology Division.

July 29 *The Subcellular Distribution of Ether Lipids in Tumors.* Ten-ching Lee, Biological Chemistry, Division staff.

1028101

FELLOWSHIPS AND TERM APPOINTMENTS

Visiting Scientist in Lipid Research
Kun Chae, M.S.
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School of Pharmacy

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Thomas F. Hogan*
Virginia Commonwealth University, Richmond

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John Kateley, M.S.
Mike Yurconic, B.A.
Elizabeth Allen, M.S.
Andrew So, M.S.

Postdoctoral Fellows
Murray Grigor
Medical Research Council of New Zealand

Kazuo Kasama
Mie Prefectural University
School of Medicine

Donald D. Barnhart
University of Wisconsin

Damon Runyon Fellows
Roger Lumb
Western Carolina University

Yukio Tateno, M.D.*
University of Chiba Hospital

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Winston Salem, North Carolina

Jessup MacLean Shively
Clemson University
Clemson, South Carolina

Ki-Hyon Kim
North Carolina Central University
Durham, North Carolina

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Immaculata, Pennsylvania

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*Terminated during 1971.

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Angwin, California

Nancy J. Hitzfelder
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San Antonio, Texas

Raymond D. Pratt
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Evanston, Illinois

Timothy Taga
Loyola University
Los Angeles, California

ORAU MEDICAL DIVISION ADVISORY COMMITTEE

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Robert H. Greenlaw (Chairman)
Marshfield Clinic

John G. Coniglio
Vanderbilt University

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Johns Hopkins Medical Institutions

Wright H. Langham
Los Alamos Scientific Laboratory

Shields Warren
Cancer Research Institute
New England Deaconess Hospital

Henry J. Winn
Massachusetts General Hospital

1971–1972

Robert M. Heyssel (Chairman)
Johns Hopkins Medical Institutions

John G. Coniglio
Vanderbilt University

Douglas A. Grahn
Argonne National Laboratory

Robert H. Greenlaw
Marshfield Clinic

Shields Warren
Cancer Research Institute
New England Deaconess Hospital

Henry J. Winn
Massachusetts General Hospital

1028102

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Charles Kochakian
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North Carolina State University

Thomas H. Siddall, III
Louisiana State University

1971–1972

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Meharry Medical College

Lowell F. Bailey
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Ernest A. Daigneault
Louisiana State University
Medical Center

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University of Arkansas
Medical School

P. R. Girardot
University of Texas
Arlington

M. J. Ohanian
University of Florida

Walter J. Peterson
North Carolina State University

Thomas H. Siddall, III
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Joseph F. Volker
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Merle Blank, B.S.
David H. Brown, Ph.D.
Billy L. Byrd, B.S.
Eddie Ruth Capshaw
James E. Carlton, B.S.
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Stuart J. Chaskes, M.S.
Martha Clevenger, B.S.
Roger Cloutier, M.S.
Shirley B. Colyer, B.S.
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Dieter Emrich,* M.D.
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Gretchen Humason, M.A.
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*Terminated 1971.

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1972

1028104



1972 RESEARCH REPORT

Medical Division

Oak Ridge Associated Universities

1028105

ORAU-122

1972
RESEARCH REPORT

for the year ending December 31, 1972



Medical Division
Oak Ridge Associated Universities

OPERATING UNDER CONTRACT WITH
THE UNITED STATES ATOMIC ENERGY COMMISSION

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COVER—The figure is taken from the ceramic mural, "Hymn to Life," by East Tennessee artist Charles Counts, which is on the facade of the Medical Division's research hospital building. The work was commissioned by the division from funds contributed by friends and patients, and is reproduced on the cover from plates prepared for a special ORAU 25th anniversary report on the Medical Division to the faculties of its member colleges and universities.

NOTICE

This report contains preliminary data and was prepared primarily to record the year's work, and is not intended as a substitute for publication in the open literature. It is subject to revision and does not represent a final report. Please do not quote from or refer to this work in open literature without permission from individual authors to reference the material as a personal communication.

ORAU-122

Requests for additional information in reference to this report may be directed to:
Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

Printed in the United States of America. Price $7.60.
Available from: National Technical Information Service, Springfield, Virginia 22151.

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CONTENTS

ORGANIZATION OF RESEARCH iv

INTRODUCTION 1

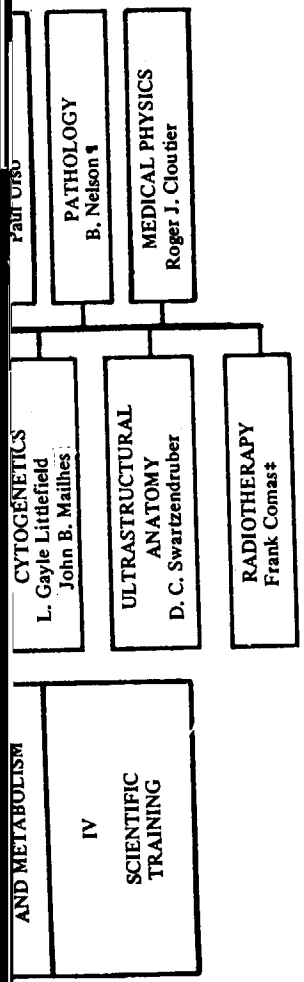
RADIATION EFFECTS AND RELATED TOPICS 4
- Summary of Accomplishments 4
- Clinical Observations 6
- Infections in Irradiated Patients 18
- Dosimetry Studies and Monitoring 29
- Applied Radiobiology 33
- Radiation Immunology and Marrow Transplantation 50
- Experimental Hematology 62

RESEARCH IN NUCLEAR MEDICINE 64
- Summary of Accomplishments 64
- Radiopharmaceutical Development 65
- Clinical Investigations in Nuclear Medicine 82
- Instrumentation and Computer Methods 95

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM 104
- Summary of Accomplishments 104
- Biological Chemistry 105
- Marmoset Research Project 129
- Ultrastructural Anatomy 151
- Induced Cytogenetic Abnormalities 161
- Miscellaneous Research Activities 178

MISCELLANEOUS 182
- Publications 182
- Medical Division Conferences - 1972 187
- Lipid Seminars - 1972 188
- Regional Lipid Meeting 189
- Fellowships and Term Appointments 192
- Research Participants - 1972 192
- Undergraduate Student Trainees - 1972 192
- ORAU Medical Division Advisory Committee 193
- ORAU Medical Division Committee on Human Studies 193
- Medical Committee, ORAU Council 194
- Medical Division Employees 195
- Consultants and Collaborators - 1972 197
- List of Non-AEC Support 202

Note: Numbered references in the text may be located in the list of publications beginning on page 182.



INTRODUCTION

This is the annual report for calendar year 1972 for the Medical Division of Oak Ridge Associated Universities. The Division conducts biomedical research, under contract with the United States Atomic Energy Commission, with some additional support from other sources, both federal and private. The main research objectives lie in three closely related areas: 1) mechanisms of radiation injury, especially in man; 2) beneficial applications of radiation for diagnosis and therapy of disease, especially cancer; and 3) basic biological and physical problems arising from ongoing research and mainly related to cancer. In addition, the staff devotes a significant effort to training activities. In this report brief summaries beginning on pages 4, 64, and 104 highlight the accomplishments of the year.

Senior Staff Changes. The sudden death of Dr. G. Charles Kingdon is marked elsewhere in this volume. Toward the end of the year arrangements were made for Dr. Richard Tyndall, formerly of the Biology Division of Oak Ridge National Laboratory, to direct our microbiology program, starting with January 1973. Dr. John Mailhes, who was formerly working on a temporary training appointment, has joined the regular staff in the cytogenetics program. Mrs. Gretchen Humason, who has made an important contribution in histologic techniques, histochemistry, and pathology research during her 9 years at the Division, has retired as a regular staff member but will continue to work on a special postretirement basis. The third edition of her excellent book "Animal Tissue Techniques" appeared during the year.

Guest Scientists and Trainees. The Division is pleased that Dr. Conrado Asenjo, of the University of Puerto Rico Medical School, is spending his sabbatical year with us; he is working in the marmoset project. Dr. Donald Barnhart is continuing his postdoctoral program for a second year, also in the marmoset project. Two Presidential Interns began their assignments, Dr. Lee Washburn in Radiopharmaceutical Development and Dr. Lewis Barber in Medical Physics. Guests and trainees in the Biochemistry Program included Kun Chae, a graduate student from the University of North Carolina School of Pharmacy; Dr. Emad A. El-Bassiouni, University of North Carolina School of Pharmacy; Charles O. Rock, graduate student from the University of Tennessee; and Dr. Murray Grigor, Medical Research Council of New Zealand, who finished his 2-year appointment during the year. Miss Ana Maria Garcia-Campos, San Marcos University, Peru, began a program sponsored by the Pan American Health Organization. Faculty participants included Dr. Barbara Howard, University of Virginia School of Medicine; Dr. Ki-Hyon Kim, North Carolina Central University; Dr. Thomas McCain, Paine College, Georgia; and Dr. George Thompson, Augusta College, Georgia.

A list of medical students, college students, and other visitors who worked for periods of 2 months or more at the Division is found on page 192.

Overseas Staff Assignment. Dr. Bill Nelson, pathologist with the Medical Division, is spending 1 year of special study at the University of Ulm in Germany, working with Dr. Theodor Fliedner and others there on problems related to transplantation. This opportunity was made possible through the help-

fulness of the USAEC, The University of Ulm, and Drs. C. C. Lushbaugh and Raph Kniseley who are covering the essential work load.

Special Staff Activities and Travel. Dr. Nazareth Gengozian, who had been serving as a consultant to the new periodical, *Journal of Medical Primatology*, has been appointed an associate editor of that publication. Dr. Raymond Hayes lectured on April 19, 1972 on "Tumor scanning with gallium-67" at a meeting of the Southern California Section of the Society of Nuclear Medicine; this lecture was designated the first in a series to honor the late Benedict Cassen who had worked for many years in nuclear medicine and is generally credited with having developed the radioisotope scanner.

Although travel funds were again severely limited in 1972, three of our staff made overseas trips. In June, Dr. Nazareth Gengozian presented two papers entitled "Identification of three blood factors in the marmoset, *Saguinus fuscicollis*" and "Laboratory observations on the breeding of the marmoset, *Saguinus fuscicollis*" at the Third Conference on Experimental Medicine and Surgery in Primates in Lyon, France. Dr. Gengozian served as cochairman of the session, Transplantation Immunology. He also attended the Fourth International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions in Dubrovnik, Yugoslavia. In October, Dr. Fred Snyder presented a paper entitled, "Detection and chromatographic resolution of labeled lipid intermediates formed in enzyme systems," at the Fifth International Symposium on Quantitative Flat-Bed Chromatography in Carlsbad, Czechoslovakia. On the return trip, Dr. Snyder visited Dr. Ivor Smith's laboratory in London, England to discuss a new type of radioassay device for chromatography known as a radiochromatogram spark-chamber imaging system. Dr. Snyder's travel expenses were shared by the Czechoslovak Chemical Society and the USAEC. Under the sponsonship of the International Atomic Energy Agency, Dr. Raymond Hayes presented a paper entitled, "New applications of tumor-localizing radiopharmaceuticals" at the IAEA Symposium on Medical Radioisotope Scintigraphy held in October in Monte Carlo, Monaco.

Sources of Financial Support. The Medical Division continues to do most of its work under contract with the United States Atomic Energy Commission. The number of outside sources of support continues to grow; in previous years we have listed them in this introduction, but this year we are presenting them in a Table which is to be found on page 202.

Investigation of Feasibility of Cancer Research Center for East Tennessee. Continued effort has gone into a cooperative plan for a possible cancer research center in the Knoxville-Oak Ridge area. A 2-year grant entitled "Planning grant for cancer research center" was funded July 1, 1972, with Drs. Amoz Chernoff and Charles Congdon as principal co-investigators for the planning effort. A professional consultant firm, JRB Associates, has been employed to aid in the planning.

Partially as an outgrowth of this effort, there has been an increasing development of cooperative research efforts with staff members of the Oak Ridge National Laboratory, University of Tennessee Memorial Research Hospital, and the University of Tennessee.

Future Plans. The year has involved more than the usual share of time given to planning efforts. In spite of limited and uncertain support, many promising potentialities are on the horizon. The Division badly needs a new major building. Meanwhile plans are being made for a modest-sized addition to make our animal care facilities more adequate.

An increasing number of inquiries are being received from utility companies concerning preparations for care of radiation-accident victims. This is an outgrowth of the expected increase in nuclear power production. Because of our long interest in radiation accidents, and special facilities for dealing with them, this is an area in which we hope to render a significant service. The one thing we have lacked for comprehensive care of radiation-accident victims is an adequate decontamination facility. It now appears likely that this deficiency will be remedied by a new facility, which may be provided by AEC to be available for its operations in the Oak Ridge area.

Gould A. Andrews
Chairman

1028109

GALE CHARLES KINGDON
September 4, 1938 — May 21, 1972

Gale Charles Kingdon worked at the Medical Division from February 1970, until a week before his sudden death from thrombotic thrombocytopenic purpura. As leader of our microbiology program he was seeking better understanding and management of the infections that commonly cause death in patients who have blood diseases or bone marrow depression caused by radiation or chemical treatment for cancer. His fine scientific qualifications were combined with a very practical approach; this was illustrated by his efforts to improve the care of patients by devising inexpensive ways of reducing the hazard of infection in our hospital.

Gale was strongly motivated to make the best use of his unusual talent and excellent training; he was a self-propelled hard worker and, had he lived, would have made major contributions to scientific knowledge.

We will remember also his effective cooperation with his colleagues and the profound integrity that was apparent in all of his relationships.

RADIATION EFFECTS AND RELATED TOPICS

A major objective of the Division's research is to develop a better understanding of the effects of total-body irradiation (TBI) in man. Moderate repeated doses of TBI are used in the control of chronic proliferative diseases of the bone marrow and lymphoreticular tissues and larger, single doses are used as a part of the immunosuppressive protocol for marrow transplantation. The information developed in these studies is correlated with that collected as a part of our long-standing project to provide better treatment for the victims of the rare radiation accidents that occur in atomic energy programs.

The work on TBI is approached from many angles including hematologic effects, the role of infection, radiation dosimetry, and physiologic monitoring.

The immunologic work on human marrow transplantation is intimately related with similar basic immunologic studies on experimental animals.

SUMMARY OF ACCOMPLISHMENTS

Additional patients were treated for chronic blood diseases by TBI at very low- and medium-dose rates. Increasing use was made of multiple, spaced fractions of radiation. Special emphasis was put on evaluating effects in chronic granulocytic leukemia, in which somewhat variable patterns of response were seen; an interesting feature was an abortive rise in the leukocyte count at about days 30 to 35 after an exposure to irradiation. An assessment of longevity and causes of death was made in this group of patients. An evaluation of marrow changes after exposure showed qualitative changes that appeared less than alterations produced in the blood would signify. Our analysis indicated that low-dose-rate radiation therapy appears to depress platelets relatively less in comparison with other hematologic effects than does the single, quickly delivered dose.

A program has been developed to look more closely for possible cataract production in our patients receiving TBI; so far we have observed none that could be attributed to the treatment.

Our bacteriologic studies of patients in laminar-flow rooms confirm the impression that we can greatly reduce but not eliminate bacteria from the patient and his environment, and that this reduction is clinically useful for patients with impaired resistance.

Studies on the previously reported patient who was given a high dose of TBI for a bone-marrow graft showed a distinct fall in serum immunoglobulins after treatment and a remarkable fall in salivary IgA. Additional observations supported the earlier report that TBI impairs the ability of human polymorphonuclear leukocytes to phagocytize and kill bacteria. In mice, it was shown that chemical radiation-protective agents act to reduce the rates of postirradiation infection.

Our information center for internal dosimetry of radiopharmaceuticals continued to enhance its collection of data and techniques for dose calculation. The "effective absorbed energy" values were calculated for 12 radionuclides commonly used in nuclear medicine. Methods were evaluated for fitting equations to data on retention curves for radionuclides.

We continued to perform physiological monitoring of patients treated with TBI to assess the effects of the treatment on ability to perform work. Decreases in exercise tolerance were seen and in some cases there was an alteration of resting pulmonary impedance. A new procedure was the measurement of alveolar CO_2 in correlation with pulmonary impedance; the two increase together during exercise but the alveolar CO_2 more rapidly returned to pre-exercise levels.

This year in animal studies on continuous low-dose-rate irradiation we focused on the lymphoreticular system in five different mouse strains. In AKR mice, adult and 40-day-old, radiosensitivity to low-dose-rate, continuous exposure appears disproportionate to single-dose lethality studies. The incidence of lymphoma (normally 90% in unirradiated AKR mice) is strikingly reduced in adult animals. In young mice exposed at 3.2 R/hr, death occurred before the usual time of onset of lymphoma, and at death atrophic thymuses and marrow were present. Another unexpected finding was an extremely high incidence of pulmonary adenomas, perhaps attributable to radiation-induced thymic atrophy and immune failure.

Also in animals, we have looked at spontaneous recovery of immune response after chronic irradiation with exposures of 1.6 R/hr and 6 R/hr. After an accumulated exposure of 2139 R (6 R/hr) the recovery of immune response to sheep red blood cells was fairly rapid. With lower exposures, the recovery was slower. Also, with a total of 5256 R (6 R/hr) the animal's immune response came back spontaneously and promptly in the postirradiation recovery period. Clearly the clinical exposures to 150 to 250 R in our low-exposure-rate total-body irradiator (LETBI) are far below those of the mouse experiment, but the data tend to indicate that our LETBI protocols do not produce permanent impairment of the immune system.

The evaluations of two additional marrow-graft procedures have been completed; in one a temporary graft was achieved, but the other failed. In reviewing our experiences to date on three patients, we believe that our immunosuppressive regimen is adequate to permit a graft, that graft-versus-host disease has not been a major problem, and that great efforts should be made to reduce the numbers of leukemic cells before grafting. The entire procedure, including antilymphocyte globulin, radiation, and infusion of marrow is well tolerated, does not materially increase the patient's morbidity, and may at least result in another hematologic remission.

In our animal work related to marrow chimerism, we made further progress in 1972. In the three-cell interaction needed for humoral antibody response we showed that allogeneic mouse chimeras in their spleens have a basic deficiency in the population of thymus-derived (T) cells. While bone-marrow-derived (B) cells are present and functioning,

1028110

we are still uncertain whether they have a deficiency. The so-called adherent (A) cells (macrophages?) behave normally. (See also later sections describing progress in the marmoset project.)

Our project on the research for granulocytic chalone has progressed. We have been able to demonstrate that incubated rat granulocytes produce a substance which inhibits the in vitro uptake of thymidine into marrow precursors of the granulocyte. The results support the hypothesis that granulocytes produce a regulatory substance which functions in the humoral control of granulopoiesis.

CLINICAL OBSERVATIONS

THERAPEUTIC TOTAL-BODY IRRADIATION

During the past year 22 patients with the following diseases received all or part of their therapy as total-body irradiation (TBI); each received one or more exposures in the low-exposure-rate total-body irradiator (LETBI) (1.5 R/hr) facility or the medium-exposure-rate total-body irradiator (METBI) (1.5 R/min) facility (C. C. Lushbaugh, C. L. Edwards, Helen Vodopick, F. A. Goswitz, G. A. Andrews, and Ann Sipe).

Chronic granulocytic leukemia (CGL)	11
Chronic lymphocytic leukemia (CLL)	3
Lymphosarcoma (LS)	2
Polycythemia rubra vera (PRV)	4
Idiopathic thrombocythemia (IT)	2
	22 total

Twelve TBI treatments, with either 100, 150, or 250 R, were given as single protracted exposures in LETBI at 30 R/day. In this treatment plan patients are exposed to 1.5 R/min for approximately 20 hr/day by living in LETBI.

LETBI — PROTRACTED EXPOSURES

1.	63-year-old man	CGL*	100 R
2.	59-year-old woman	PRV	100 R
3.	67-year-old woman	PRV	100 R
4.	67-year-old man	IT	100 R
5.	66-year-old man	PRV	150 R
6.	67-year-old man	IT	150 R
7.	54-year-old man	PRV	150 R
8.	66-year-old man	PRV	150 R
9.	50-year-old man	CGL	250 R
10.	49-year-old woman	CGL	250 R
11.	47-year-old woman	CGL	250 R
12.	62-year-old woman	CGL	250 R

* This patient may have had primary myelofibrosis instead of CGL.

1028111

Three patients received daily exposures of 30 R in METBI, giving them comparable daily exposures as the patients treated in LEBTI, but at a higher dose rate and shorter (~20 min) daily exposures. These patients are mostly treated as outpatients.

METBI — FRACTIONATED EXPOSURES (30 R/day)

1.	75-year-old man	IT	150 R
2.	68-year-old woman	CGL	150 R
3.	73-year-old man	CGL	250 R

The treatment goals were at least partially, although temporarily, achieved with each of these exposures, which were well tolerated with only minor symptoms, chiefly fatigue, lethargy, anorexia, or occasionally mild nausea.

These treatments combined with the 62 reported last year at the same dose-schedules brings our total experience to 57 in LETBI and 20 fractionated treatments in METBI.

In evaluating responses for chronic leukemia and these other blood and lymphoproliferative diseases treated with radiation, there are many important

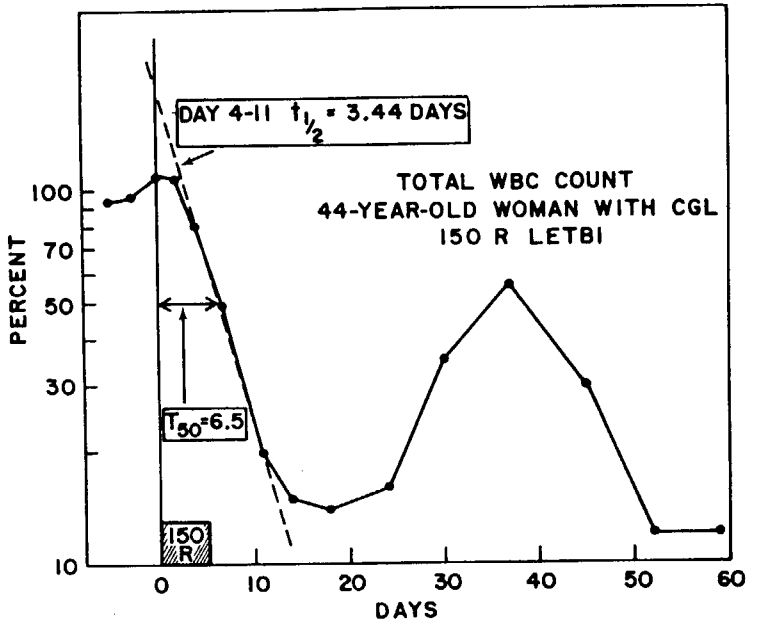


Fig. 1. The WBC response of a 44-year-old woman with CGL treated with 150 R TBI in LETBI (Treatment No. 22, Table 1) expressed as percentage of the average of the three pretreatment counts. The curve shows an exponential decrease between day 4 and 11, an abortive rise peaking out at day 37 and a second decrease to a late nadir of 12% occurring after day 50.

considerations other than the blood counts. However, the cell counts are more readily expressed quantitatively and for CGL, at least, reducing the absolute number of circulating white blood cells (WBC) is an important objective.

In our patients with CGL, exposures of 30 R/day either in LETBI or as fractionated exposures in METBI decrease the WBC, usually beginning within a day or two after the start of therapy (Fig. 1, 2, and 3). After a few days with continued treatment up to 150 or 250 R, the decrease in WBC quickens. Between days 4 and 11, the fall is nearly exponential with an early nadir coming at about day 14 to day 20 (Fig. 1), after which there is often a plateauing of the count, followed by a temporary increase. This increase or abortive rise peaks at about day 30 to 35, and then is followed by a second fall in the WBC count, often to levels lower than that of the early nadir. Blood counts of all patients treated with TBI have not followed this sequence. In some of the patients as shown in Fig. 2 the early decrease was quite slow, so the WBC did not reach 50% of the pretreatment

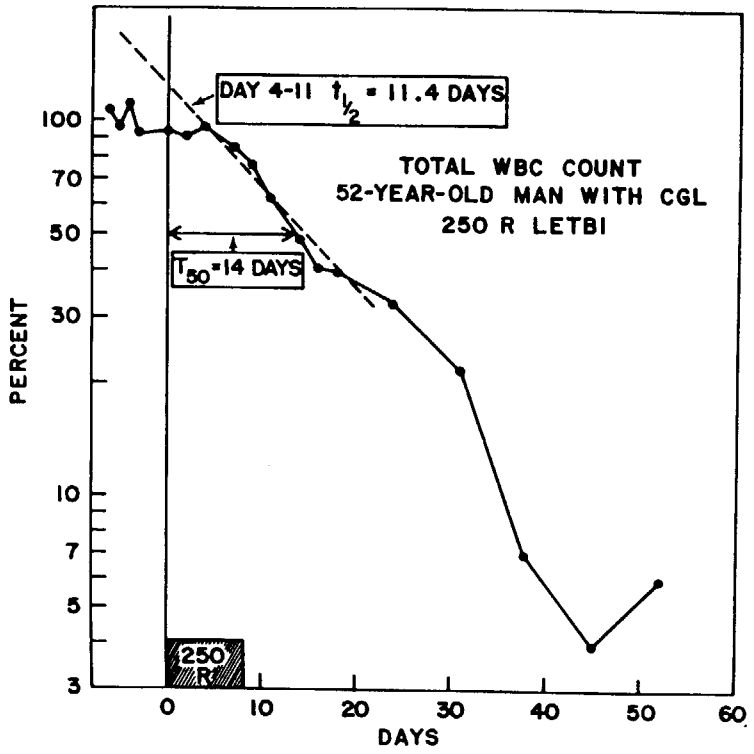


Fig. 2. The WBC response of a 52-year-old man with CGL treated with 250 R in LETBI (Treatment No. 3, Table 1) showing a fairly gradual decrease in the number of cells. The curve only faintly suggests an abortive rise, being diverted only slightly from the exponential decrease to a late nadir of 4% at 45 days.

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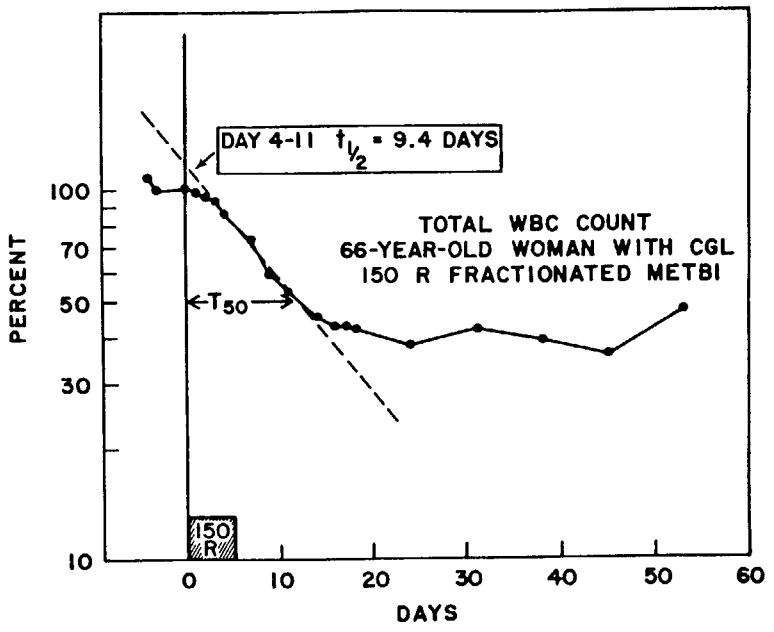


Fig. 3. The WBC response of a 66-year-old woman with 150 R given in five daily fractions of 30 R in METBI (Treatment No. 16, Table 1) showing a gradual decrease and an early plateauing of the response at 40% of the average of the pretreatment count.

value until 2 weeks after starting treatment. Table 1 shows a range of values for half time (T_{50}) of from 4.5 to 14 days. The half-time value for the exponential drop between days 4 and 11 ($t_{1/2}$) varied between 2.2 and 18.9 days.

We did not see an early nadir in every patient (Fig. 2). In some, values may more gradually decrease to a nadir at 6 to 8 weeks after beginning the irradiation. Likewise the abortive rise was also not always seen and when present its magnitude was quite variable although it always seemed to be maximum at about 30 to 35 days after the start of therapy (Fig. 3).

The level of the nadir, whether early or late, was also quite variable in this group of patients. As shown in Table 1 the lowest WBC value obtained without further therapy ranged from 1 to 59% of the pretreatment value. Of the 27 patients treated with 150 to 250 R, either continuously in LETBI or with daily fractions of 30 R in METBI, we failed in only two to achieve a 50% reduction in the WBC; we failed to achieve a 75% reduction in 10. On the other hand, in eight the count was decreased by more than 90% of the pretreatment value. This extreme variability and our inability to predict the response before completing the exposure has limited our use of these doses to patients with high WBC counts (>100,000).

While the numbers are still too small for meaningful survival statistics, it is still worth looking at the status of these patients to determine if their longevity has been significantly altered by this choice of therapy. Table 2 lists the CGL

TABLE 1

RESPONSE OF WBC COUNT IN PATIENTS WITH CGL TREATED WITH TBI*

Rx. No.	Patient Age	Sex	Pretreatment counts† (Average)	Day 0	T_{50} day	$t_{½}$ day 4-11	Early Nadir Percent	Early Nadir Day	Day 31	Late Nadir Percent	Late Nadir Day
				250 R, FRACTIONATED, METBI							
1	73	M	210.0	140.8		1.7	3	12	4‡	No late nadir	
				250 R, LETBI							
2	41	F	142.7	117.6	7.5	5.1	17	18	34	10	52
3	52	M	273.1	257.6	14.0	11.7	—	—	23	4	45
4	53	M	246.3	245.2	7.0	3.7	14	16	60	18	49
5	47	F	123.3	124.0	5.5	2.3	9	11	39	15	50
6	49	F	136.5	143.6	5.0	2.2	9	13	9	3	59
7	50	M	158.2	162.4	9.5	4.5	25	14	32	No late nadir	
8	62	F	155.0	156.6	5.0	2.4	7	14	106	Blastic phase	
				150 R, FRACTIONATED, METBI							
9	44	F	252.4	245.2	7.5	3.5	7	16	8	2	52
10	68	F	277.2	261.2	8.5	8.1	35	11	32	18	37
11	41	F	132.6	156.2	6.5	4.4	22	14	26	12	45
12	66	F	279.5	262.8	12.0	9.4	38	24	42	36	45
13	53	M	297.2	262.0	6.0	5.7	25	14	40	19	45
14	52	F	124.7	144.0	6.0	5.0	21	14	28	26	38
15	53	M	369.6	417.2	7.0	4.3	29	10	50	39	44
16	66	F	425.1	437.6	7.0	14.6	47	10	25	1	48
17	41	F	106.4	89.2	—	9.5	53	10	Rec. Add. Rx Day 24 →		
18	62	F	174.2	160.6	9.0	7.4	49	9	117	60	50
19	68	F	82.1	89.9	6.0	3.3	10	18	No Data		
				150 R, LETBI							
20	28	M	65.9	72.2	4.5	3.2	12	11	—	15	52
21	68	F	157.7	132.6	6.0	7.2	32	14	41	3	52
22	44	F	147.5	162.4	6.5	3.4	14	18	35	12	52
23	52	M	81.9	86.5	8.0	11.6	33	14	37	21	38
24	44	F	285.7	296.0	6.5	3.9	11	24	19	9	45
25	38	F	165.3	142.4	—	18.9	59	16	76	71	52
26	65	M	111.3	106.6	5.0	2.5	12	17	24	18	52
27	52	M	79.9	78.7	6.0	6.7	27	14	48	32	38

* This table (parts a and b) summarizes the response of the WBC count in patients with CGL treated either with 150 to 250 R continuously in LETBI or with divided daily fractions of 30 R in METBI. The pretreatment WBC is the average of at least three counts within 10 days of the start of therapy. The WBC Day 0 is the count taken on the morning before starting therapy. The T_{50} is the time in days from the start of the therapy to when the WBC count reached 50% of the pretreatment WBC. The $t_{½}$ day 4-11 is the half-time value of the exponential decrease between days 4 and 11. Early and late nadirs are the lowest value expressed in percent of the pretreatment WBC before and after day 31, the date when the abortive rise most often occurs.

† Times 10^3.

‡ Day 32.

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patients treated with TBI in LETBI or with fractionated doses in METBI, including those who have died and those presently being observed. The survival times from the date of diagnosis range from 9 to 76 months for the deceased (mean 36.3 months and 37 months median). Four of the nine patients still living have already survived longer than the median above. These figures do not differ greatly from the values published by Galton (Brit. Med J. 1, 201, 1968) and Bergsagel (Can. Med. Ass. J. 96, 1615, 1967) (170 weeks and 3.3 years respectively) as the median survival in patients treated with busulfan alone.

As with other reported series most of our patients with CGL die after their disease has entered an accelerated or blastic phase, now regarded as a terminal phase. Because of the leukemogenic property of radiation, a reasonable concern is whether TBI accelerates the blastic change. Three of our patients did not show blastic transformation before death. One patient (No. 8, Table 2) died of liver failure and at autopsy had an atrophic liver. Although she had received blood transfusions, a test for Australian antigen was negative during this illness. Conceivably the liver failure could be related to the earlier hepatic irradiation, or the busulfan therapy. The patient's disease had not become blastic even though she had been our longest survivor (76 months) and had received over 2,600 R of TBI. Another patient (No. 7, Table 2) died as a result of severe hemorrhage in the intra-abdominal wall, probably due to his large spleen tearing away from its supportive structure after being jostled as he rode on a tractor in a cornfield all

TABLE 2

SURVIVAL OF PATIENTS WITH CGL
TREATED WITH FRACTIONATED OR PROTRACTED TBI

Rx. No.	Patient Age	Sex	Survival (months)	Cumulative exposure (R)	Splenic irradiation dose (rads)	Drugs and other therapy*	Cause of death
1	12	M	48	100	500	—	Blastic
2	53	F	32	100	1,356	—	Blastic
3	82	F	29	100	2,313	—	Unknown, but not blastic
4	28	M	9	150	—	—	Blastic
5	66	F	44	400	1,168	—	Blastic
6	52	M	21	550	—	—	Blastic
7	53	M	15	640	—	—	Hemorrhage
						1,270 rads, liver	
8	44	F	76	1,100	2,685	Busulfan	Liver failure
9	41	F	47	670	—	—	Blastic
10	52	F	42	150	598	Busulfan	Blastic
					Surviving		
11	38	F	78	830	416	Busulfan	
12	68	F	38	450	—	—	
13	65	M	37	281	—	Busulfan	
14	62	F	15	582	1,196	—	Now in blastic phase
15	50	M	56	280	1,303	—	
16	49	F	8	250	—	—	
17	47	F	3	250	—	—	
18	73	M	10	700	—	—	
19	70	M	6	90	—	—	

*This does not include chemotherapy during terminal (blastic) phase of the disease.

day. The other elderly patient (No. 3, Table 2) died of unknown causes; her leukemia seemed to have been under good control.

Survival statistics, however, fail to tell the complete story. Also of importance is the morbidity of the patients during their illness and the time are they able to live productive and pleasurable lives. The TBI at these exposures (100, 150, and 250 R) are well tolerated, producing only minimal and transient anorexia, mild fatigue or malaise, and rarely some mausea. These symptoms are often indistinguishable from the symptoms the patients are already experiencing, and nearly always subside soon after the end of irradiation. Nevertheless, the treatment pattern of allowing the disease to relapse to levels "requiring therapy" with WBC counts of 100,000/mm^3 or more before reinstituting therapy subjects the patient needlessly to periods with increased symptoms and perhaps to increased risk of hemorrhage or ischemia. In an effort to avoid this oscillating, we have been using smaller single exposures at intervals up to several months to keep the WBC down to desired levels.

A total of 10 patients with lymphoproliferative or myeloproliferative diseases received one or more exposures of 10 to 30 R in METBI at irregular intervals on an "as needed" basis attempting to keep the blood counts or basic disease process suppressed. These treatments as a rule, were given on an outpatient basis as part of the routinely scheduled clinic visit. The intervals between treatments varied from a few days to 9 months. In three patients 30-R METBI exposures at biweekly or monthly intervals failed to reduce the blood count to optimum levels, perhaps because of too much time between treatments. Adding splenic irradiation for three produced a more satisfactory response in blood values, but this, too, was brief in one patient whose disease soon became refractory and then entered the blastic phase.

Only patients with quite high blood values and hypercellular marrow are suitable for protracted or fractionated exposure such as we have been using (150 R to 250 R) and, as has been shown, such exposures alone often fail to achieve the desired or optimal suppression. Clinically we need to be able to titrate the treatments more precisely to the individual patient's response before attempting to drive the blood count down further to normal or optimal levels. This is especially true for patients with lymphoproliferative disease in whom the response seems to be even more variable and the risk of oversuppression greater.

Because highly fractionated exposures with smaller and less frequent fractions may allow us to observe minimal untoward effects in time to alter the treatment plan, we have begun therapeutic trials with prolonged schedules and more slowly accumulated doses. Other possible advantages include: 1) enhanced relative effectiveness on malignant cells as compared to normal cells, 2) relative ineffectiveness on the different lines of normal blood cells (platelet sparing), 3) a prolonged beneficial effect, and 4) further reduction in symptoms.

As shown on Table 3 and in the following list, three patients were treated with four courses of irradiation at 10 R/day, 3 days a week (Monday, Wednesday, and Friday) in METBI. Each treatment lasted less than 7 min and was easily worked into their schedule, making it a convenient outpatient therapy.

METBI — FRACTIONATED EXPOSURES (10 R/DAY, 3 DAYS/WEEK)

1.	73-year-old woman	CLL	150 R
2.	72-year-old man	LS	150 R
3.	67-year-old woman	CLL	120 R
4.	72-year-old man	LS	90 R

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In three of these treatments (No. 2, 3, and 4 in the above list) the results were gratifying, with a profound effect on the patient's tumor or number of abnormal blood cells and on the patient's symptoms, together with relatively little effect on normal blood cells especially on the platelets. In the third patient neither the platelet-sparing effect, nor the desired effect on the abnormal cells was seen.

Other modifications of treatment schedule have been used in individual patients. A 50-year-old man with disseminated LS, who previously was treated

TABLE 3

CUMULATIVE TBI TREATMENTS THROUGH 1972

Exposure (R)	Single acute exposures	METBI (1.5 R/min) 10 R/day (3 days/week)	METBI (1.5 R/min) 10 R/day	METBI (1.5 R/min) 30 R/day	LETBI (1.5 R/hr) 10 R/day	LETBI (1.5 R/hr) 30 R/day	Totals
Chronic lymphocytic leukemia							
50	15						15
100	20				1	4	25
120		1					1
150		1					1
	35	2			1	4	42
Lymphosarcoma							
50	5		1				6
100	22	1*	1		1	3	28
150		1		1	2†		4
250				1		4	5
	27	2	2	2	3	7	43
Chronic Granulocytic leukemia							
50	8						8
100	4			1‡		6§	11
120						1	1
150				11	1	8	20
200					2		2
250				1		7‖	8
	12			14	3	21	50
Polycythemia rubra vera							
100	7					5	12
150				2		8	10
250						4¶	4
	7			2		17	26
Idiopathic thromobcythemia							
100	1					1	2
150				2		7	9
	1			2		8	11
TOTAL	82	4	2	19	7	58	172

* Patient received only 90 R.
† One patient received only 140 R.
‡ 25 R/day.
§ This patient may have had primary myelofibrosis instead of CGL.
‖ One patient received only 222 R.
¶ One patient received 230 R.

with drugs and radiation but was showing progressive marrow involvement and suppression of the normal blood elements, was treated with 5 r/day. After 6 days this was abandoned because of the rapid progression of his disease. No effect on the size of tumor was noted clinically. A 67-year-old woman with CLL of more than 6-years duration was treated with 60 R at 10 R/week in METBI for 6 weeks achieving a good reduction in the total lymphocyte count to approximately 20% of the pretreatment value by 42 days after the first exposure. However, this was accompanied by a temporary decline in her hemoglobin from 10.6 to 9.5 g/100 ml and a drop in the platelet count to 25% of the pretreatment values.

CHANGES IN HUMAN MARROW AFTER TREATMENT WITH TOTAL-BODY IRRADIATION

Last year in the Research Report we gave an account of the marrow changes in 30 patients after continuous irradiation with the low-dose rate of 1.5 R/hr. During 1972 we (F. A. Goswitz, C. C. Lushbaugh, and Helen Vodopick) studied the marrow changes in 12 patients with malignant hematologic disorders who were treated with total exposures of 30 R/day.

The patients, treated in the LETBI and METBI facilities, were in a protocol that investigated the therapeutic usefulness of TBI. The radiation field is uniform to within ±10%. The treatments, given at average exposure rate of 30 R/day, were scheduled to last from 3 to 8 days to provide a total protracted exposure of 100, 150, or 250 R. Twelve treatments were administered to 10 patients given 1.5 R/hr in the LETBI facility and 3 treatments were prescribed to 3 patients exposed to 1.5 R/min in the METBI facility. One PRV patient treated with 150 R had no marrow aspirated at the posttreatment time designated by the study, and therefore, his data were excluded. We compared the marrow at approximately 18 days after the start of treatment with the marrow taken before therapy. We evaluated the cellularity, fat, granulocytic:erythroid (G:E) ratio, maturation of the granulocytic and erythroid elements, number of lymphocytes, megakaryocytes and platelets, and concentration of iron in histiocytes.

The treatments were given to 12 patients with myeloproliferative disorders: 7 CGL, 3 PRV, and 2 IT. Of the 7 CGL patients, 4 were untreated and newly diagnosed. The 3 PRV and the 2 persons with IT had been previously treated. During the past year no patients with lymphoproliferative disease were treated daily at a dose rate of 30 R/day to 100, 150, or 250 R total exposure.

To the 12 patients with myeloproliferative disorders, 14 treatments (7 CGL, 4 PRV, and 3 IT) were given. Of these 14 treatments, 4 were given with a total exposure of 250 R, 5 with 150 R, 1 with 222 R, and 4 with 100 R. Two patients (1 with PRV and 1 with IT) were treated twice in the LETBI facility.

The calculated radiation-absorbed dose to the marrow is 60 rads per 100 R, 90 rads per 150 R, and 150 rads per 250 R. Generally, we aspirated marrow from the iliac spine; only twice did we use the sternum. If the sternum was the site for the preirradiation sample, it was also used for the postirradiation specimen. If satisfactory aspirates were not obtained, we biopsied the marrow with a Westerman-Jensen or a Jamshidi needle.

Marrows were interpreted in the same manner as described in the 1971 Research Report.

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Table 4 reports the marrow changes in those patients treated with 250 R. One patient who received only 222 R is included in this table. All 5 patients have CGL. Four patients were treated in LETBI, 1 in METBI. The most consistent change is the increase of fat. The other striking change is the moderately pronounced reduction in the G:E ratio. Less immaturity in the granulocytic series was observed also. Although only 1 patient (patient 4) was treated in METBI with 250 R, it appeared that the effect on his marrow was less pronounced than that seen in the marrows of patients treated with the same dose in LETBI.

TABLE 4

MARROW DATA IN PATIENTS WITH CGL (250 R)*

Patient number	Cells		Fat		G:E ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
	b	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*
1	↑↑↑	↑	↓↓↓	↓↓	32.3	23.0	N	N	L↑↑	L↑↑	A	A	↑↑↑/↑↑	↑/↑↑↑	A	A
2	↑↑↑	↑	A	↓↓↓	99.0	2.2	N	N	L↑	L↑	↓↓	↓↓↓	↑↑/↑↑	↑/↑	A	↑
3	↑↑↑	↑↑	A	↓↓	20.0	6.7	N	N	L↑↑	L↑↑	↓↓	↓↓↓	↑↑↑/↑↑	↑↑/↑↑	A	↓↓
4†	↑↑↑	↑↑	↓↓↓	↓↓	99.0	10.6	L↑↑	N	L↑↑	L↑↑	A	↓↓↓	↑↑/↑↑	↑↑/↑↑	A	A
5‡	↑↑↑	↑↑	A	↓↓↓	25.0	13.2§	N	N	L↑↑	L↑↑↑§	↓↓↓	↓↓↓	↑/N	↑/N	A	A

* Symbol abbreviations
↑ = increase, ↓ = decrease
b = before, *a* = after irradiation
A = absent, N = normal, L = left shift

† Treated in METBI.

‡ Received only 222 R.

§ Suggestive changes of blast-cell crisis with more immaturity, myeloblasts noted.

Table 5 shows the marrow data after 5 treatments to 4 patients (1 CGL, 1 PRV, and 2 IT). The interval between the two treatments of the patient with PRV was 9 months. The patient with CGL had an excellent response to treatment with 150 R; she previously had received 150 R in METBI in November 1969, and 150 R in LETBI in February 1970 as her only treatment. She had been in remission almost 28 months before additional treatment was indicated. The 2 patients with IT had normal cellularity, but an increase in megakaryocytes and platelets, of course, prior to therapy. In the patient treated in METBI, the number of megakaryocytes and platelets became subnormal. This patient had been treated with 150 R in LETBI 10 months earlier so that re-treatment with this dose was not judged excessive.

Table 6 demonstrates the findings after 100 R. Little change is seen in the marrow after this dose is given to patients with active PRV, or in a relapse phase of CGL. Good control of the blood-cell counts, however, can be achieved for a short period in these disorders. The patient with IT had been treated only 3 months before with 150 R; treatment with 100 R has maintained a "remission" for him now for 8 months.

In summary, 59 treatments with TBI (58 in LETBI; 1 in METBI) have been given at the rate of 30 R/day in continuous exposures of either 100, 150, 222, or 250 R to 38 patients with a proliferative hematologic disorder. The marrow studies

TABLE 5

MARROW DATA IN PATIENTS TREATED WITH 150 R*

Patient number	Cells		Fat		G:E ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
	b	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*
							Chronic granulocytic leukemia									
1†	↑↑	↑	↓↓	↓	97.5	11.5	N	N	L↑	N	↓↓	↓↓↓	↑↑/↑↑	N/N	↓↓	A
							Polycythemia rubra vera									
1‡	↑↑	N	↓↓	N	2.6	0.9	N	N	N	N	N	N	↑/N	↓/N	A	A
1§	↑↑	↑	↓↓	N	0.8	1.6	N	N	N	N	N	N	↑/N	N/N	A	A
							Idiopathic thrombocythemia									
2‡	N	↓	N	↑	1.1	2.1	N	N	N	N	N	↓	↑↑↑/↑↑	↑↑/↑	A	A
3	N	↓↓	↑	↑↑	0.9	1.0	N	N	N	N	N	↑	↑↑/N	↓/↓↓	↓↓↓	A

* Symbol abbreviations
↑ = increase, ↓ = decrease
b = before, *a* = after irradiation
A = absent, N = normal, L = left shift

† Treated in METBI.
‡ Treated fourth time in LETBI.
§ Treated fifth time in LETBI.

TABLE 6

MARROW DATA IN PATIENTS TREATED WITH 100 R*

Patient number	Cells		Fat		G:E ratio		Erythroid maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
	b	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*
							Chronic granulocytic leukemia									
1†	↓↓↓	↓↓↓‡	↑↑	↑↑↑	3.6		N		L↑		↓		↑/↑↑	↓↓/↓↓	N	A
							Polycythemia rubra vera									
1§	↑↑	↑	N	N	0.9	2.3	L↑	R↑	N	N	N	↓↓↓	↑/N	N/N	A	A
2§	↑↑	↑↑	↓↓	↓↓	2.8	3.1	N	N	L↑	N	↓↓	↓	↑↑/↑↑	↑/↑	A	A
							Idiopathic thrombocythemia									
3‖	↑	↓	N	↑	2.0	2.0	N	N	N	N	↓	N	↑↑/N	↑/N	A	A

* Symbol abbreviations
↑ = increase, ↓ = decrease
b = before, *a* = after irradiation
A = absent, N = normal, L = left shift, R = right shift.

† This patient may have had primary myelofibrosis instead of CGL.
‡ Needle biopsy.
§ Treated for second time in LETBI.
‖ Treated fifth time in LETBI.

after 57 treatments to 36 patients reveal changes that were usually less than we had anticipated, even in patients who showed the greatest reductions in blood-cell counts at the time of the posttreatment marrow specimen. The basis for this discrepancy is not clear. Possibly the influence of radiation on cell-survival times in the circulation is greater than it is on the production rates in the marrow. Possibly the mass of functioning marrow is affected more than its qualitative composition. Possibly the irradiation produces its effects through slowing of maturation and release processes, changes that would not be demonstrated by

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this static study. Kinetic studies correlated with our morphologic data would help to quantitate marrow changes and thereby show the full significance of radiation-produced alterations. In an effort to note the ideal therapeutic effect in a particular hematologic malignancy, data are now being compiled to compare marrow changes after medium-dose-rate irradiation to changes after low-dose-rate irradiation in these continuous exposures ranging from 100 to 250 R.

PLATELET EFFECTS OF LOW-DOSE-RATE IRRADIATION

We are continuing our program analyzing hematologic and clincal data retrospectively to obtain radiobiologic information potentially useful in managing occupational radiation exposures. This year we are studying in detail the relation of maximum platelet depression to protracted total-body exposure of patients with chronic leukemia, lymphoma, or polycythemia. Unlike the curves reflecting total granulocytes, the platelet curves in these patients show no abortive rise and, in addition, show an earlier recovery and often a lesser response when the level at the nadir expressed as percent of pretreatment value is used as the measurement. This phenomenon of comparatively greater resistance, which has not been described previously by others, remains unexplained. It is apparently related to dose rate and stands in distinct contrast with the effect of single, prompt exposures where platelet depressions are often greater than those of total WBC. Not infrequently this greater sensitivity of platelets to single TBI at more rapid-dose rates than used in LETBI has created undesirable thrombocytopenia particularly in patients with lower than normal initial platelet counts. In radiation accidents, also, hemorrhagic manifestations secondary to severe platelet depressions have created therapeutic problems that augment the hazards of granulocytopenia and infection. Low-dose-rate TBI therapy in most cases avoids such serious platelet depressions and its continued trial in our hospital reveals this preferential sparing of platelets as one of the most beneficial results of this modification of TBI therapy. To date we have not been able to fit the data satisfactorily with a unique mathematical model. A preliminary attempt, however, suggests that the percentage survival curve for platelets has a shoulder similar to that of in vitro cellular systems irradiated with small doses or low-dose rates, an observation suggesting that below 50 R at 1.5 R/hr (or less) human megakaryocytes have either a greater than usual capability for repairing radiation damage or a reserve compartment capable of compensating for damage by increased platelet release. Since a significant amount of daily platelet utilization in man is determined by insensible vascular trauma during physical activity, another explanation for this platelet-sparing effect might be that it merely reflects a lesser demand for platelets caused by a reduction in radiation-induced capillary and other vascular damage. This thesis, however, does not have the experimentally obtained radiobiologic basis of the others.

THE PROBLEM OF RADIATION-INDUCED CATARACTS

One of the questions that often arises during the use of cytotoxic agents in therapy is whether undesirable damage is being produced in nondiseased cellular systems. In our clinical studies of TBI we have been concerned with the

adiation of the ocular lens might result in
response relationship is not known for this
The best clinical data in the literature,
institutions who received local therapy to
s that 150 and 620 rads given in a 4-hr period
d one, respectively, for causing cataracts.
period as is done in our LETBI patients de-
e required for 50% probability (ED_{50}) for this
ads (750 to 900 R). While our patients have
single treatment, some have accumulated
R in multiple treatments over 5 to 6 years
the ocular lens is its unshielded location (3
ssible presence in the LETBI radiation field
s which are capable of causing lens damage
have attempted unsuccessfully on several
of such scatter-radiation contamination of
cluded that the amount of scattered radia-
o detect, we have continued on the alert for
ggest its presence. Our routine eye examina-
failed to reveal either any physical findings
This year we enlisted the aid of Dr. George
les being a practicing ophthalmologist, has
program in radiation cataractogenesis.
microscope the eyes of 16 patients who were
been so treated in the past. Accumulated
the majority were in the 300-to-350-R range.
nged from 6 years to less than 1 year. The
and 2 years after their first exposure. The
radiation-induced type of cataracts but
advanced age of these patients) that many
s around the lens periphery, which are easily
ts that have a central-posterior location. Dr.
y positive findings was largely the result of
te of their accumulation fortified our thesis
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DIATED PATIENTS

of sublethal total-body irradiation (TBI) and
pid course and a high mortality rate. The
ry animals to experimental or spontaneous
n studied extensively; less is known of the
ased susceptibility to infections in irradiated

The major objectives of the microbiology group are to study postirradiation infection in patients undergoing radiation therapy by characterizing the pathogens responsbile for infection, by defining factors involved in the increased susceptibility of the host, and by seeking suitable methods for the prevention and treatment of infections in those patients.

BACTERIAL FLORA IN PATIENTS IN AN ULTRACLEAN ENVIRONMENT

The Medical Division maintains a laminar air-flow (LAF) facility which provides an ultraclean environment for patients with unusual susceptibility to infection. We have continued microbiological studies (G. Charles Kingdon* and Jean McIntyre) on the ultraclean environment during patients' occupancy. The microbial levels on surfaces, furnishings, fixtures, and medical personnel in the LAF rooms and preparation room were determined by contact plate at least once a week. The microflora of the patient's nose, throat, mouth, skin, and feces were cultured two or three times each week; blood and urine samples were also examined.

Two patients were studied for a total of 79 days while they were housed in the LAF facility. Detailed data on one are presented here. A woman with acute granulocytic leukemia, given 500 R TBI (40 R/min) and a marrow transplant, was confined to this environment for 39 days. Visitors were allowed in the room on day 32 following irradiation, and reverse isolation was maintained for 26 days thereafter. This period of modified clean environment was considered necessary for establishing a reasonably normal microflora before she left the LAF facility.

The bacterial microflora of this patient was reasonably well controlled with Betadine and pHisoHex skin scrubs and nonabsorbable antibiotics (Mycostatin, colistin, paromomycin, polymyxin B). *Staphylococcus albus* and diphtheroid species (sp) were present on the patient's skin beforehand. Skin scrubs eliminated the diphtheroids almost entirely, but the *S. albus* remained. *Staphylococcus aureus* was isolated from the patient's skin on days 8 and 22, but caused no apparent infection problems. *Klebsiella-Enterobacter* group (gp) and *Proteus* sp were first cultured from the skin 12 days postirradiation and persisted.

The bacterial flora of the patient's nose and throat was normal before confinement except for *S. auerus* in the nose (Table 7) which persisted in the nares throughout the isolation and which was also cultured from the throat after day 28. *Candida* sp were isolated only once from the throat, occurring after the ultraclean procedures had been modified and the patient's husband admitted.

The bacteria of the patient's mouth before irradiation were unremarkable except for low numbers of *S. aureus*. Betadine mouthwashes were used daily and, except for *Lactobacillus* sp and *Bacteroides* sp, no bacteria were grown from the mouth washings until day 29. By day 47 the oral microflora was the same as before exposure except for the absence of *Fusobacteria*.

Prophylactic nonabsorbable antibiotics were given to reduce the microbial load in the intestinal tract. Her intestinal microflora was normal before therapy

* Deceased.

except for *S. aureus* in moderate numbers (Table 8). After antibiotics, no bacteria were cultured from feces through day 26, although *Klebsiella-Enterobacter* gp, *Lactobacillus* sp, *Bacteroides* sp, and *Proteus* sp were consistently cultured from rectal swabs. After antibiotics were discontinued the patient's fecal microflora again became normal except for the absence of *Escherichia coli*. A suspension of *E. coli* (10^9 bacteria), grown from the patient's original strain, was therefore administered by rectal tube on day 42. Cultures from the patient's feces and rectal swabs on day 50 demonstrated a normal intestinal flora.

TABLE 7

MICROORGANISMS ISOLATED FROM NOSE* AND THROAT† OF PATIENT DURING OCCUPANCY OF LAF ROOM‡

Day	*S. aureus*	*S. albus*	*S. salivarius*	*S. mitis*	*Micrococcus*	*Lactobacillus*	*Veillonella*	*Fusobacterium*	*Bacteroides*	Diphtheroids	*Candida albicans*
-8	x		✓	x✓	✓	✓	✓	✓			
-2	x	x				✓			✓		
2	x					✓			✓		
5	x	x				✓			✓		
8	x					✓					
12	x	x				✓					
15		x				✓					
19		x				✓					
22	x	x				✓					
26	x	x				✓			✓		
29	x✓					✓					
33	x✓					✓			✓	x✓	
37	x					✓			✓	✓	✓
40	x✓	x		✓		✓					
43	x✓			✓		✓					
47	x✓	x	✓	✓	✓	✓	✓				
50	x		✓	✓	✓	✓	✓		✓		

* x = Cultured from nose swabs.
† ✓ = Cultured from throat swabs.
‡ Modified clean environment after day 31.

Genitourinary tract contamination was a continual problem in this patient. Her urine samples contained small numbers of *S. albus* and *Enterococci* before she entered the LAF facility and although these species were eliminated or greatly reduced, *Klebsiella-Enterbacter* gp and *Proteus* sp were cultured on day 2 and persisted despite antibiotic therapy. *Proteus* sp were consistently cultured from vaginal swabs, and *S. aureus* was isolated once from this site on day 27.

1028118

TABLE 8

MICROORGANISMS ISOLATED FROM FECES OF A PATIENT DURING OCCUPANCY OF LAF ROOM*

Day	Lactose negative	*Klebsiella-Enterobacter* gp	*S. salivarius*	*S. mitus*	*S. aureus*	*Lactobacillus*	*Bacteroides*	*Proteus*	*Clostridium*	*Enterococcus*	*E. coli*
-8	5.48†	5.70	5.00	8.00	4.60	9.95	9.84				
-2‡											
2‡											
5‡											
8‡											
12‡											
15‡											
19‡											
26‡											
33	8.00					8.70		6.30			
40	9.00	8.70			5.08	7.87	8.29	7.90	8.70		
47	6.95	7.80				7.00	9.84		7.30	6.60	
50		5.60				7.18	9.40	5.00	7.00	7.48	6.08

* Modified clean environment after day 31.
† Log_{10} per ml.
‡ No growth for any of the organisms.

The most consistently isolated contaminates in the LAF rooms were *Bacillus* sp and *S. albus* (Table 9). *Pseudomonas* was cultured three times and saprophytic fungi and diphtheroids were cultured once during the patient's occupancy. The presence of *S. albus* on many surfaces was not surprising, since this organism was continually present on the patient's skin. The isolation of the other bacterial species, particularly *Bacillus* sp, indicates that our initial decontamination procedure was not completely effective.

TABLE 9

MICROORGANISMS CULTURED FROM LAF ROOM DURING PATIENT'S OCCUPANCY

Organism	Date of Occupancy						
	11-23	12-2	12-8	12-14	12-21	12-29	1-5
Bacillus	11	10		3	15	13	3
S. albus	9	29	19			34	3
Pseudomonas	4				1		1
Diphtheroids		2					
Fungi (saprophytic)					2		
Positive samples (+/T)	8/19	8/20	7/20	2/20	5/20	6/20	4/20
Avg. colonies/inch^2	0.36	0.61	0.28	0.04	0.27	0.70	0.07

These data indicate that we cannot yet completely eliminate bacteria from the patients' environment. However, the numbers and varieties of organisms are greatly reduced. The clinicians on our staff believe that patients with unusual susceptibility to infection can be cared for with greatly reduced risk under these conditions.

EFFECT OF TBI ON HUMORAL DEFENSE — SERUM IMMUNOGLOBULIN, SECRETORY IGA LEVELS, AND OTHER SERUM PROTEINS

Bactericidal and agglutinating antibody titers decline in the sera of some patients after TBI. We are continuing our work (G. Charles Kingdon and Stuart Chaskes) to determine if altered serum immunoglobulin levels after irradiation indeed reflect reduced natural antibody titers and diminished de novo antibody synthesis.

The previous study completed in 1971 included 42 patients treated with 100 to 350 R irradiation in either the low-exposure-rate total-body irradiator (LETBI) or the medium-exposure-rate total-body irradiator (METBI). The present study is limited to patients receiving high doses (greater than 350 R) of TBI, and as yet only three such patients have been studied. Serum immunoglobulins, secretory IgA levels, and other serum proteins are being quantitated.

Detailed data from the woman with acute granulocytic leukemia are presented here. Serum albumin and gamma globulin fractions decreased gradually and consistently throughout the period of study. The beta globulin fraction also decreased sharply but only after day 74. Table 10 shows changes in specific serum protein levels. Serum immunoglobulins (IgG, IgA, and IgM) decreased, finally reaching minimum levels of 55, 56, and 65% of preirradiation levels respectively. Complement (C'3) levels varied and were reduced after antilymphocyte globulin (ALG) was given on days −7 through −1 and days 78 through 84. The level of C'3 in the final sample assayed was only 43% of the original level. Transferrin levels declined throughout the study, reaching a minimum level on day 92. Increased C-reactive protein (CRP) values often reflect an inflammatory type response, and three distinct peaks occurred. We suggest that the first increase (day 12) corresponds to an episode of otitis and pharyngitis, the second peak (at 5 weeks) to hepatitis, and the third peak (days 83 through 92) to blast cell crisis and pneumonia.

Secretory IgA is known to play an important role in the host's defense system against infection. The amounts of IgA in the saliva or stools during treatment are recorded in Table 11. The level of salivary IgA decreased drastically after irradiation and bone marrow transplantation to 15% of preirradiation levels. A transient recovery occurred on days 33 through 40, and salivary IgA levels remained stable until day 54, when IgA again decreased. On day 81 (last saliva sample obtained) the salivary IgA was 25% of the original level. Secretory IgA levels in the stools varied widely with no apparent pattern.

EFFECT OF TBI ON CELLULAR DEFENSE — PHAGOCYTOSIS AND KILLING OF BACTERIA BY BLOOD GRANULOCYTES

These studies (G. Charles Kingdon, Shirley Colyer, and William G. Tankersley) were done on six patients exposed to TBI. Five patients were being

1028119

TABLE 10

IMMUNOGLOBULINS (IgG, IgA, IgM), C'3, TRANSFERRIN, AND CRP LEVELS IN A PATIENT'S SERUM FOLLOWING 500 R TBI AND BONE MARROW TRANSPLANTATION

	Serum Proteins (mg/100 ml)					
Day	IgG	IgA	IgM	C'3	Transferrin	CRP
-8	1100	210	71	140	145	0.70
-7	1000	190	65	110	152	0.81
-2	1100	195	73	110	170	0.87
-1	1340	238	77	130	208	0.92
0	1200	280	77	130	198	0.60
1	1060	270	71	122	195	3.00
2	1140	220	61	110	152	5.80
5	1180	238	65	160	158	2.70
6	1140	212	64	155	152	6.10
7	1060	195	58	173	125	6.10
8	1000	190	61	155	130	7.55
9	1060	220	65	192	125	8.05
12	950	230	64	160	125	16.75
15	860	205	57	150	125	11.70
16	880	195	64	210	121	11.00
19	980	205	64	187	121	1.38
21	860	230	61	173	130	1.10
26	880	212	57	120	104	1.00
29	785	167	40	114	116	1.06
33	755	180	49	105	116	4.38
35	785	180	51	118	113	13.50
37	710	150	47	98	90	13.00
40	640	172	47	105	98	3.38
42	730	167	51	—	135	2.25
43	640	167	42	120	128	2.52
44	690	150	42	167	120	2.35
47	630	160	40	100	165	1.55
50	640	137	42	120	140	2.38
54	610	165	43	135	165	1.75
56	730	155	40	145	165	1.40
60	725	155	43	140	165	1.12
69	865	173	55	127	150	0
74	835	195	69	137	210	0.40
76	865	177	68	127	200	—
78	740	167	68	115	170	1.95
81	780	205	90	115	175	5.00
83	670	172	73	95	112	41.00
85	640	150	47	60	112	30.75
88	640	145	45	60	105	16.50
92	655	167	50	60	85	31.65

TABLE 11

EFFECT OF 500 R TBI (40 R/min) AND BONE MARROW TRANSPLANTATION ON THE LEVEL OF SECRETORY IgA IN A PATIENT'S SALIVA OR STOOLS

Day	Secretory IgA*	
	Saliva (mg/100 ml)	Feces (mg/100) g)
-8	96	212
-2	68	34
-1	---	37
1	---	115
2	---	52
3	78	---
5	---	103
8	117	344
9	40	---
12	14	150
15	17	330
19	21	195
23	---	114
26	8	66
29	---	114
33	180	360
40	66	---
42	---	276
44	---	414
47	27	258
50	96	318
54	84	54
57	24	50
60	8	36
69	10	---
74	6	---
81	24	---

* Calculated as 11S.

treated for polycythemia rubra vera, and one was being treated for chronic granulocytic leukemia. Our data continue to support the earlier interpretation that phagocytosis and killing of bacteria by polymorphonuclear leukocytes are reduced after irradiation; the mechanism of this reduction has not been explained.

Figures 4 and 5 show results from a single patient and are representative of the changes in phagocytosis and killing that occur in most patients. On the day of irradiation (day 0), 70% of the *Pseudomonas aeruginosa* were removed from the supernatant fluid during the initial 30-min incubation. Phagocytosis continued after 30 min, and by 180 min only 0.27% of the bacteria remained in the supernatant fluid. The number of viable *P. aeruginosa* recovered from the lysed pellet after 30-min incubation was 0.065% of the original number. Viability decreased sharply after 30 min to less than 0.01% of that at 0 time.

Significant changes in phagocytosis and killing occurred by 7 days after irradiation. Phagocytosis was greatly reduced between 0- and 30-min incubation with 77% of the *P. aeruginosa* still remaining in the supernate at 30 min; it

1028120

increased slightly after 30 min, and by 60 min the rate of removal equalled day 0. After 180 min, 3.2% of the original number of bacteria remained in the supernate. Bacterial killing on day 7 was temporarily increased. At no time during incubation was more than 0.001% of the original number of *P. aeruginosa* recovered from the pellet.

Samples of the blood obtained after day 7 showed progressively decreased phagocytosis and killing. From day 17 forward no removal of *P. aeruginosa* from the supernatant fluid was detected during the first hour of incubation; no

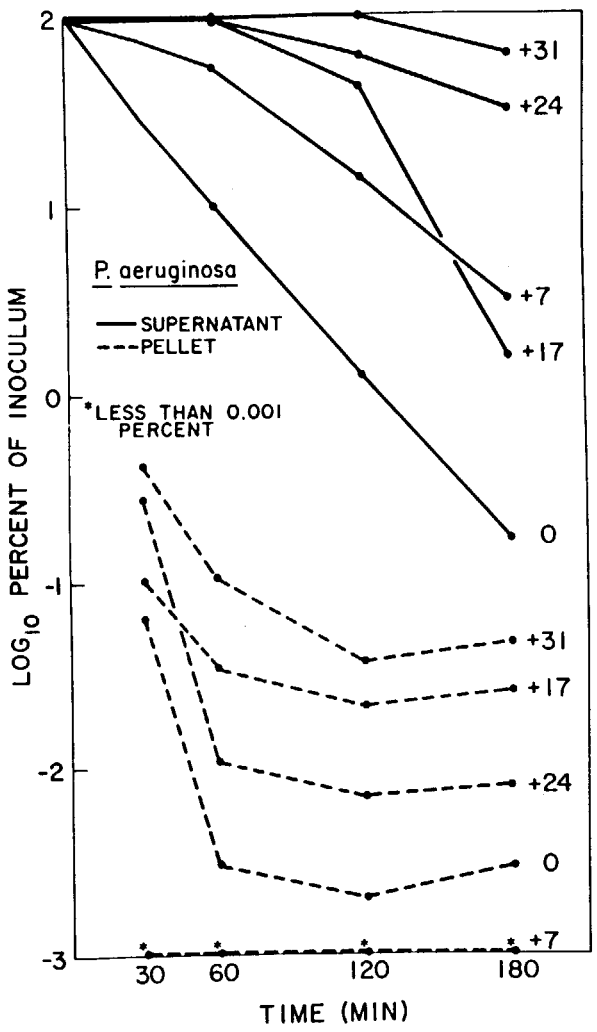


Fig. 4. Plagocytosis and killing of P. aeruginosa *by blood leukocytes after 100 R TBI.*

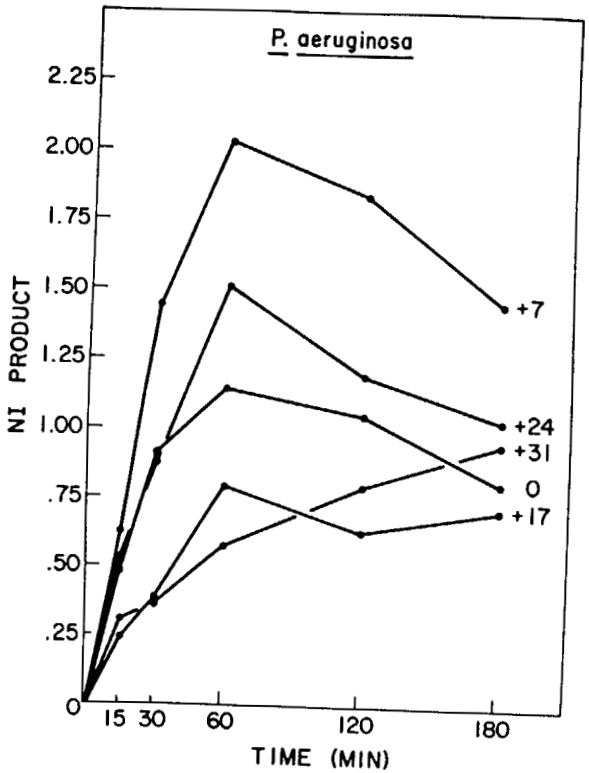


Fig. 5. NI product for **P. aeruginosa** *after 100 R TBI.*

removal was seen during the first 2 hr on day 31. The delayed phagocytosis observed after day 17 resulted in significantly increased numbers of bacteria remaining in the supernatant fluid; 33% remaining on day 24 and 64% remaining on day 31. The number of viable bacteria in the pellet decreased immediately after exposure but increased significantly on days 17 and 31, indicating decreased killing activity. The changes in the number of bacteria in the pellet after irradiation have not been as consistent as those changes observed in the supernatant fluids.

From the data on viable counts in the supernate one would expect decreases in the phagocytic number (the number of granulocytes participating in phagocytosis) or the phagocytic index (the average number of bacteria in each phagocyte) or in both. To the contrary most patients show progressive increases or no changes in these values after irradiation; no significant changes occurred in the phagocytic numbers or phagocytic indices during the study. Interpreting phagocytic activity based on either the phagocytic number or phagocytic index alone is usually impossible, since an increase in the phagocytic number may be balanced by a corresponding decrease in the phagocytic index. The total phagocytic activity is more easily studied if the NI product (numerical product of phagocytic number and phagocytic index) is used as in Fig. 5. The finding of no

1028121

significant change in the NI products after irradiation is not consistent with the observed decrease in removal of bacteria from the supernatant fluids. This apparent paradox — greatly reduced bacterial removal with no change or even increases in NI product values — occurs after irradiation in virtually every patient studied and has not been fully explained.

Granulocyte counts were made at various times during the incubation period to determine if the granulocytes were adhering to the sides of the rotating test tube or were lysing during incubation (Fig. 6). A slight decrease in granulocyte count was observed, the greater part of which occurred during the first 60 min. A similar decrease occurred in normal blood samples incubated simultaneously, which indicates that the decreased phagocytosis cannot be explained by the loss of granulocytes.

During these studies the irradiation caused gradual decreases in the patients' total white blood counts (WBC), usually to a minimum of 50 to 30% or less of the preirradiation value. Since the number of bacteria was held constant, a proportional increase in bacterium/granulocyte ratios resulted. Duplicate experiments were done using bacterium/granulocyte ratios of 1:1 (preirradiation ratio), and 2:1 (usually the maximum postirradiation ratio) to determine if the higher ratios after irradiation were responsible for the greatly reduced phagocytic and killing activity. No significant differences in phagocytosis or killing were found when the bacterium/granulocyte ratios differed by a factor of 2.

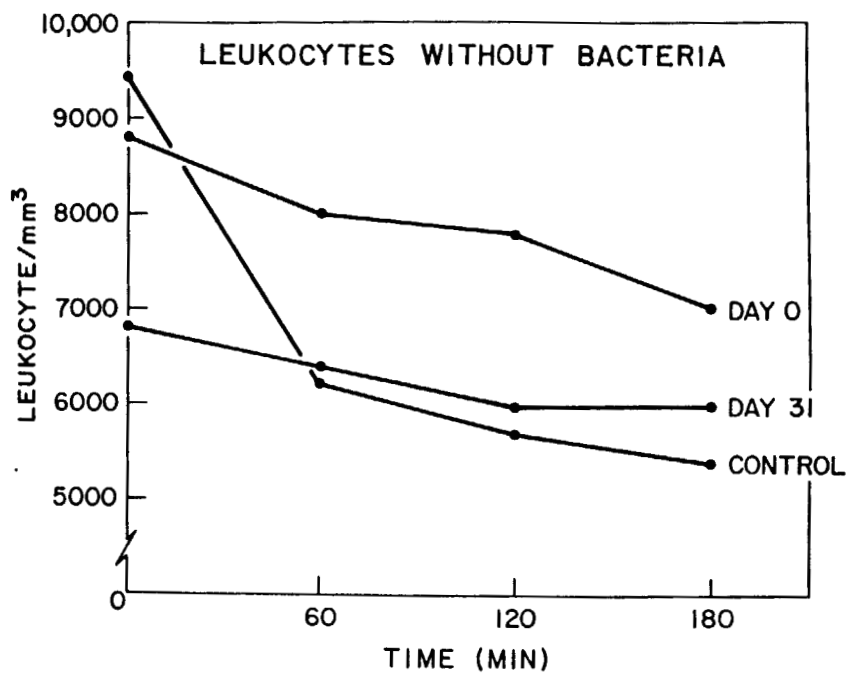


Fig. 6. Changes in leukocyte number during rotation of blood at 37°C.

CHEMICAL PROTECTION OF NATURAL DEFENSE MECHANISMS DURING TBI

Because infections occurring after irradiation at times have a rapid course and high mortality rate, it is important to protect or enhance the host's natural defense system during exposure. A number of chemicals experimentally can modify the effects of irradiation, including acute lethality, splenic atrophy, intestinal mucosal cell damage, or leukopenia.

We have continued studies (G. Charles Kingdon and William G. Tankersley) on the efficacy of radioprotective chemicals in reducing postirradiation infection rates. In these studies mercaptoethylamine HCl (MEA) or aminoethylisothiouronium HBr (AET) are used to protect female Swiss Webster pathogen-free mice (20 g) during exposure to a ^{60} Co source.

Preliminary work, including determination of irradiation LD_{50} values and infection LD_{50} values, chemical analyses, and evaluations of animal infection test procedures, is completed. The experimental irradiation $LD_{50(30)}$ using groups of 10 animals at each dose has averaged 753 R and is very consistent.

Since the basis of this project is to detect differences in infection rates with and without chemical radioprotector, it is mandatory to determine the accuracy and sensitivity of our infection assay procedures including the frequency of contaminated viscera in untreated mice. With extensive tests using our procedure, we can detect as few as 50 to 100 bacteria (aerobic or anaerobic) per liver and spleen. To date no bacteria have been grown from cultures of the homogenized livers and spleens from 30 untreated control mice; a total of 50 normal control mice will be examined.

Both MEA and AET significantly protect against spontaneous infection in CD-1 mice exposed to 800 R(~50 R/min) TBI. Table 12 shows the infection rates in untreated mice, in unprotected irradiated mice, in mice injected with different doses of MEA or AET immediately prior to exposure, and in mice receiving radioprotectors only. Unprotected irradiated mice showed a low rate of spontaneous infection during the first 8 days after exposure. The percent of infected animals increased to 48% on day 11 and remained between 35 and 60% through day 18; 14% of the surviving animals were infected on day 20. The period of high spontaneous infection rates corresponds closely with the time of maximum death rates for unprotected animals. Sixty to 80% of the animals died between 10 and 19 days after irradiation, with most deaths occurring in the 6-day period between 12 and 18 days. In contrast, mice treated with 100 to 400 mg MEA or AET/kg of body weight before irradiation showed significantly less spontaneous infections. Greater protection was provided by approximately 400 mg MEA/kg or 300 mg AET/kg, and experiments are continuing to determine more precisely the optimal protective doses. Neither chemical has clearly demonstrated a higher level of protection. Mice receiving MEA or AET but no irradiation showed no spontaneous infection.

Toxicity has proven to be a problem with most radioprotective chemicals and is important when considering these drugs for use in humans. We administered varying doses of MEA and AET to untreated normal mice to determine the effects of the drugs alone on spontaneous infection rates, morbidity, and mortality. The $LD_{50(1)}$ levels for MEA and AET are approximately 500 mg/kg and 525 mg/kg respectively. Fortunately, the doses of MEA and AET which produce toxic

1028122

TABLE 12

PERCENT OF INFECTED LIVERS AND SPLEENS IN CHEMICALLY PROTECTED OR UNPROTECTED MICE AFTER 800 R TBI (50 R/min) AND IN CONTROL MICE

Treatment	Days Postirradiation								
	0	4	6	8	11	13	15	18	20
None	0	0	0	0	0	0	0	0	0
MEA (200 mg/kg)	0	0	0	0	0	0	0	0	0
AET (200 mg/kg)	0	0	0	0	0	0	0	0	0
800 R TBI	0	4	24	24	48	60	44	35	14
MEA (20 mg/kg) + TBI	0	0	20	0	40	60	80	0	0
AET (20 mg/kg) + TBI	0	0	0	0	40	60	40	40	20
MEA (50 mg/kg) + TBI	0	20	40	0	40	40	40	40	20
AET (50 mg/kg) + TBI	0	20	20	0	60	40	40	40	0
MEA (100 mg/kg) + TBI	0	0	0	20	0	40	0	0	0
AET (100 mg/kg) + TBI	0	20	0	0	0	20	40	0	0
MEA (200 mg/kg) + TBI	0	0	0	7	20	0	0	0	0
AET (200 mg/kg) + TBI	0	0	7	7	13	0	7	0	0
MEA (300 mg/kg) + TBI	0	0	0	0	10	10	20	0	0
AET (300 mg/kg) + TBI	0	10	0	0	0	0	0	0	0
MEA (400 mg/kg) + TBI	0	0	0	0	0	0	0	20	0
AET (400 mg/kg) + TBI	0	0	0	20	0	20	0	0	0

morbidity or death in the CD-1 mice are considerably higher than the doses which have provided significant protection against spontaneous infection.

Our preliminary studies with MEA and AET suggest that these chemicals can afford significant protection against postirradiation infection in CD-1 mice. Either of the drugs (150 to 400 mg/kg) provided excellent protection against spontaneous infection in mice exposed to 800 R (^{60}Co) TBI. In contrast up to 80% of the unprotected animals were infected sometime during the 21-day study. Our studies also suggest a low toxicity at the dose levels we have chosen. Many unanswered questions remain, however, before chemical radioprotectors can be used in humans receiving radiotherapy. We have not shown that the drugs will permit the use of higher doses of irradiation without substantially higher risk of infection, although we anticipate this to be true. We must also study other radioprotectors such as WR 2721 (S-2-[3-aminopropylamino]ethylphosphorothioic acid) which possibly offer greater protection with less toxicity.

DOSIMETRY STUDIES AND MONITORING

INFORMATION CENTER FOR INTERNAL RADIATION DOSE FROM RADIOPHARMACEUTICALS

Activities of Center. The staff (Roger J. Cloutier and Evelyn E. Watson) of the Information Center for Internal Radiation Dose from Radiopharmaceuticals (work supported in part by the U. S. Food and Drug Administration) continues to

collect and analyze data related to calculating the radiation dose and has developed several analytical programs to assist in this task. These computer programs permit rapid solving of the mathematical equations required for dose calculations, manipulation of data for exponential-curve fitting, and calculation of the effect of bladder-filling time on the radiation dose to organs from radioactivity in the urine.

During the year the Center has answered many requests for information from the United States as well as from Israel, Japan, Canada, and Germany.

A closer tie developed with the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine when Mr. Cloutier became chairman of the committee. Several Medical Division staff members also serve on MIRD task groups responsible for preparing dose-estimate reports on specific radiopharmaceuticals.

ORLOOK, A Data-Retrieval System. The Center was established to collect, evaluate, and analyze data pertaining to the radiation dose to the patient from administered radionuclides. To facilitate the rapid retrieval of information, the Center uses ORLOOK, a computer data-retrieval system developed by the Oak Ridge National Laboratory Environmental Systems Office to handle large quantities of information and to permit rapid data searches (Roger J. Cloutier, Evelyn E. Watson, Robin A. Wilkins, and G. U. Ulrickson*). The data are divided into data elements or fields, such as title, author, publication information, abstract, etc. There are special fields for comments about retention and distribution.

A special feature of the ORLOOK system permits a search of all stored information or of select data fields. Such a search is not limited to key words but can be for any word stored in the text material. Because the system permits adding information to a specific computer entry at any time, additional data may be entered into the bank as articles are read or reread. The total number of entries in the data bank now exceeds 600 and is increasing rapidly.

Calculation of Effective Absorbed Energy. The average radiation dose, $\bar{D}$, to the whole body or a specific organ from a radionuclide in the body may be calculated by using the equations suggested by the MIRD Committee (Loevinger, R and Berman, M. MIRD Pamphlet No. 1, J. Nucl. Med, 9, Supplement No. 1, 7, 1968):

$$\bar{D}_{(t\leftarrow s)} = \frac{\tilde{A}_s}{m_t} \sum_i \Delta_i \phi_{i(t\leftarrow s)} \text{ rad}$$

where

$\tilde{A}$ = cumulated activity (μCi−hr)
m = mass (g)
Δ = equilibrium dose constant for type i radiation (g−rad/μCi−hr)
ϕ = absorbed fraction for type i radiation
subscript $_t$ -- indicates target organ
subscript $_s$ -- indicates source organ.

* Environmental Information Systems Office, Oak Ridge National Laboratory.

1028123

The summed terms $\sum_i \Delta_i \phi_{i(t\leftarrow s)}$ are called the "effective absorbed energy" and have a specific value for each radionuclide and target-source combination. We (Lewis R. Barber,* Roger J. Cloutier, and Evelyn E. Watson) have calculated this value for 12 radionuclides common to nuclear medicine by using several target-source organ combinations. These combinations were selected because of either their tendency to contain high concentrations or their radiosensitivity. Most values of ϕ for penetrating radiation were obtained by interpolating tabulated values prepared by the MIRD Committee (Snyder, W. S. et al., MIRD Pamphlet No. 5, J. Nucl. Med 10, Supplement No. 3, 5, 1969). When absorbed fractions were not available, we calculated the value by using the reciprocity principle which states that the ratio of absorbed fractions is equal to the ratio of the organ masses:

$$\frac{\phi_{(t\leftarrow s)}}{\phi_{(s\leftarrow t)}} = \frac{m_t}{m_s}.$$

Table 13 gives a partial listing of the calculated effective absorbed fractions for one of the 12 radionuclides.

TABLE 13

TECHNETIUM-99m EFFECTIVE ABSORBED ENERGY

$\sum_i \Delta_i \phi_i$ (g-rad/μCi-hr)*

	Source Organ				
Target	Total body	Kidney	Bladder	Ovaries	Testes
Total Body	1.31E-1†	1.52E-1†	1.66E-1†	1.78E-1†	1.74E-1†
Kidney	4.62E-4	5.69E-2†	8.7E-4	2.55E-4	1.7E-5
Bladder	8.88E-4	1.37E-4	6.97E-2†	3.46E-3	3.01E-3
Ovaries	1.74E-5‡	---	---	4.41E-2†	---
Testes	7.29E-5‡	---	---	---	4.99E-2†

* The digits following the symbol E indicate the power of 10 by which the number is to be multiplied. Example: 2.31E-2 is equivalent to 2.31 x 10^{-2} or 0.0231.
† Includes both penetrating and nonpenetrating radiation.
‡ Calculated by the reciprocity method.

ANALYSIS OF EXPONENTIAL RETENTION DATA

Radionuclide retention data for most biological systems can be fitted by a series of exponential equations. In examining several exponential curve fitting methods, we (Barbara Y. Howard,† Roger J. Cloutier, and Evelyn E. Watson) found no single method entirely free of disadvantages; however, advantages of several of the techniques make them useful for many applications in analyzing retention and other biological data. We have grouped the techniques into analytical methods and minimization methods.

* Presidential Intern.
† Research participant, summer of 1972, University of Virginia Medical School, Charlottesville.

Analytical Methods. The curve-stripping method which provides starting values for most other techniques is performed by plotting the log of the dependent variable, y, versus the independent variable, x, on semilogarithmic graph paper, choosing a line that best represents the y-values for large values of x, and extrapolating this line to x = 0. If the experimental data cannot be fitted by a single exponential, the points for small values of x will deviate from this line. The second exponential term can be found by subtracting the y-values of the line from the experimental y-values for a given x and plotting the resulting y-values. If these values yield a straight line, two exponential terms make up the curve. This relatively simple procedure is accurate enough for many purposes if the slopes of the two components differ significantly. However, if the subtraction results in a curve, there are more exponential terms and this process must be repeated. At each repetition the problem of how to draw the line becomes more acute and the results less certain.

Many analytical methods require the data points to be evenly spaced and have little variability, or the answers may be inaccurate. Evenly spaced data points are not required if the sum-of-exponential method is replaced by a difference-equation method; however, this method can give highly erratic results.

Sine and Fourier transforms of y = f(x), in conjunction with examination of graphs of these functions, are the basis for other analytical methods of fitting exponential data. When a sine transform is used, the data must be smoothed and the ratio between exponential factors should be at least four in order to separate the terms. The Fourier transformation has the problem that its infinite limits cannot be treated in a computer. If limits are set, the particular method of integration becomes critical and the choice of starting values affects the results.

Minimization Methods. The simplest minimization method, the linear-least-squares fit to a line, serves as a model for all the minimization methods. When a set of data is being fitted with the line, $y = mx + b$, this method is applied to determine the best m and b. Basically the procedure involves the calculation of the sum of the squares of the differences between the y-values of the data and the calculated y-values:

$$S = \Sigma (y_i - mx_i - b)^2 .$$

This sum, S, is minimized with respect to m and b by setting the partial derivatives equal to zero:

$$\frac{\partial S}{\partial m} = 0 \quad \frac{\partial S}{\partial b} = 0 .$$

The resulting equations are solved for m and b, which are then given in terms of sums of combinations of the x- and y-values of the data.

A linear-least-squares fit can be performed for a single exponential by taking the natural logarithm of both sides of the equation,

$$y = Ae^{-\lambda x}$$

to give a linear relationship between log y and x. If there is more than one exponential term, the expression for S and the resulting partial derivative expressions are too complicated to solve by inspection and another method is needed. The method chosen depends on the problem and the kind of computational

1028124

equipment available, but all methods have in common the goal of minimizing the quantity S, that is,

$$S = \sum_{i=1}^{n} \left[y_i - \sum_{j=1}^{m} \left(A_j e^{-\lambda_j x_i} \right) \right]^2 ,$$

where there are n data points and m terms in the sum of exponentials. The number of exponential terms must be specified since the computer does not determine the value for m, and an estimate must be given for each of the parameters as a starting point for the minimization.

Experimental Experience. Several sets of data were prepared for comparison and processed on the Oak Ridge National Laboratory IBM 360 and the ORAU IBM 1800 and PDP-8 computers. The programs for the IBM computers are written in FORTRAN while the program for the PDP-8 is in BASIC and can be used with other Digital Equipment Corporation computers such as the PDP-11. Each program was tested with several perfect sets of data, with "good" x- and y-values, and with actual animal retention data having both two- and three-term exponential sums. All of the programs were versatile and achieved answers of acceptable accuracy.

The program for the PDP-8 is easiest to use because fewer steps are involved in making a run. The IBM 360 program is the most powerful, but our data are not good enough to warrant using it routinely. A precaution that must be observed with all programs is that the best estimates of the input parameters must be used or the resulting curves may not fit the actual data.

APPLIED RADIOBIOLOGY

PHYSIOLOGICAL MONITORING

Measurement of Radiation-Induced Performance Decrement. In the past year our efforts continued on methods of assessing radiation-induced performance decrement after total-body irradiation (Robert C. Ricks, Ann Sipe, and C. C. Lushbaugh). In addition to using repetitive physiological monitoring of additional patients to improve our detection system for radiation-induced gastrointestinal distress, we have studied changes in exercise capability of normal men. For this we used pulmonary responses to controlled exercise in order to have a data base for isolating the effects of radiation exposure. Previously we found that increased respiratory efforts were required during controlled post-irradiation (1 to 2 weeks) exercise periods when exposures were protracted (30 R/day), while similar but much more subtle changes occurred when the treatments were fractionated at 10 R/day. Regardless of the temporal format of the exposure protocol, when the treatment had caused increased pulmonary effort during controlled exercise it had also produced an elevation in resting pulmonary effort when measured just prior to exercise testing.

To compare the response of irradiated men and unirradiated volunteers, normal males (age 30 to 40 years) were monitored during controlled bicycling at workloads comparable to those used for patients. In a typical study, each of five

normal males, who had not had previous experience with bicycle ergometry, exercised under controlled ergometry two or three times weekly for 4 consecutive weeks. The results in Fig. 7 show that normal man typically responds to moderate exercise by an increase in pulmonary impedance (as measured by its average variance). A decrease in the elevation of pulmonary impedance during controlled work developed progressively during the first week and a half after which the exercise-induced response was relatively constant, probably due to adaptation to the exercise workloads. This effect is similar to "training" but differs in that training would probably require 8 to 10 weeks during which the work levels would be progressively increased. Nearly all persons monitored show this decrease during their second and third exercise period (first week of controlled exercise testing). Irradiated subjects, however, in contrast to the normal volunteers, all demonstrated an increase in pulmonary impedance at some time during or after radiation exposure. Normal males also showed less variable profiles of immediate recovery from exercise (4 to 30 min postexercise) as compared to irradiated patients whose profiles were highly variable.

The first profile of exercise tolerance was obtained this year on a patient undergoing treatment in the medium-exposure-rate total-body irradiator (METBI) (30 R/day, 1.5 R/min). She received a total exposure of 250 R and is the first woman who has participated in extensive exercise testing. The results (Fig. 8) show that the variance of pulmonary impedance increased when 60 R were accumulated and remained amplified for 10 days after irradiation. Exercise

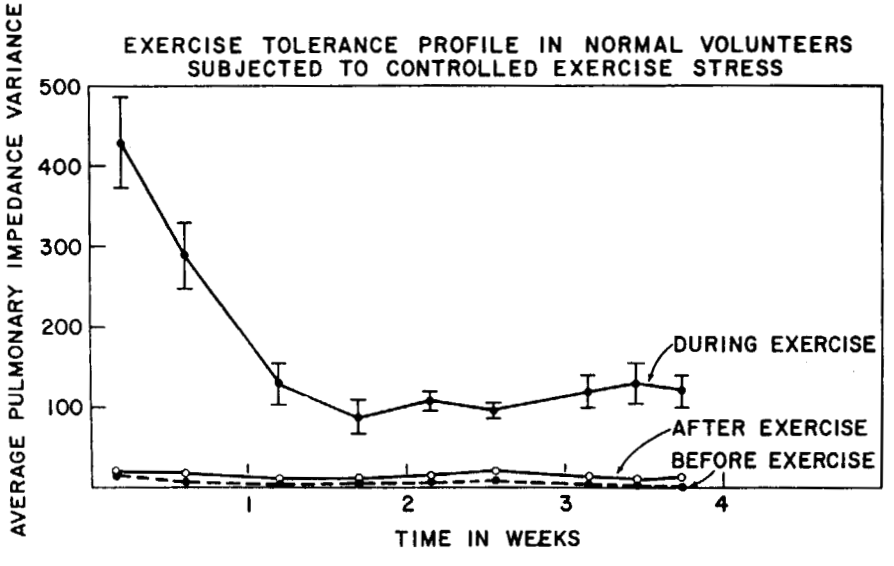


Fig. 7. Graphic representation of normal man's response to controlled exercise on a bicycle ergometer. The progressive early reduction in average pulmonary-impedance variance during exercise is indicative of adaptation to the controlled ergometry. No significant changes are notable in the response before and after exercise.

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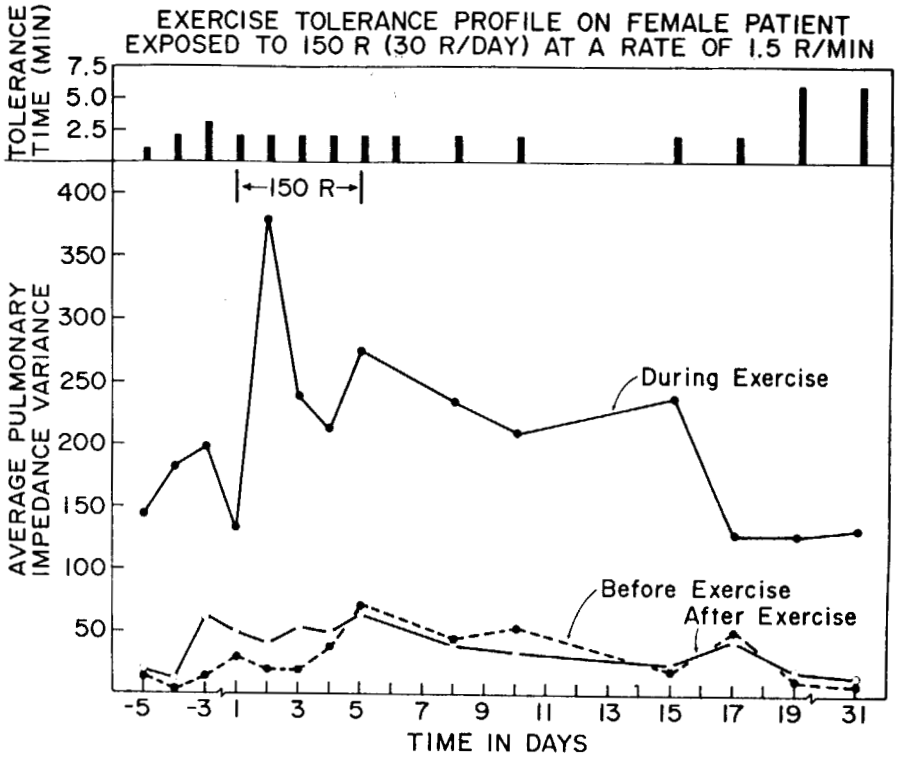


Fig. 8. Pulmonary-impedance power-spectral analysis from a leukemic patient who exercised to tolerance on a bicycle ergometer before, during, and after exposure to fractionated therapeutic radiation. Note increases in response to controlled stress when ∿60 R are accumulated and continuing for 10 days postirradiation. These amplified pulmonary-impedance responses, not seen in normal volunteers, are indicative of dimished exercise capacity.

tolerance time was low (~2 min)through 18 days of controlled testing but had increased threefold when measured at 11 and 22 days after irradiation. Variance of pulmonary impedance recorded before each exercise period (baseline data) rose slightly but significantly when 150 R had been accumulated and remained elevated until 7 days after irradiation. The significance of a slightly amplified preexercise baseline is not clear but it could be due to anxiety, pain, or some unknown physiologic phenomenon that may increase basal pulmonary-demand. Study in all patients exposed in the low-exposure-rate total-body irradiator (LETBI) (protracted exposures) shows an elevated baseline pulmonary-impedance variance, most pronounced in patients exposed to 250 R (30 R/day). While patients exposed to 150 R and 100 R show slightly elevated baselines, in these instances the elevation does not seem dose-rate dependent. Figure 9 shows that the pulmonary impedance of resting patients is highly variable compared with that of normal unirradiated men.

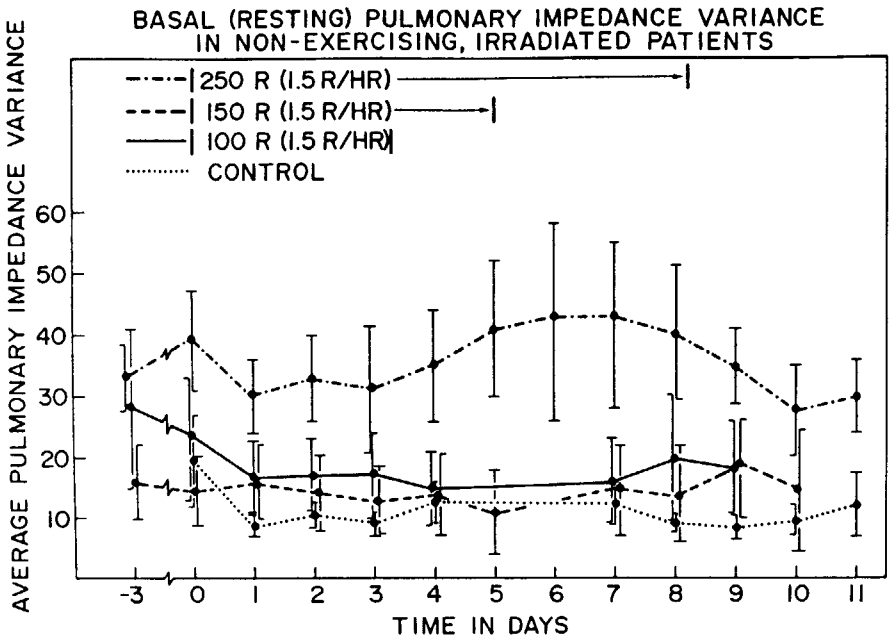


Fig. 9. Pulmonary-impedance power-spectral analysis from nonexercising, irradiated patients before, during, and after protracted total-body exposures. Except for patients receiving 250 R (whose higher variance may be due to splenomegaly) those exposed to 100 R or 150 R demonstrate little pulmonary-impedance change in the absence of controlled exercise stress.

Each subject was monitored in a sitting position for 15 min to assure basal physiologic conditions and then for periods up to 30 additional min. These data indicate that the performance-decrement effects of total-body irradiation are not expressed until the individual is stressed (although only low levels of exercise are required).

Diminished exercise-capacity (DEC) continues to be studied by physiological monitor techniques with exercise stress in patients exposed to therapeutic levels of total-body ionizing radiation. The number of patients studied is still too small to suggest a dose-response model for quantitation. Results to date indicate that 1) protracted exposures (LETBI) of 100 or 150 R result in DEC within 1 to 2 weeks after exposure; 2) fractionated exposures (10 R/day, METBI) result in DEC during the exposure when ~40 R have been accumulated and subsiding after 100 R; 3) fractionated exposures of 30 R/day (METBI) produce early in the exposure period (~60 R accumulated) a DEC that remains evident during the entire fractionation period (e.g., 5 days for 150 R). The severity of DEC (based on pulmonary changes) appears to be greater after fractionated than continuous exposures. Changes in exercise capacity in the radiation accident victim reported last year were more comparable to patients receiving 30 R/day (1.5 R/min) than to any other group of patients studied in our laboratory.

These conclusions are based upon comparison of normalized response curves on irradiated patients participating in controlled exercised testing (Fig. 10).

1028126

Serum Creatine Phosphokinase Studies in the Normal and Irradiated Man. All persons (patients and normal volunteers) demonstrated significant increases in serum creatine phosphokinase (SCPK) levels when subjected to controlled exercise stress and/or irradiation. Serum creatine phosphokinase levels (shown in Fig. 11) in normal volunteers exercising under our conditions showed increases in this enzyme comparable to those for exercising men reported in recent literature (Hunter, J. and Critz, J., J. Appl. Physiol. 31, 20, 1971). Generally, SCPK levels rose 2 to 2.5 times the preexercise levels and although they remained elevated throughout the 4-week test period, they appeared to be returning to normal during the fourth week, possibly indicating an adaptation to the constant workload.

Serum creatine phosphokinase levels were determined in 1) irradiated patients who did not exercise and 2) patients who were similarly exposed and tested by controlled exercise. In nonexercising patients exposed to 100 R (30 R/day, 1.5 R/hr) significant increases in enzyme activity occurred during the exposure periods. The enzyme activity returned to normal within 2 weeks after exposure in all but one patient studied (Fig. 12). Similar changes in SCPK activity occurred in nonexercising patients exposed to 150 R in LETBI (Fig. 13). Only one of the patients exposed to 100 or 150 R failed to demonstrate the rise in

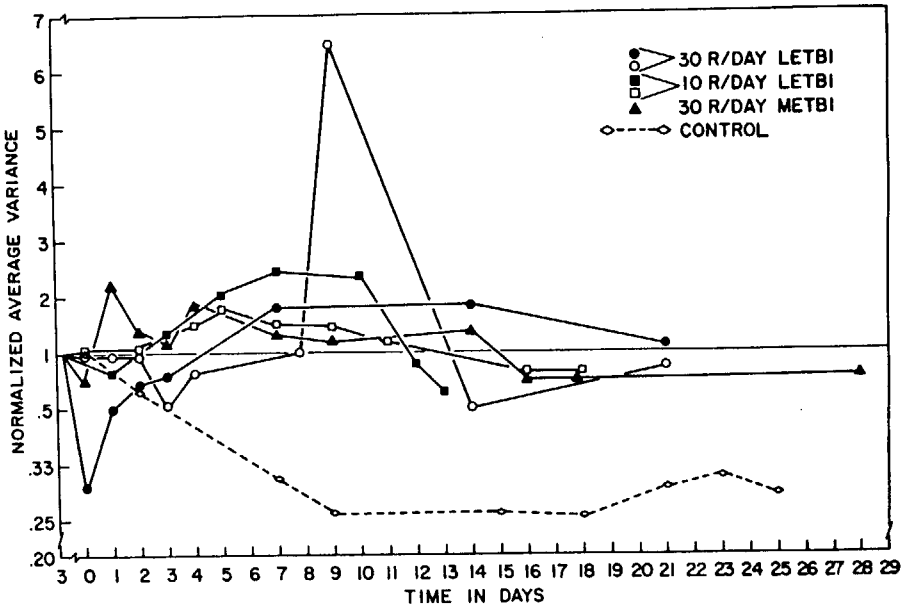


Fig. 10. Exercise-tolerance profiles from patients receiving protracted or fractionated irradiation therapy and who were subjected to controlled exercise stress. Each patient performed the same workload on successive exercise trials. Results are normalized to preirradiation values and demonstrate the difference in the response of the normal, unirradiated and irradiated man to exercise stress. Normal man rapidly adapts to stress whereas the irradiated man shows periods of performance decrements dependent upon exposure.

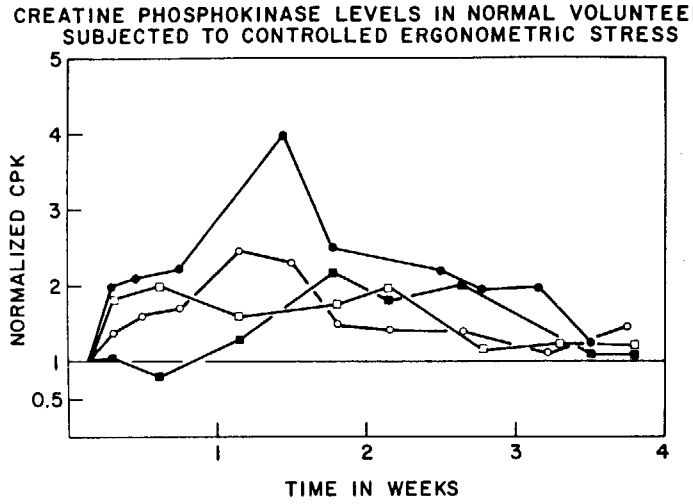


Fig. 11. Serum creating phosphokinase levels from four normal male volunteers performing in two or three controlled exercise periods per week over a 1-month period. (Pulmonary-impedance response shown in Fig. 7.) Values are normalized to the pretest sample value. Results are typical for expected SCPK levels for nontrained individuals stressed at submaximal levels for short periods.

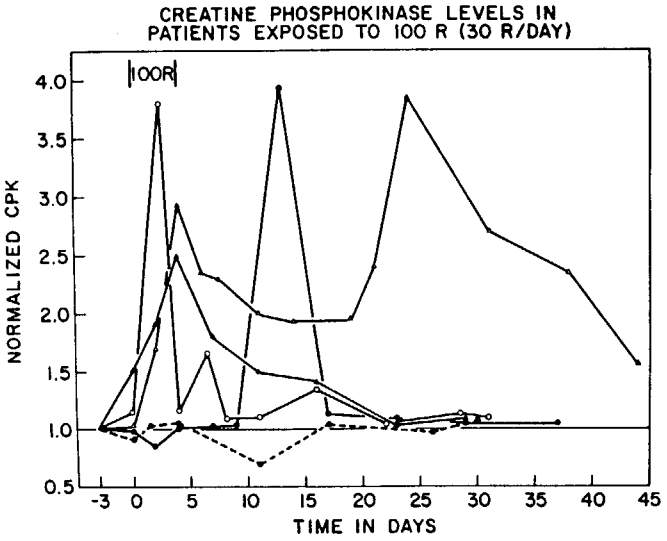


Fig. 12. Serum creatine phosphokinase levels in four nonexercise-stressed patients exposed to 10u R protracted irradiation therapy and normalized to preexposure levels. Although no exercise stress was administered, these patients responded as stressed; the radiobiological mechanism is not understood at this time.

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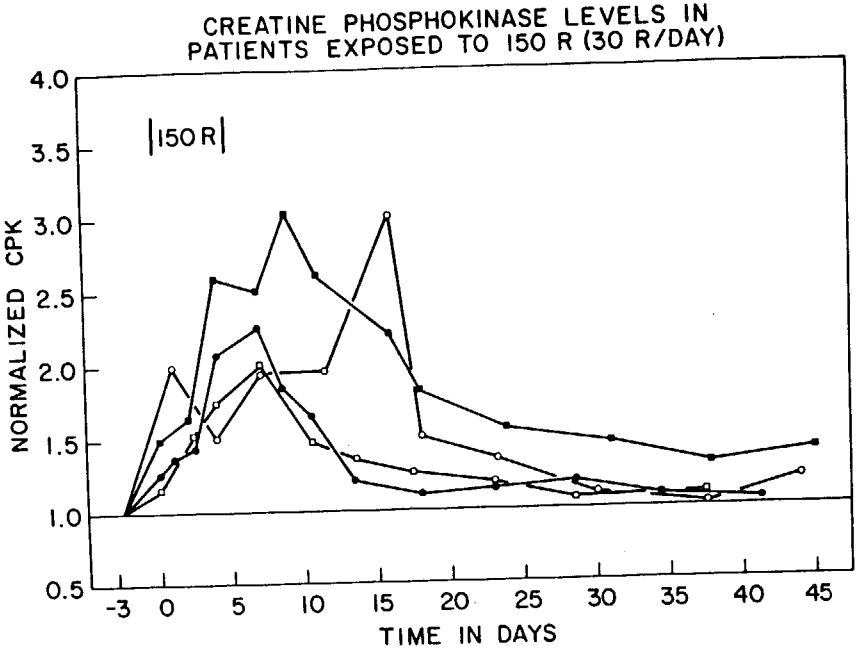


Fig. 13. Serum creatine phosphokinase levels in four nonexercise-stressed patients exposed to 150 R protracted irradiation therapy and normalized to preexposure levels. The magnitude and timing of the response is similar to those seen when 100 R is administered (Fig. 12).

SCPK levels. In nonexercising patients receiving fractionated exposures (10 R/day, LETBI) SCPK levels rose significantly in those receiving 150 R but not in one who received only 100 R (Fig. 14). This increase in SCPK activity was not evident until a few days (<1 week) after irradiation and was higher than in patients with similar but protracted exposures.

Our limited data on changes in SCPK levels suggest that some relationship exists between exercise response and energy output levels that are creatine-phosphokinase dependent. It is not yet clear which is cause and effect. For example, in one patient DEC during fractionated (10 R/day) irradiation therapy was followed by a significant increase in SCPK and then by recovery from radiation-induced fatigability (Fig. 15). We feel that the increase in circulating SCPK level may be a systemic response to stress that feeds back to prevent or delay fatigability, but further studies will be required before any such role can be substantiated.

Our studies show well that an irradiated patient does not respond to controlled exercise in the same way that normal unirradiated man does. The differences noted in irradiated patients could be due to their disease state but this seems highly unlikely since 1) none of the patients studied were acutely ill and 2) the patients tended to demonstrate degrees of DEC proportional to their irradiation exposure.

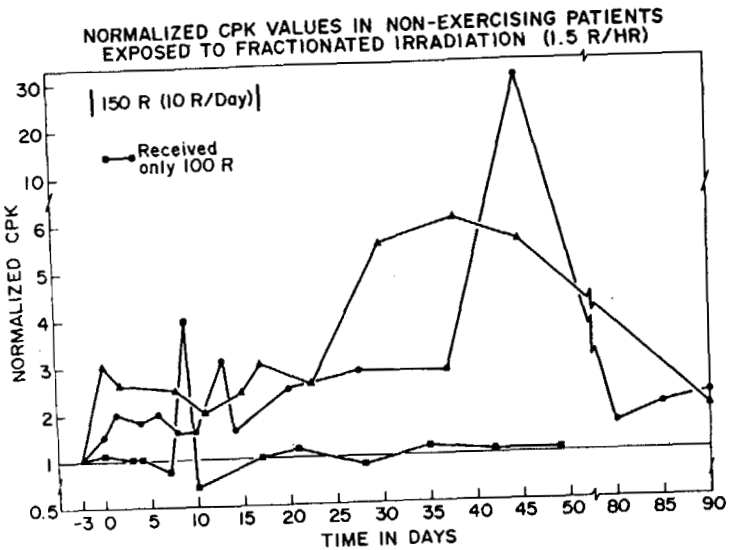


Fig. 14. Serum creatine phosphokinase levels in three nonexercise-stressed patients exposed to 100 R or 150 R fractionated irradiation therapy and normalized to preexposure levels. Individual responses are similar to those seen during protracted exposures.

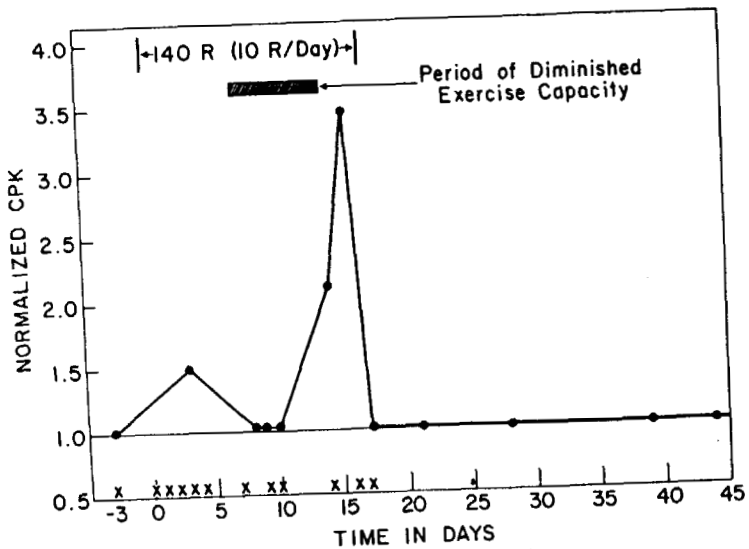


Fig. 15. Graphic representation of the relationship between SCPK levels and DEC in a patient exposed to 140 R total-body therapeutic irradiation and controlled exercise stress. Values for SCPK are normalized to preexercise levels. These isolated data indicate that rising creatine phosphokinase levels may reverse the physiological process responsible for radiation-induced fatigue. However, the mechanism is not explained by this limited data.

1028128

Correlation of Alveolar CO_2 Concentration with Pulmonary Impedance. After several unsuccessful attempts, measurements of CO_2 in the expired air of exercising subjects are now being made in our laboratory. We thought it likely that CO_2 levels might correlate with changes in pulmonary impedance during controlled exercise stress. In preliminary studies (Robert C. Ricks and Ann Sipe) expired CO_2 levels before controlled exercise were within normal levels reported for man (4.8%). Within less than 30 sec after initiation of controlled exercise the alveolar CO_2 rose ~25% to a value of 5.9%. Expired CO_2 levels remained elevated throughout the remainder of our exercise test period and when exercise was stopped, returned within 2 min to preexercise levels. The increases and decreases in CO_2 levels correlate directly with those in pulmonary impedance during and immediately following exercise. The expired CO_2 level, however, reached the preexercise level before pulmonary impedance did in the subjects tested so far. Increased concentration of alveolar CO_2 also correlates well with elevated heart-rate during exercise. However, exhaled CO_2 concentration returned to the preexercise level in about 1/12 the time required for heart rate. Table 14 illustrates these correlations between alveolar CO_2 concentration and heart rate in a normal volunteer subjected to controlled exercise testing. Figure 16 shows graphically the relationship between pulmonary impedance and CO_2 in one study.

TABLE 14

RELATIONSHIP BETWEEN PULMONARY IMPEDANCE, ALVEOLAR CO_2, AND CARDIAC RATE IN A NORMAL VOLUNTEER EXERCISING ON A BICYCLE ERGOMETER

Monitoring period	Pulmonary impedance variance	Ratio	
		CO_2/ basal CO_2	Heart rate/ basal heart rate
Preexercise*	41	1.00	1.00
During exercise	497	1.45	1.70
End exercise + 4 min	301	1.25	1.20
End exercise + 8 min	61	1.01	1.13
End exercise + 12 min	58	1.00	1.12
End exercise + 16 min	46	1.00	1.07
End exercise + 20 min	64	1.00	1.08
End exercise + 24 min	51	1.00	1.08
End exercise + 28 min	65	1.00	1.05

* Data measured at 4 min prior to bicycle ergometry (50 watt workload).

Studies in progress on two patients receiving 250 R (30 R/day, 1.5 R/hr) who are receiving controlled exercise stress indicate that the increase in alveolar CO_2 appears greater than expected after initiation of irradiation therapy. Alveolar CO_2 levels remain elevated 1 month after the end of therapy. We do not yet know the full signigicance of the relationship between pulmonary-impedance variance and alveolar CO_2 levels.

Pulmonary-Impedance Power-Spectral Analysis of the Irradiation Prodromal Syndrome. We have previously shown that pulmonary-impedance power-spectral analysis could recognize the onset and severity of gastrointestinal distress in patients undergoing total-body therapeutic irradiation.

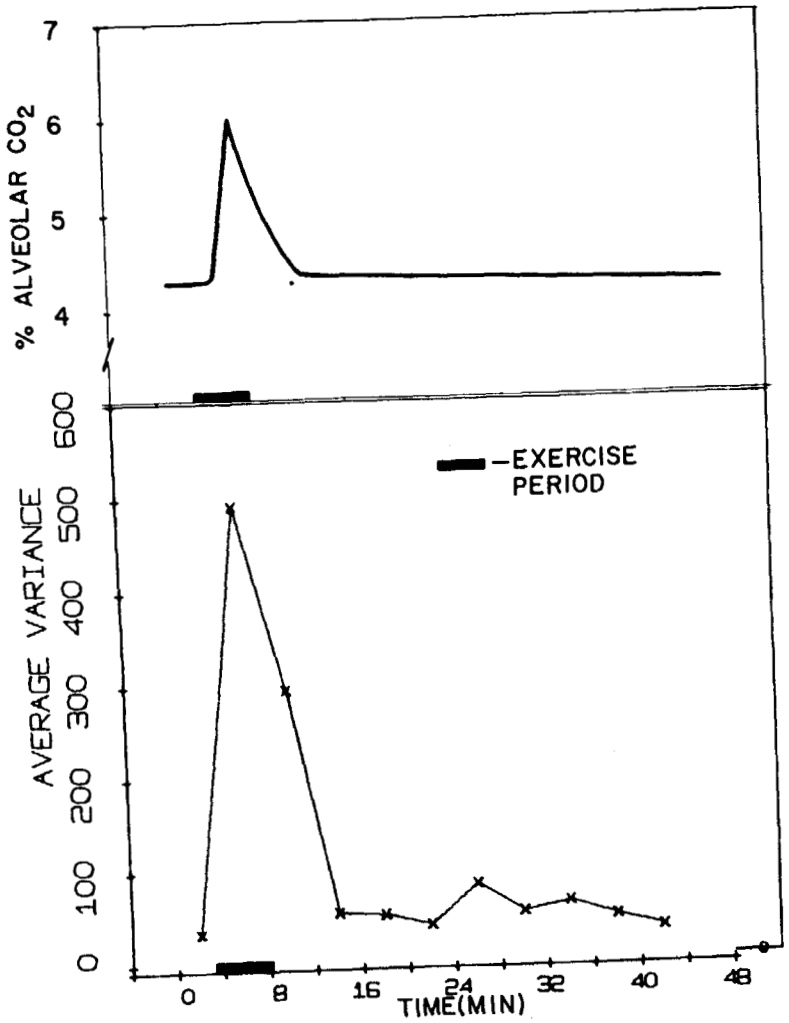


Fig. 16. Graphic representation of the relationship between variance of pulmonary impedance and simultaneously measured alveolar CO_2 concentration in a normal male volunteer stressed under controlled exercise conditions. Data for CO_2 recorded breath-by-breath on a Beckman LB-2 medical gas analyzer and averaged every 15 sec. These data can be used to further determine the difference in exercise response between the irradiated and normal man.

1028129

Periods of gastrointestinal distress were accompanied by high-power, low-frequency shifts in the power spectra.

The effect of nausea and vomiting on pulmonary impedance is vividly demonstrated in Fig. 17 and represents data taken from the radiation accident victim who received 260 rem (350 R/min) total-body irradiation. Physiological monitoring commenced 4 hr postirradiation and periods of amplified average variance of the pulmonary impedance correlated well with nausea and emesis. The accident victim had vomited several times before the initiation of physiological monitoring.

The radiation prodromal syndrome is also well demonstrated in the physiological data taken at various times over several days after irradiation preparatory to bone marrow transplantation. Figure 18 shows data from a patient who received 500 R (40 R/min) total-body gamma irradiation. Average variance of the pulmonary impedance was very stable over a period 10 days before and during irradiation. However, by 50 min postirradiation the patient demonstrated a shift in this variance that correlated with nausea and emesis. The changes subsided with 24 hr.

General Conclusions and Proposals Related to Biochemical and Cardiopulmonary Studies on Irradiated Patients. We are continuing our efforts to correlate enzymatic, respiratory, and cardiac changes with periods of radiation-induced fatigue. Current software is constantly being updated and new modifications are being studied to allow for three-dimensional correlation between pulmonary impedance, cardiac rate, and CO_2 production. In addition, through a cooperative project with UT-AEC Agricultural Research Laboratory, we will this year be able to study radiation-induced fatigue in exercise-conditioned ponies and therefore determine the effect of training on the response mechanisms to total-body irradiation. Six teams of conditioned, matched ponies will be exposed to radiation comparable to that our patients receive. Initial physiological monitoring under exercise stress indicates that the pony responds similarly to man. Figure 19 illustrates the exercise-response profile in one pony monitored before, during, and after pulling a cart against a workload of three-quarter horsepower. Heart rate changes and SCPK levels will also be monitored in these ponies as part of our efforts to evaluate radiation-induced performance decrement.

THE EFFECTS OF CONTINUOUS LOW-DOSE-RATE IRRADIATION ON VARIOUS MOUSE STRAINS

Our studies on the mechanisms of survival of mice during continuous irradiation at 1.6 R/hr, our total-body exposure rate for human therapy, suggest that length of survival depends upon reparability of marrow damage and the reestablishment, particularly, of erythropoiesis. During such low-dose-rate exposures damaged intestinal mucosa is easily repaired, bacterial invasion at that site is thereby prevented, and need for increased granulocytopoiesis and platelet production is obviated. Death seems to result simply from the anemia that becomes increasingly severe as red blood cell (RBC) production rate finally declines and the new RBC become small and perhaps short-lived. It is still unclear whether this effect, which is highly dose-rate dependent, is due to decreasing numbers of marrow stem cells, their decreasing proliferative rates, or to

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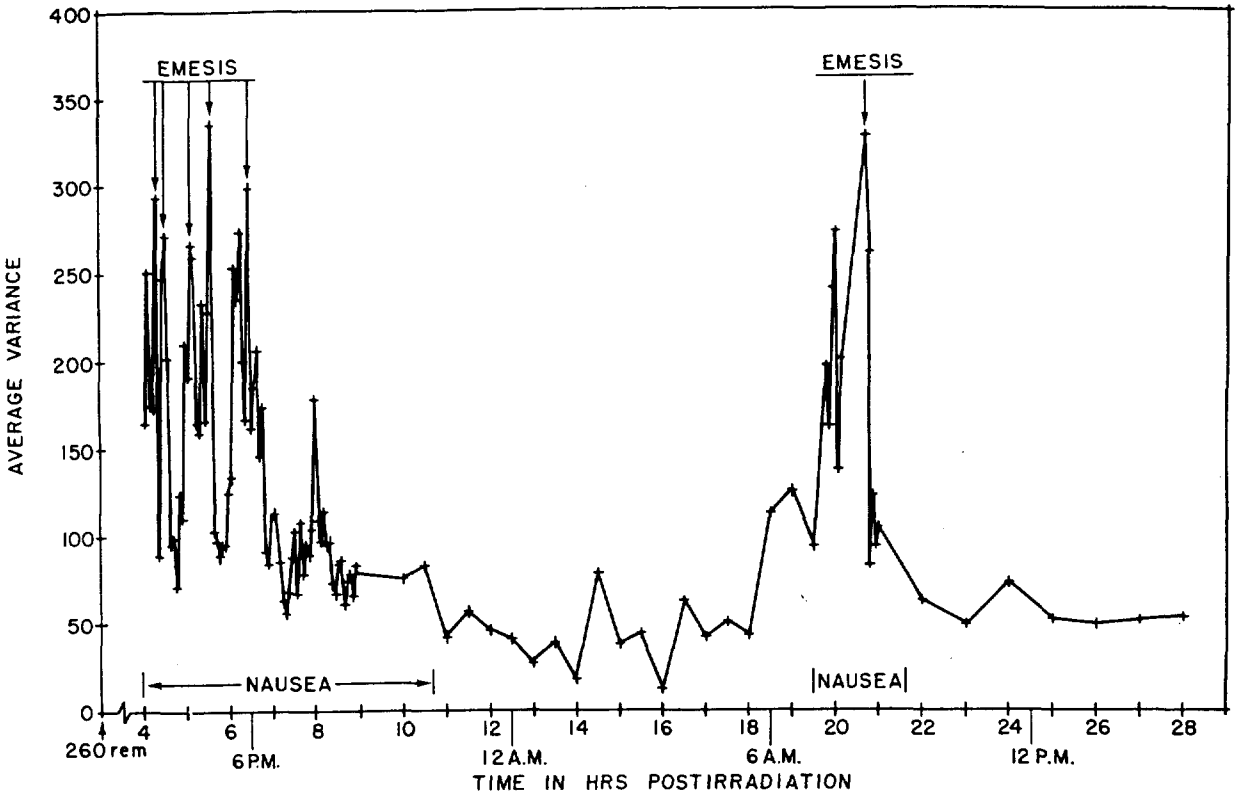


Fig. 17. The effect of nausea and vomiting on pulmonary impedance in a radiation accident victim exposed to 260 rem total-body gamma irradiation at the rate of 350 R/min.

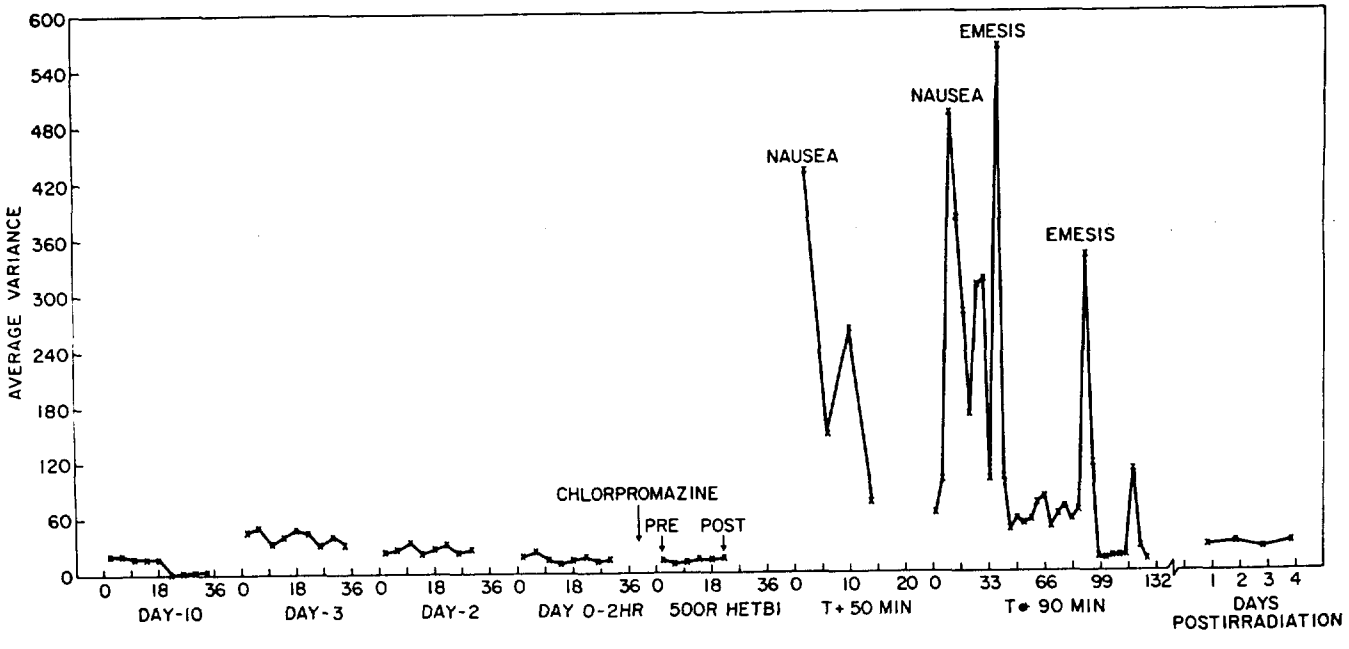


Fig. 18. Effect of nausea and vomiting on pulmonary impedance in a female patient exposed to 500 R (40 R/min) total-body gamma irradiation prior to bone marrow transplantation.

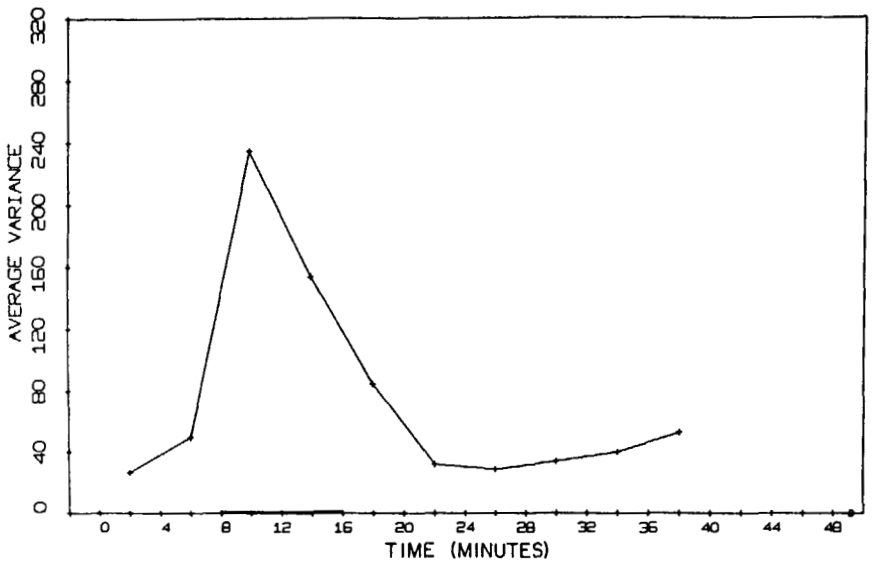


Fig. 19. Profile of exercise tolerance from a Shetland pony working against a workload of three-quarter horsepower. Workload was controlled by requiring a trained pony to pull a cart equipped with a variable-resistance wheel. Recording electrode configuration was similar to that used in human controlled ergometric testing. These data will allow studies to determine the effect of exercise conditioning (in ponies) and irradiation fatigue. This pony data can then be compared to that from the untrained irradiated man to see if training alters the radiation fatigue syndrome.

their increasing difficulties with protein synthesis. Previously we showed in collaboration with Dr. Lorraine Kraus* and Dr. S. Rasad* that an abnormal hemoglobin is produced in this syndrome. It is unusually fast moving on electrophoresis, unlike most fetal and embryonic hemoglobins. The thesis, as yet unproven, is that this hemoglobin is a "stress" protein reflecting a radiation-induced enzymatic lesion in erythroblastic stem cells rather than a purposeful reversion to the manufacture of an "embryonic" hemoglobin. Hübner's observation reported in the 1971 Medical Division Research Report that splenic immune competence is also depressed late in such exposures suggests that our focus upon RBC production is too narrow and needs to be broadened to consider also failure of both humoral and cellular immune mechanisms as contributing lethal factors in this syndrome.

We had not previously studied lymphopoietic repair during low-dose-rate exposures, although it was well known that extensive lymphoid atrophy of all organs develops. We chose as a first approach to the problem of lymphoreticular repair to study the survival time patterns of several mouse strains under continuous irradiation at rates used clinically in total-body irradiation therapy

* University of Tennessee Medical Units at Memphis.

(T. R. Stokes, C. C. Lushbaugh, Gretchen Humason J. Yuhas*, and D. C. Swartzendruber). In addition to obtaining the lethality curves in relation to accumulated "doses" (R), we are studying histologically the survival and regeneration of splenic and thymic lymphocyte reservoirs in five strains of mice: $C3BF_1$, C57BL/6 Cum, C57BL/Cum, BALB/c, and AKR. Our studies on radiation suppression of erythropoiesis have been done principally on C57BL/6 Cum, because it is a long-lived radioresistant strain and upon C57BL/Cum, its radiosensitive parent strain. We included this year $C3BF_1$ because of the superresistence of this hybrid and BALB/c because of its well-known radiosensitivity. Our inclusion also of AKR strain mice was suggested by the recent study of Perkins et al. (J. Nat. Cancer Inst. 46, 845, 1971) who showed that the AKR mouse had an unusually low level of immunohematopoietic competence. This strain, well known for its 90% incidence of a virus-associated thymic lymphoma, has an exceedingly short (approximately 275-day) mean life-span. It has a low resistance to promptly given single doses of radiation ($LD_{50(30)} = \sim 600$ R). For unknown reasons BALB/c is more radiosensitive even though much longer lived. Perkins et al. showed that the immunoincompetence of the AKR mouse is expressed in part by an inability to sustain the growth of exogenous colony-forming units in its spleen, a capability that we believe is required for compensatory erythropoiesis during continuous low-dose-rate irradiation. The AKR mouse seemed therefore to offer a unique opportunity in our system to study whether human therapy levels of irradiation would 1) alter the high lymphoma incidence of this strain, 2) affect the leukemic process once it was initiated, and 3) decrease mean survival time by some process other than aplastic anemia. If the unusual immunoincompetence of this strain was worsened by irradiation, one might expect an earlier onset of thymic lymphoma in some animals along with a decrease in its incidence because of radiation-induced thymic destruction in others. Possibly also the reported immunoincompetence of this strain might be expressed as poor splenic production of endogenous erythroblasts with early death from aplastic anemia.

Others have shown that small, fractionated doses of radiation (150 R at three weekly exposures) can shorten the latent period and increase the incidence of a similar leukemia in C57BL/6 mice and that 24 R/day (our lowest human therapeutic dose per day is approximately 10 R in LETBI) given to this mouse strain will increase the leukemia incidence from about 12% to about 60%. In AKR mice, however, previous studies of leukemogenesis have included only single, prompt radiation exposures which produced a shortened leukemia induction time without alteration of the high incidence.

Two ages of AKR mice were available to us: 40-day-old females and "retired-breeder" females of uncertain age (about 120 days old). The two age groups were divided into three subgroups: unirradiated controls, irradiated mice at 1.6 R/hr or 37 R/day and irradiated mice at 3.2 R/hr or 74 R/day. We observed the mice closely to obtain prompt necropsies for histologic study of the thymus, lungs, liver, spleen, and sternal bone marrow; yet about half were too autolyzed for histologic study but contributed data for determining the length of survival.

* Oak Ridge National Laboratory.

Figure 20 is a graph of the percentage mortality of the AKR, C57BL/Cum, BALB/c, C57BL/6 Cum, and $C3BF_1$ strains under continuous irradiation at 1.6 R/hr, as a function of accumulated dose. The steeply rising curves of the other four strains stand in marked contrast with the slowly rising curve of the two age groups of AKR. At this low-dose rate surprisingly there was no difference in apparent sensitivity between the 40-day-old and the "retired-breeder" AKR. This relatively slow-rising curve for number of deaths per dosage-increment indicates a wide variance in radiosensitivity among different animals, and suggests that more than one lethal mechanism may be operative. The first differentials of the other sigmoid curves are single-peaked distributions indicative of only one lethal process. The apparently sharp lower thresholds and the steepness of both seem to be related to homogeneity of the mice, here expressed as radiosensitivity. While the data are too few for mathematical differentiation of the AKR mortality curve, this irregularly rising line is roughly the sum of two separate subgroups of deaths that occur with peak incidences at quite different accumulated doses.

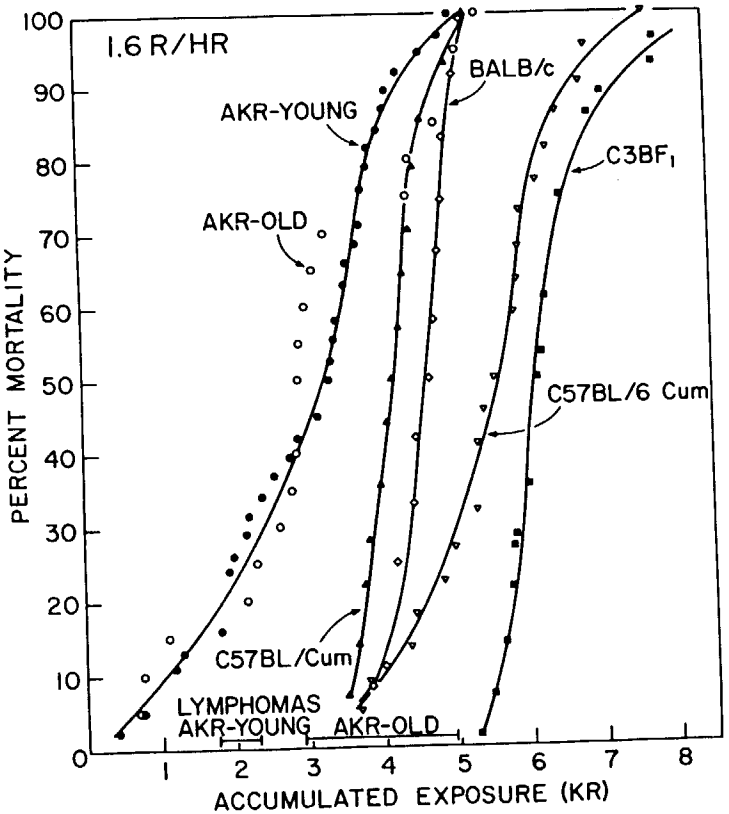


Fig. 20. Percent mortality as a function of accumulated dose for five strains of mice irradiated continuously (23+ hr/day at 1.6 R/hr).

1028132

Gross anatomical and histologic study is not complete for all groups. So far, in all groups all deaths occurring after more than 4000 R have been accumulated have been due to splenic and marrow atrophy. Comparative evaluations of lymphocyte reservoirs have not been completed in sufficient numbers for comment at this time.

The data from the histologic study of irradiated AKR mice at death have been obtained for the young and adult groups exposed continuously at 1.6 and 3.2 R/hr. The data from 48 necropsies and histologic studies are summarized in Table 15 as percent incidence of lymphoma, thymic atrophy, lung adenosis and adenomas, complete splenic atrophy, and presence of splenic erythroblastic colonies. The approximate mean accumulated doses for the four groups are shown along with the average survival time. Unirradiated AKR mice have a mean life-span of about 275 days and their natural incidence of lymphoma is about 90% for females. Here the highest incidence of lymphoma was 40% and

TABLE 15
INCIDENCE OF HISTOPATHOLOGIC ENDPOINTS AT DEATH
OF AKR MICE FROM CONTINUOUS IRRADIATION AT 1.6 AND 3.2 R/HR

R/hr	Age (days)	MAD* (R)	Average survival time (days)	Lymphoma	Atrophy: Thymus	Atrophy: Spleen	Splenic erythroblastosis	Lung adenoma
				Percent				
1.6	40	3100	84 (124)†	17	82	53	39	44
1.6	120	3700	100 (∿220)	40	40	20	40	30
3.2	40	2600	35 (75)	0	100	77	23	55
3.2	120	2700	36 (∿150)	27	72	72	10	18

* MAD = accumulated dose at average survival time.
† Numbers in parentheses represent age at average survival time.

occurred in the adult mice at the lower exposure rate. We completely suppressed lymphoma in the 40-day-old mice exposed to 3.2 R/hr but this may simply mean that they did not live long enough to show it. The AKR mice of both age groups given 3.2 R/hr were the most radiosensitive that we have observed; mean accumulated dose at death was about 2600 R and complete thymic atrophy occurred within their 35-day survival period. Seventeen percent of these young mice irradiated at 1.6 R/hr developed lymphoma between 50 to 60 days of continuous irradiation, when they were 90 to 100 days old. The 27% of adult mice, on the other hand, that developed leukemia did so between accumulated doses of 1500 and 2500 R at 3.2 R/hr (20 to 34 days). However, accumulated doses between 2900 and 5000 R at 1.6 R/hr (78 to 135 days) were associated with the induction of lymphoma in 40%. Some of these late lymphomas could have been spontaneous rather than augmented by irradiation because these animals after 135 days of exposure were approaching their normal life-span of 275 days. It is noteworthy that the thymus of only one mouse was nonleukemic and nonatrophic, in an adult female dying for unknown reasons after only 765 R with an apparently effective erythropoietic response of marrow and spleen. All other thymuses were either enlarged and lymphomatous or extremely small due to loss of lymphoid and

histiocytic cells, sometimes with only the primitive epithelium remaining. This atrophy of the thymus was unrelated to presence or absence of a splenic erythropoietic response. Splenic erythropoiesis was quantitatively poor in all animals compared to similarly irradiated C57BL/6 Cum mice. When thymic lymphoma was present, its metastatic spread masked any normal splenic response to radiation-induced marrow damage. The reduced lymphoma incidence in the adult mice was not accompanied at the lower exposure rate by a decrease in induction time but was when they were exposed at the higher rate. The majority of mice at both ages and rates of exposure, that were free of lymphoma, appeared to die from complete marrow and splenic atrophy. For the first time in our experience in these low-dose-rate studies, we noted that some of the early nonlymphomatous deaths were associated with bacteremias.

The occurrence of pulmonary adenomas (Table 15) was completely unexpected and, as far as we know from a cursory search of the literature, has not been reported in AKR mice even though this strain was derived originally from strain A mice having an extremely high incidence of these tumors. An atrophic thymus (or the absence of lymphoma?) seemed to be a necessary condition for this process to occur. Previously others (Trainin, N. and Linker-Israeli, M. In: Immunological Parameters of Host-Tumor Relationships, D. W. Weiss, ed., Academic Press, Inc., New York, 1971, p. 36) have shown in young Swiss mice that after thymectomy there is an increase in both the spontaneous incidence of lung adenoma and the ease with which it is induced by carcinogens. This effect has been attributed to the impairment of immunocompetence that follows thymectomy and the observation may explain why the incidence of pulmonary adenomas was less in our adult AKR mice than in the young, since it is well known that thymic ablation has much less effect upon immune mechanisms in adult mice than in young ones. These lung tumors were minute and obviously just beginning to develop in many instances. Many would have been overlooked if only gross examinations had been made. In no instance were the tumors large enough to cause morbidity or death, although an occasional one filled a single lobe completely.

Tentatively these observations at two dose rates of continuous radiation exposure suggest that: 1) AKR thymic lymphoma, if it develops during the treatment, is not suppressed by the irradiation, 2) if radiation-induced thymic atrophy develops quickly enough, leukemogenesis is prevented, and 3) radiation-induced thymic atrophy is similar to surgical thymectomy in releasing pulmonary tumor formation from some normally constraining mechanism. The quantitative and qualitative differences in the responses of the two age groups probably reflect the well-known age differences in immunocompetence.

RADIATION IMMUNOLOGY AND MARROW TRANSPLANTATION

ALLOGENEIC MARROW TRANSPLANTATION IN MAN: EVALUATION OF CASE NO. 2 AND NO. 3

In two patients with acute leukemia we attempted therapeutic allogeneic bone marrow grafts using the immunosuppression protocol, described in our 1970

1028133

Medical Division Research Report, featuring high-dose-rate total-body irradiation (TBI) and eight daily infusions of antilymphocyte serum before the transplant (N. Gengozian, C. L. Edwards, Helen Vodopick, and K. F. Hübner).

The second patient of our transplant series was a 53-year-old woman with acute granulocytic leukemia. Following the failure of a chemotherapeutic attempt to achieve a second remission, she was prepared for a bone marrow transplant. The donor was a 50-year-old brother who was HL-A identical and compatible by mixed lymphocyte culture (MLC). In this patient we were unable to give horse antihuman thymocyte globulin, (H-AHTGG) (Upjohn Company, Michigan) because on skin testing she showed immediate hypersensitivity to whole horse serum and horse immune globulin G. Therefore, immunosuppression was started with goat antihuman lymphoblast globulin from the University of Minnesota, Department of Surgery. This antibody, produced against cultured human lymphoblasts, was well tolerated giving no systemic reactions. However, within 2 to 4 days after the first infusion a superficial phlebitis developed in both forearms. After eight daily infusions the patient received TBI, approximately 370 rads (at 40 R/min) from ^{60}Co gamma rays. The TBI was again performed at the facility and with the help of the staff of the UT-AEC Agricultural Research Laboratory. During the exposure there were no noteworthy symptoms and no significant changes in the patient's EKG and pulmonary impedance. The patient vomited, however, 2 hr after the TBI and again several times during the following night.

Within 24 hr after irradiation she received 14.9×10^9 bone marrow cells intravenously without untoward effects. Because of the immunosuppressed state and the increased susceptibility to infections, she was housed in the laminar air-flow unit 1 week before and 8 weeks after the transplant. Continued immunosuppression was maintained with intermittent doses of methotrexate.

The hematological response following the graft indicates a take of the infused allogeneic bone marrow cells (Fig. 21). Evidence for recovery of platelet, white cell, and red cell values is seen within 2 weeks after the procedure. A red cell graft (100% at 8½ weeks) was well documented by a red-cell marker (MN-antigens), and direct chromosome preparations from bone marrow cells showing 49 out of 50 metaphases to be Y bearing on day 20 following the transplant and on day 33, of 56 chromosome sets 54 showed a Y chromosome (data from Dr. L. Gayle Littlefield).

We have no laboratory evidence for a T-cell graft. This could account for a clinical absence of graft-verus-host (GvH) disease that still could have developed even in view of identical HL-A types and a good histocompatibility according to MLC tests. Phytohemagglutinin-stimulated peripheral blood (presumably thymus-derived or T-cells) showed 41 out of 50 metaphases to be of the female type on day 20, and 30 out of 35 were female metaphases on day 35 after the transplant. According to these chromosome analyses an erythrocytic and a granulocytic graft, but not a T-lymphocytic graft, were achieved. However, from observations of the serum allotypes performed before and after the transplant, a partial transient conversion from allotype Gm(+1), Gm(-2), Gm(+12) recipient) to Gm(-1), Gm(-2), Gm(-12) [Donor: Gm(-1), Gm(-2), Gm(+12)] occurred. This indicates a graft of B-lymphocytes, the antibody globulin-producing cells.

The posttransplant immunosuppression with intermittent doses of methotrexate to prevent GvH disease was modified for this patient because of previous

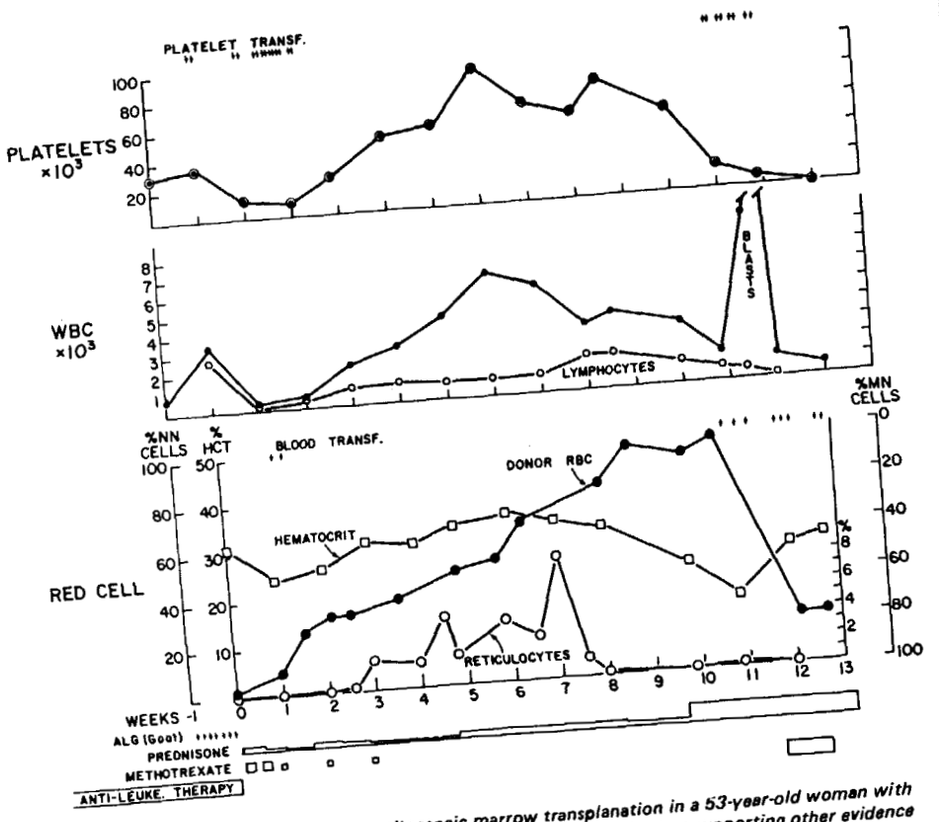


Fig. 21. The hematologic response to allogeneic marrow transplanation in a 53-year-old woman with acute granulocytic leukemia. Note early recovery of blood values supporting other evidence for a take.

liver damage and laboratory evidence of hepatitis during the posttransplant period. The last dose of methotrexate was given 21 days posttransplant. A total of only 140 mg was given and it is doubtful that this or the moderate doses of prednisone could have prevented GvH disease. On the other hand, stopping the methotrexate prematurely on day 21 with the resulting reduction of immunosuppression could have allowed the host to reject the graft. Graft rejection may have occurred at approximately 10 weeks after the transplant when platelet, white cell, and red cell values dropped (Fig. 21) but cytotoxic tests using the patient's lymphocytes and donor-type skin fibroblasts (from the donor) demonstrated no host-versus-graft activity. She developed a prompt relapse of her leukemia, with infiltration of the marrow and leukemic cells circulating in the blood at 11 weeks posttransplant. At the time of her death from leukemia and preumonia, 92 days after the transplant, she had lost the graft and we were preparing to attempt a re-graft.

1028134

The second patient (third in our series) was a 41-year-old man with acute granulocytic leukemia. One year after diagnosis his disease had become resistant to chemotherapy. Histocompatibility testing with his siblings, unfortunately showed only a D match with a brother (Patient: HL-A 3, 5, 22; Donor: HL-A 1, 3 8, 22) and there was bidirectional stimulation in MLC. The combination indicated that the patient was probably homozygous for HL-A 22 and that he had one antigen (5) which was foreign for the donor. But concerning the host-versus-graft situation, two antigens were found in the donor (HL-A 1 and 8) not present in the recipient. A severe GvH disease appeared less likely than rejection and in view of the patient's extremely poor prognosis and the fact that in our two previous transplants we had seen no signs of GvH disease with our immunosuppressive protocol, we felt that an attempt was justified. The patient and family were informed of the probability of early rejection or even nonacceptance of the graft but they were anxious to pursue the trial.

The patient was prepared with H-AHTGG, 500 mg intravenously daily for 8 days, and TBI (500 R, or approximately 370 rads, ^{60}Co gamma rays) was administered 1 day thereafter. This patient reacted with severe chills and fever to almost every dose of the H-AHTGG, despite the concomitant use of aspirin, phenhydramine, heparin, and millipore filtering of horse globulin. However, the TBI was tolerated well, without nausea or vomiting. Likewise, the infusion of 18×10^9 bone marrow cells from his brother was accepted without immediate or delayed difficulties or reactions. He was housed in the laminar air-flow unit and received continued immunosuppression by intermittent doses of methotrexate (total of 195 mg). The last dose of methotrexate was given 60 days after the transplant.

The hematological response of this patient is shown in Fig. 22. In contrast to the course of the patient just described, blood cell values failed to recover and he continued to need platelet and blood transfusions. The reticulocytosis reported during the third week after the transplant was only minimal and the rise of donor-type red cells at that time was only slight and transient. At best a very temporary red cell graft was achieved. There was no other biological marker available to substantiate this assumption and one must conclude that the bone marrow graft failed to take. It is a noteworthy addition to our data base that a GvH disease did not develop in this patient, despite the histoincompatibility. The difference in the HL-A antigens, by preventing implantation of the transfused bone marrow cells or causing very early rejection, may also have prevented GvH disease.

As may be seen in Fig. 22, numerous leukemic cells were in the blood at the time of the procedure. Although an antileukemic effect of the irradiation would be expected, the size of the leukemic cell population had not been adequately diminished by the antileukemic therapy administered before the marrow infusion, and leukemia prevailed through the posttransplant period. A potential graft-versus-leukemia effect can only be expected if the malignant cell pool is at a minimum. We resumed antileukemic chemotherapy in the fifth week posttransplant, which, however, did not appear to change the course of the disease. The patient died of septicemia and persisting leukemia 60 days after the transplant attempt.

In reviewing the three marrow transplant cases we have the following views:

1) The immunosuppressive regimen used in these cases makes it possible to graft

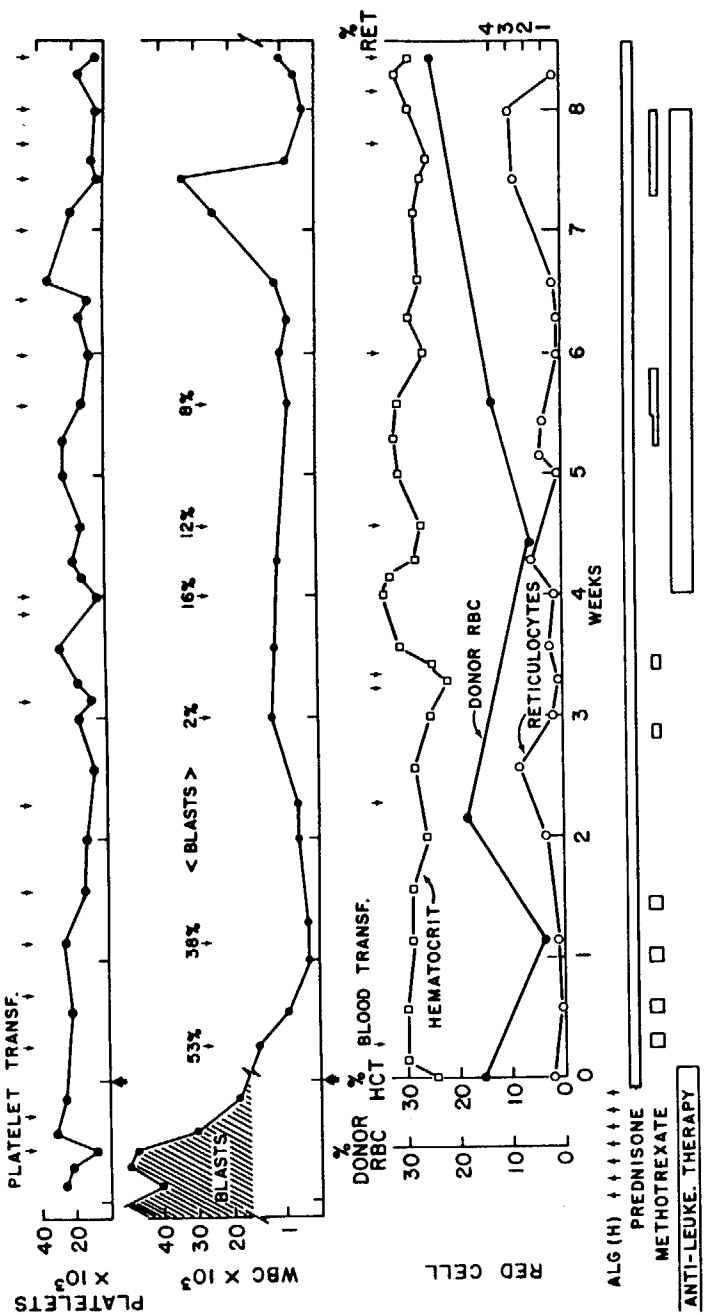


Fig. 22. The hematologic response of a D-match allogeneic marrow transplant attempt in a 41-year-old man with acute granulocytic leukemia. The blood values showed no response to the procedure, and the graft failed to take.

1028135

HL-A compatible allogeneic bone marrow. 2) Severe or fatal GvH disease has not been a major problem. 3) The number of leukemic cells should be reduced to a minimum prior to grafting and the antileukemic effect of the methotrexate immunosuppression may be an important factor in the management of these patients. 4) Further, we believe increased immunosuppression by a higher irradiation dose might assure better, more complete grafts while exerting an additional needed antileukemic effect. 5) The entire procedure, including the anti-lymphocyte globulin, the radiation, and infusion of marrow cells, is well tolerated, does not materially increase the patient's morbidity, and may at least result in another hematological remission for these patients.

RECOVERY OF THE IMMUNORESPONSE OF MICE AFTER CHRONIC EXPOSURE TO LOW-DOSE-RATE GAMMA (COBALT-60) IRRADIATION

We have previously shown that chronic irradiation of mice at rates of 1.6 R/hr will lead to profound immunosuppression at total accumulated exposures of 2,819 R and 2,138 R respectively. With the hemolysis-in-agar technique, measuring an immune response at the cullular level, we could demonstrate that the immunosuppressive effect is dose dependent and that differences in the dose rates can be detected even at these low dose rates. We also showed that immunosuppressive effects could be more easily detected with the hemolysis-in-agar technique than by determining circulating antibodies. Definite reduction of the number of plaque-forming cells (PFC) was seen after exposure of 1,005 R at 1.6 R/hr, which reduced the number of PFC per spleen from approximately 400,000 to roughly 60,000. At the higher exposure rate, 6 R/hr, suppression of PFC was obtained after a total of only 468 R. A total of 2,138 R at 6 R/hr reduced the number of PFC per spleen from roughly 400,000 to 12.5 PFC per spleen.

Since chronic irradiation at approximately these dose rates is used as a therapeutic measure for some of the patients at the ORAU Medical Division, we felt it important to determine whether spontaneous recovery of the immune response is possible after severe immunosuppression induced by chronic irradiation (K. F. Hübner, Thomas A. Gilman, and N. Gengozian).

For these recovery studies mice were exposed to chronic irradiation in the low-exposure-rate total-body irradiator (LETBI) (^{60}Co gamma rays) at a rate of 1.6 R/hr (36 R/day) or 6 R/hr (144 R/day). In the high-dose-rate group, animals were continuously irradiated until they had accumulated total exposures of 1,005 R, 1579 R, and 2,138 R respectively. In the low-dose-rate study only one group was studied to determine their recovery potential. In that group all mice were exposed to a total dose of 5,256 R.

When the desired irradiation dose was completed, the mice were allowed to "recover" for 6, 13, 20, 28, 30, or 60 days. After the chosen recovery times the animals were immunized with sheep red blood cells (SRBC) and 4 days later the PFC response of their spleens was determined. Figure 23 shows the recovery of the PFC response from different accumulated total doses. It is remarkable that after a total-body exposure to 2,138 R (curve A), spontaneous recovery is possible and fairly rapid. After a 28-day period, 265 PFC were found in the spleens of these animals, and at 123 days there were 34,355 PFC per spleen (latter data not shown in this report). It is interesting to note that recovery from the total exposure of 1,579 R (curve B) was apparently starting later and only after 28 days does curve

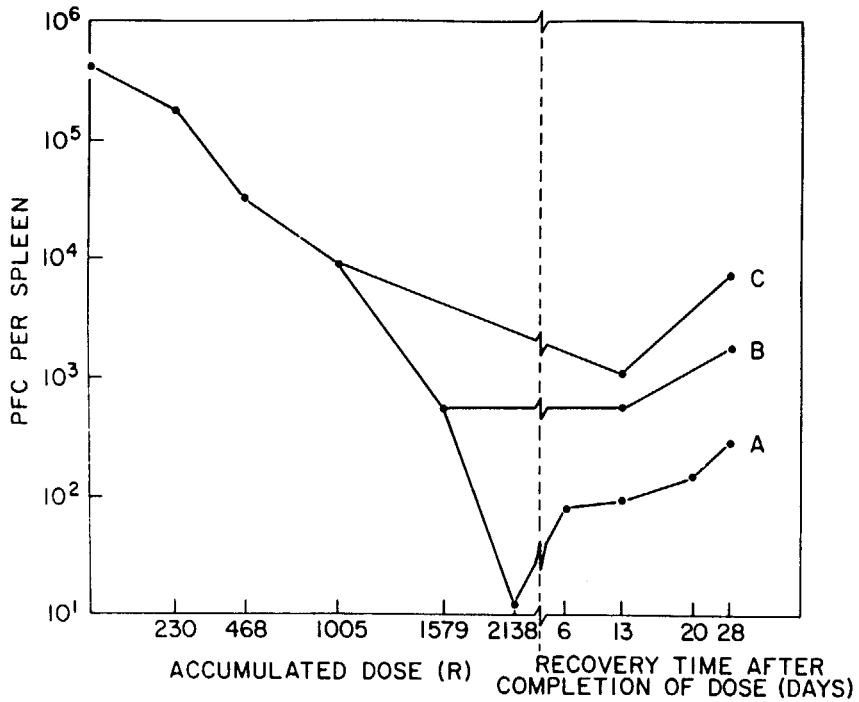


Fig. 23. Day 4—PFC response to SRBC after continuous irradiation (6 R/hr) and recovery from total exposures of 2,138 R (curve A), 1,579 R (curve B), and 1,005 R (curve C).

B approximate the slope of curve A. After a total of 1,005 R, the recovery was not only delayed, but 13 days after discontinuation of the irradiation, the PFC response was lower than immediately after completion of the dose. Again, however, after day 13 the PFC response recovered fairly rapidly. These observations suggest that extreme damage to the immune system is followed by a relatively faster recovery than occurs after less severe damage.

Figure 24 shows the recovery potential in mice exposed to 5,256 R at 1.6 R/hr. The maximal depression of the PFC response was not determined for these animals; we assume that such an exposure would be comparable in its immunosuppresive effect to a total dose of 2,138 R at 6 R/hr. As shown in Fig. 24, the recovery after 5,256 R was also spontaneous and quite rapid and after 30 days approximately 1,000 PFC were found in the spleen of such animals.The survival and recovery potential of these mice after 5,000 R is remarkable. Indeed, mortality data, not discussed in this report, showed that approximately 25% of these mice can survive a total of 7,800 R exposure at 1.6 R/hr.

We have no data that would explain the recovery patterns observed. One might assume that slow-dose-rate irradiation permits regenerative cell proliferation and repair. Under the conditions we have described this cannot be the case because the response to SRBC continues to decline as the irradiation is continued

1028136

and eventually the response approaches zero. Nevertheless, recovery begins promptly when irradiation is stopped; therefore concomitant repair must occur. At least the precursors of antigen processing and antibody-forming cells are being recruited even after accumulated doses so large that only a minimal response can be obtained when the antigen is given without a recovery period.

On the other hand, the recovery of the immune response occurs without delay and relatively faster after the highest accumulated dose. After 2,138 R at 6 R/hr the number of PFC increased tenfold within a 28-day recovery, whereas after 1,579 R the number of PFC increased only five times during the same period (Fig. 23). After 1,005 R, recovery was even slower and at 28 days the PFC response was below the immediate posttreatment level. This indicates that the immunosuppressive effects of irradiation can continue briefly after irradiation is stopped and that with increased irradiation, repair mechanisms are more and more stimulated, resulting in a relative fast recovery.

In the mouse the spleen is not only an important part of the immune system, but also a hematopoietic organ. After seeing the recovery capacity of splenic immunocytes subsequent to these chronic irradiation exposures, we examined the same spleens for their hematological potential. Animals were irradiated

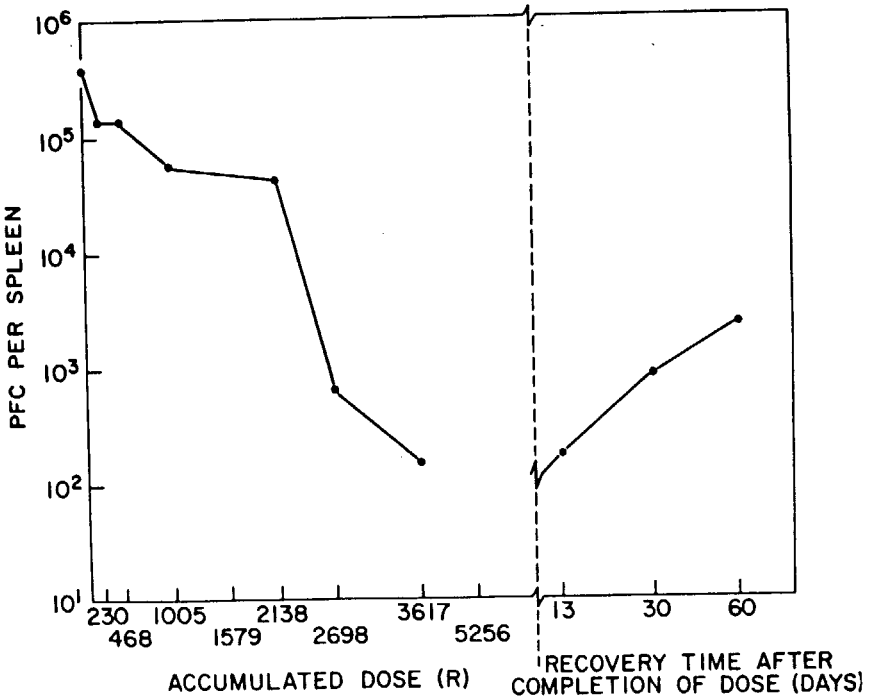


Fig. 24. Day 4—PFC response to SRBC after continuous irradiation (1.6 R/hr) and recovery after total exposure to 5,256 R.

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the PFC response of chimeric cells (Table 17). However, cells from normal *donors* and the parental *DBA/2* strain of $CD2F_1$ donors, were ineffective; bone marrow cells from any source also lacked any restorative effects.

TABLE 16

ANTIBODY RESPONSE BY MIXTURES OF ADHERENT AND NONADHERENT CELLS FROM ALLOGENEIC RADIATION CHIMERAS AND NORMAL ANIMALS AGAINST SRBC IN VITRO*

Number of nonadherent cells x 10^6	PFC/10^6 culture cells						
	CAd + CN	CAd + HN	CAd + DN	HAd + CN	DAd + CN	HAd + HN	DAd + DN
2.5	79	508	2432	98	40	725	892
10.0	50	---	---	114	23	707	1372

* Symbol abbreviations:
C = Chimera ($CD2F_1 \rightarrow C3BF_1$)
H = Normal hosts ($C3BF_1$)
D = Normal donor ($CD2F_1$)
Ad = Adherent cells
N = Nonadherent cells.

TABLE 17

THE EFFECT OF THYMUS OR BONE MARROW CELLS FROM NORMAL HOSTS, DONORS, VARIOUS STRAINS OF MICE, AND RATS ON THE IN VITRO ANTIBODY RESPONSE OF $CD2F_1 \rightarrow C3BF_1$ CHIMERIC SPLEEN OR NONADHERENT CELLS* TO SRBC†

Exp.	PFC/10^6 culture cells of chimeric cells cultured with SRBC and:											
	None	Host T	Donor T	C3H T	BALB/c T	DBA/2 T	CBA T	Rat T	Host BM	Donor BM	C3H BM	BALB/c BM
1	1	133	---	628	318	---	---	59	9	4	---	---
2	31	658	---	570	106	29	---	---	50	28	---	21
3	35	296	19	1957	---	---	---	---	---	---	---	---
4	18	191	15	1672	410	26	390	174	10	3	6	1
5	45	240	15	---	673	16	677	---	---		---	9
6	20	317	17	---	86	20	---	---	---		---	---

* The nonadherent cells were cultured with adherent cells from $CD2F_1 \rightarrow C3BF_1$ chimeras, normal hosts, or donors.
† Symbol abbreviations:
T = Thymic cells
BM = Bone marrow cells.

In further experiments we studied the ability of thymic cells from $CD2F_1 \rightarrow C3BF_1$ chimeras and the reciprocal combination, i.e., $C3BF_1 \rightarrow CD2F$, to improve the PFC response of nonadherent cells from these two allogeneic combinations. Table 18 shows that thymic cells of chimeras were unable to improve the PFC response of either combination while normal, host thymus again was effective and normal, donor thymus was not.

1028138

TABLE 18

THE EFFECT OF THYMIC CELLS FROM ALLOGENEIC CHIMERAS, NORMAL HOSTS, OR DONORS ON THE IN VITRO ANTIBODY RESPONSE OF CHIMERIC NONADHERENT CELLS TO SRBC

Chimera	PFC/10^6 culture cells of chimeric adherent and nonadherent cells cultured with SRBC and:				
		Thymic cells of:			
	None	$CD2F_1 \rightarrow C3BF_1$	$C3BF_1 \rightarrow CD2F_1$	$C3BF_1$	$CD2F_1$
$CD2F_1 \rightarrow C3BF_1$	56	89	---	240	17
$CD2F_1 \rightarrow C3BF_1$	19	8	---	---	---
$CD2F_1 \rightarrow C3BF_1$	13	1	3	191	15
$C3BF_1 \rightarrow CD2F_1$	23	---	16	1	102
$C3BF_1 \rightarrow CD2F_1$	12	4	0	---	---

The subnormal immune response in allogeneic chimeras is primarily due to a defective T-cell population. The A cells behave normally in the three cell-type interactions required for the immune response to SRBC, and B cells are present and functional in the spleen. The effectiveness of genetically incompatible (to donor-type chimeric cells) thymic cells suggests that an allogeneic effect is operating in reconstituting immunocompetence in chimeras. Normal donor cells being of the same genetic specificity as the chimeric cells may become involved in maintaining unresponsiveness of donor-type chimeric cells against host antigens and thus would fail to restore the response of the chimeric cells against SRBC. Failure by the genetically incompatible parental DBA/2 (donor) cells in $CD2F_1 \rightarrow C3BF_1$ chimeras may involve genetic factors other than the established H-2 alleles that play a significant role in the recognition mechanism between host and donor. At any rate, the contrasting influences of normal host, donor, and parental (donor) thymus (T) cells in reconstituting the immune response of chimeras deficient to a third party antigen supports our contention that tolerance as applied to radiation chimeras is not complete (Gengozian, N. et al., Science 149, 645, 1965). Thus, tolerance in chimeras apparently involves a unique association among immunocompetent cells which, until recently, has been ignored or unrecognized.

STAFF MEMBER ON SABBATICAL LEAVE STUDIES HEMOPOIETIC RECOVERY IN LETHALLY IRRADIATED DOGS

Beginning June 1972 Dr. B. Nelson, ORAU pathologist, is spending a sabbatical year as guest professor with Dr. T. M. Fliedner at the University of Ulm, Germany. Dr. Fliedner's group is using a canine model to demonstrate the feasibility of restoring hemopoiesis and lymphocytopoiesis after otherwise lethal irradiation by giving allogeneic mononuclear blood cells. Dr. Nelson's major activity is in the autopsy and histopathologic study of the irradiated beagles, with particular interest in evaluating the repopulation of lymph nodes, spleen,

tonsil, and intestinal lymphoid tissue, and in early GvH reactions. In connection with the project he has visited Dr. Hans Cottier and colleagues at the University of Bern, Switzerland, and plans trips to other centers working on problems of marrow transplantation lymphocytopoiesis and GvH reactions.

The beagle was chosen for this work because the techniques used for collecting sufficient numbers of mononuclear cells from the blood of such a large animal, using the IBM centrifuge, are applicable to humans, and could obviate procuring large amounts of marrow from suitable donors to treat accidentally irradiated persons, or other patients needing hemopoietic tissue. In addition, the dogs lend themselves to individualized care comparable to that required for humans in the critical postirradiation period, with careful management of antibioltics and administered fluids and electrolytes. Records of breeding and large litters, with techniques for tissue typing and matching, allow correlations of genetic differences with compatibility of the grafts. The study of the prophylaxis and treatment of the GvH reactions in the canine model provides histopathologic examples that contribute to the understanding of similar processes in the human.

EXPERIMENTAL HEMATOLOGY

STUDIES ON CHALONE

In support of the humoral control of granulopoiesis, two regulatory substances called chalone and antichalone have been isolated and tested by others. Rytömaa (Cell Tissue Kinet. 1, 329, 1968) proposed specific criteria for chalone: 1) inhibitor of cell mitosis, 2) tissue specific but not species specific, 3) reversible action, and 4) no cytotoxicity. Antichalone, a stimulator of cell production, reverses or negates the action of chalone. Supposedly chalone is produced by the tissue whose production it regulates, while antichalone of unknown source is found in serum.

In our search for granulocytic chalone (Helen Vodopick and Elizabeth Rupp), we have initially used the rat as an animal model. In our assay system, rat marrow cell suspension is used as a target tissue. The incorporation of tritiated thymidine into cells synthesizing DNA in short-term culture is measured by liquid scintillation counting and autoradiography. A cell count of the marrow suspension, pre- and postincubation, is necessary to show that cytotoxicity is absent.

Rat granulocytes harvested from peritoneal exudate produced by intraperitoneal injection of sterile polyvinylpyrrolidone served as a source of chalone. Washed granulocytes, free of red blood cell and lymphocyte contamination, were incubated in Hanks buffered salt solution at 37°C for 1 hr. The supernatant cell-free liquid was fractionated on Sephadex G-25. Immediately after collection the fractions were lyophilized to both concentrate and preserve the active component. Each of these 10 to 15 fractions was individually assayed as previously described. Fractions having inhibitory properties caused a 40 to 60% decreased incorporation of tritiated thymidine into marrow precursors with no evidence of cellular damage when compared to control samples (Fig. 26). Total cell counts in the pre- and postincubation samples remained unchanged. The

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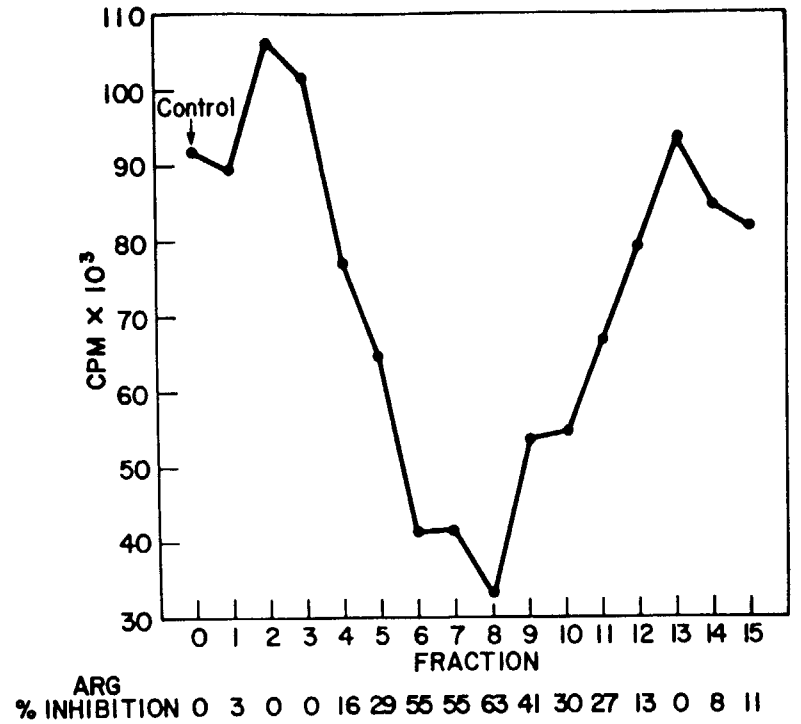


Fig. 26. The numbers on the abscissa represent the sequential fractions obtained from a single sample separated by gel filtration. Each fraction was tested individually for its ability to affect the incorporation of tritiated thymidine into the in vitro cultured rat marrow cells. Radioassay was expressed as cpm on the ordinate. Fraction 8 produced greatest inhibition while increased incorporation over control counts occurred when fraction 2 was used. The autoradiographic (ARG) data tabulated below the fraction number correlated well with the other bioassay procedure.

same inhibitory fractions produced a 40 to 60% decrease of labeled granulocytes as seen on autoradiography.

Slightly more tritiated thymidine (10 to 12% over control values) was incorporated by rat marrow cells when fraction 2 was used. Specific assay for antichalone has not been performed as yet.

We have been able to demonstrate that rat granulocytes produce a substance which reduces or retards incorporation of tritiated thymidine into rat granulocyte precursors. In previous investigations utilization of tritiated thymidine by precursor cells has been equated with cellular DNA synthesis, a step necessary prior to mitosis. Therefore in the present experiments the reduced utilization of tritiated thymidine would suggest that DNA synthesis has been impaired. Although this action cannot be conclusively attributed to chalone, the results thus far are compatible with its presence.

RESEARCH IN NUCLEAR MEDICINE

Our mission is to advance the diagnostic and therapeutic applications of radionuclides. The primary current emphasis is placed on radioactive tumor-localizing agents, exploiting the special resources in Oak Ridge for isotope production and for instrument development.

SUMMARY OF ACCOMPLISHMENTS

By special cascade zonal centrifugation on animal tumors and tissues, we have advanced our understanding of the nature of subcellular binding sites of ^{67}Ga in tissue. Binding is surprisingly fast to subcellular organelles, occurring mainly within an hour of injection into animals; data suggest it is an active metabolic process rather than passive transfer. In the time-course studies we find ^{67}Ga early is bound to much smaller intracellular particles (endoplasmic reticular vesicles?), probably precursors of the larger gallium-binding granules. We have continued work on the nature of the gallium-binding molecules and find by gel filtration that the major fractions have molecular weights of approximately 1×10^5 and 5×10^4; they are probably protein, although initial attempts at enzymatic hydrolysis have been only partly successful. We have also found that ^{111}In apparently binds to the same type of subcellular organelles as ^{67}Ga and appears in fractions of similar molecular weight.

Preliminary trials in humans, seeking to show whether ^{67}Ga uptake is enhanced when stable scandium was administered, revealed an unexpected hemolysis after small doses of scandium. We therefore have postponed further clinical trials pending additional animal experiments. In vitro studies with human red blood cells (RBC) show hemolysis, not seen with dog RBC (and not seen in vivo after high doses given to rabbits and rats). Furthermore human RBC bind scandium in vitro to a remarkable degree manyfold more than RBC of the rabbit, rat, dog, or marmoset. Whole-body retention of ^{46}Sc with and without stable carrier has revealed a longer third-term compartment for animals receiving stable scandium.

In other tumor-scanning developments, trials with ^{14}C-labeled cycloleucine in rat hepatomas have confirmed the findings of Sterling that maximum tumor incorporation occures at 30 min with tumor-to-nontumor ratios of approximately 8. These results support the feasibility of our proposal to use ^{11}C with its 20-min half-life as a tag for this amino-acid analog.

Preliminary progress is reported on methodology for preparation and quality control of ^{35}S-labeled WR-2721, a promising radioprotective agent. This ground work is essential before planned human trials can be initiated.

1028140

In the work of the Cooperative Group to Study Localization of Radiopharmaceuticals significant progress in ^{67}Ga data collection and encoding has been accomplished. More than 1100 scan studies have been accessioned, including approximately 600 previously untreated cases. Programs have been written for frequency tables permitting analysis of computer printouts for a number of variables including clinical status, morphology of the tumor, anatomic site, size of lesion, scan interpretation, other evidence of tumor, and so on. Subcommittees have initiated analyses of results in lung cancer, lymphoma, and Hodgkin's disease. In the course of the work sessions two scan-reading exercises have been conducted to measure variability in skilled, experienced observers viewing the same scan. An attempt has been made to evaluate the scope and nature of these significant differences.

In instrumentation and computer activities our instrumentation staff and collaborating clinicians have tested our computer-interfaced four-probe counter in patients receiving radioactive colloids. It is possible to simultaneously record the radioactivity levels with time in four organs. Progress has also been made in the complex chore of constructing an instrument system for transmitting the counting-rate data from our dual-head whole-body scanner to our computer. Our design emphasizes flexibility to permit operation with most on-line computers. A new, reliable, accurate, and sensitive whole-body counter for measuring therapeutic and diagnostic levels of radioactivity has been designed and field tested. The instrument has value in our retention studies on radiopharmaceuticals and in answering dosimetry questions.

RADIOPHARMACEUTICAL DEVELOPMENT

STUDIES OF THE SUBCELLULAR DISTRIBUTION OF ^{67}Ga

Zonal Centrifugal Studies. Studies to determine the nature of the subcellular binding sites of ^{67}Ga are still in progress (David H. Brown, B. L. Byrd, J. J. Rafter, J. E. Carlton, and R. L. Hayes). In our earlier work in which we isolated gallium-binding granules (GBG) from the tumor and liver of the same animal, the absolute uptake of ^{67}Ga by tumor tissue was over twice that of the liver; however, it was not apparent that the distribution of ^{67}Ga related to size or type of particles isolated, i. e., the liver and tumor had similar ^{67}Ga profiles when their subcellular particles were fractionated by our six-stage, sequential-product-recovery (SPR) zonal-centrifugation procedure (23). Recently after modifying the SPR procedure, we have found another class of particles that binds much more ^{67}Ga (~ 50%) than the GBG particles previously isolated (~ 30%). The banded in a 45% sucrose cushion outboard of a 20 to 30% sucrose gradient after a centrifugal integral of $4 \times 10^{10}\omega^2t$ indicating that they are considerably smaller than those GBG isolated by our previous procedure ($8 \times 10^9\omega^2t$). On an absolute basis we also found much more activity in the $4 \times 10^{10}\omega^2t$ fractions isolated from hepatomas than in those isolated from normal liver. The fractions from the $4 \times 10^{10}\omega^2t$ particle zone have a

relatively high specific activity for acid phosphatase (a lysosomal enzyme), but we have not yet determined the relationship between the bound ^{67}Ga and the enzyme activity in these fractions. The particles in the zone may be heterogenous, and consequently further purification will be necessary to determine the nature of the particles binding ^{67}Ga.

As a result of these experiments the isolation of GBG has evolved into a four-stage procedure which gives near quantitative recovery of the ^{67}Ga present in the intact tissue. In the first stage the nuclei are removed by zonal centrifugation of homogenate in a Sorvall SZ-14 zonal rotor. The supernatant from the first stage (containing over 92% of the ^{67}Ga present in the homogenate) is then fractionated by our new SPR centrifugation procedure in a B-XXIX zonal rotor. A comparison of the ^{67}Ga profiles of Buffalo rat liver and Morris 5123C hepatoma obtained with this new SPR procedure is shown in Fig. 27. Note that the percentage of ^{67}Ga activity contained in Stages III ($8\times10^9\ \omega^2t$) and IVp ($4\times10^{10}\ \omega^2t$) were obstensibly reversed for the liver and tumor.

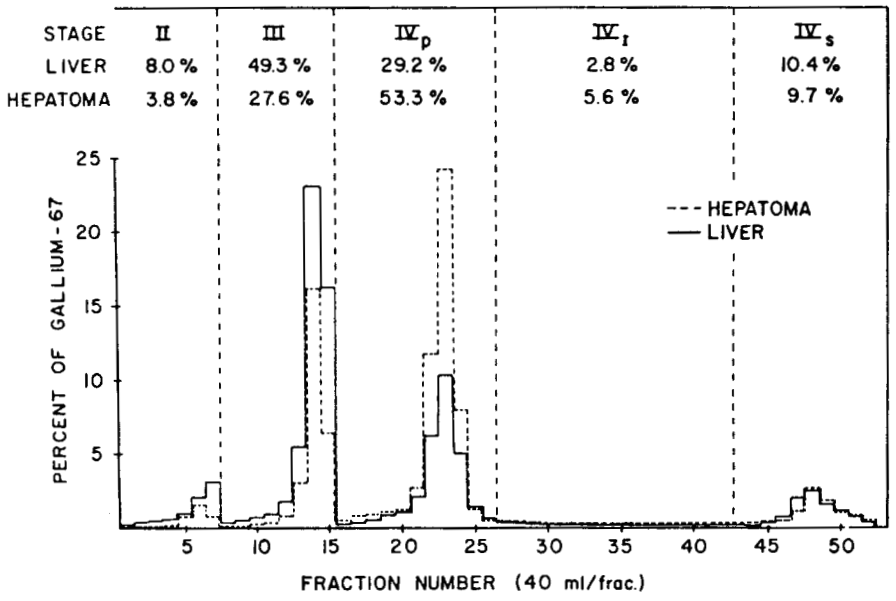


Fig. 27. Gallium-67 distribution (24 hr) in particle profiles of homogenates of Buffalo rat liver and Morris 5123C hepatoma. These were fractionated by rate-zonal density-gradient centrifugation in a three-stage separation after the initial removal of cell debris and nuclei. The particles in Stages II, III, and IV shown in the figure were isolated in a B-XXXIX zonal rotor. The data indicate that hepatoma homogenates contain fewer large (Stage III) and proportionately more smaller GBG (Stage IV$_p$) than does normal liver. Stage III particles are lysosomal; Stage IV$_p$ particles are suspected to be precursors of lysosomes although sufficient evidence has not yet been obtained to confirm this hypothesis. The origin of the Stage IV$_p$ particles is not known.

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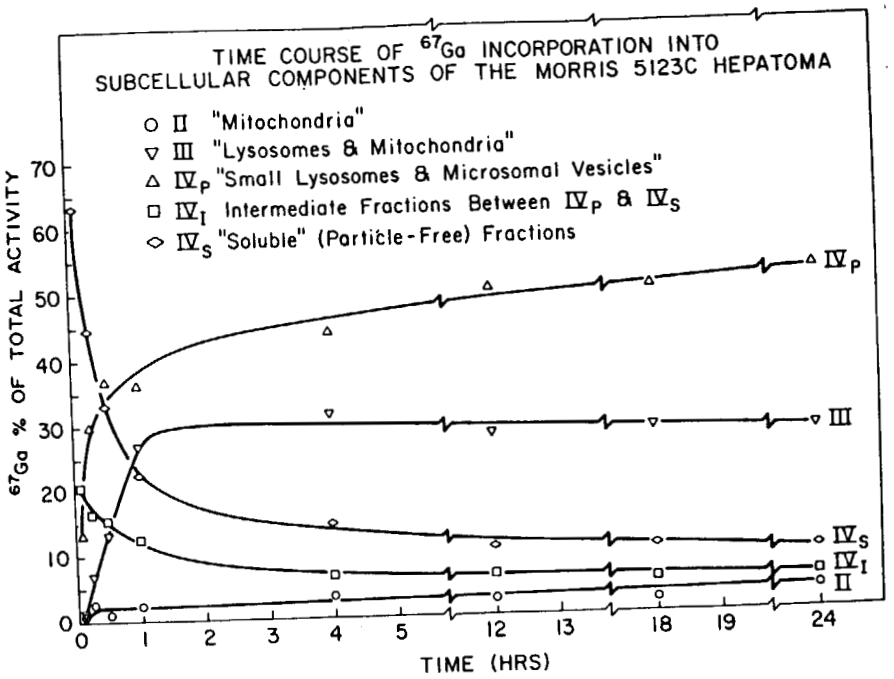


Fig. 28. Distribution of ^{67}Ga in zonal-centrifuge fractions of tissue exercised from rats at various times after ^{67}Ga administration, homogenized, and then fractionated by zonal centrifugation as described in the previous figure. Fractions at each stage of the centrifugation procedure, indicated by Roman numerals, are zones in the gradient recovered after various intervals of centrifugation. This series of experiments was designed to follow the path of ^{67}Ga uptake in cells in vivo. Since very little ^{67}Ga is bound by the nuclei (Stage I particles) at 24 hr postinjection, these data are not shown in the figure. The most striking phenomenon observed is the rapidity with which the cell organelles take up ^{67}Ga, particularly those of the tumor cells. The most striking difference between these two tissues is in the rate of disappearance of the radionuclide from the cell sap (Stage IVs) and its complimentary rate of appearance in the Stage IVp particles. Not only does hepatoma tissue bind more ^{67}Ga than normal liver, but ^{67}Ga is also bound predominantly in populations of small organelles.

In spite of the fact that the nature of the $4 \times 10^{10}\omega^2t$ ^{67}Ga-binding particles (Stage IVp) has not been resolved, we have proceeded with this cascade centrifugation approach to determine the association of ^{67}Ga with various subcellular fractions as a function of time. Morris 5123C hepatomas and normal Buffalo rat liver are under investigation. At each period we determined the percent of ^{67}Ga in: the nuclear fraction (Stage 1), the mitochondrial and large lysosome fraction (Stage II), the lysosome and/or GBG fraction (Stage III), the fraction containing $4 \times 10^{10}\omega^2t$ particles (Stage IVp), the zone between the Stage IVp particles and the "soluble" zone (Stage IV$_I$), and the "soluble" zone itself (Stage IVs). A plot of some of these data is shown in Fig. 28.

The most surprising observation was the rapidity with which ^{67}Ga was bound to subcellular organelles. In the tumor, Stage III GBG ($8 \times 10^9\omega^2t$) were

labeled almost to their maximum (29% of the ^{67}Ga present) within an hour after injection. The 4 x $10^{10}\omega^2$t Stage IVp particles were in turn labeled with 33% of the activity but continued to increase in amount of label for 24 hr to over 50% of the ^{67}Ga present. Also the rates of ^{67}Ga uptake of the liver and tumor in the Stage IVp organelles differed strikingly. Within 15 min the Stage IVp 4 x $10^{10}\omega^2$t particles in the tumor cells had bound 30% of the ^{67}Ga while the comparable liver organelles contained but 12% of the activity (Table 19). The differences are essentially reflected in the levels of ^{67}Ga in Stage IVs of the two cell types. (The Stage III GBG at 15 min for both the tumor and liver were still low and equal in activity.)

TABLE 19

COMPARISON OF SUBCELLULAR DISTRIBUTION OF ^{67}Ga IN MORRIS 5123C HEPATOMA AND BUFFALO RAT LIVER

Time	Percent ^{67}Ga in different SPR zonal stages*									
	II		III		IV_p		IV_I		IV_s	
	Tumor	*Liver*	*Tumor*	*Liver*	*Tumor*	*Liver*	*Tumor*	*Liver*	*Tumor*	*Liver*
15 min	2.7	5.6	6.9	5.8	30.0	11.9	16.6	11.9	43.8	64.9
60 min	5.8	8.3	27.3	21.9	32.8	18.3	6.5	9.5	28.5	41.6
24 hr	3.8	8.3	28.2	49.2	52.7	28.7	5.6	3.9	9.7	9.8

* See text and Fig. 2 for explanation of stages.

These data suggest to us that the binding of ^{67}Ga involves a metabolic process and is not the result of a passive transfer of ^{67}Ga into the organelles. We gained further evidence for this conclusion by "spiking" unlabeled tumor homogenates with ^{67}Ga just before fractionating by SPR centrifugation. We found that 96% of the ^{67}Ga remained "behind" in the supernatant fractions (Stage IVs), indicating that little binding or uptake by simply diffusion had occurred in the 8 × $10^9\omega^2$t or 4 × $10^{10}\omega^2$t particles during the process.

In an attempt to determine the nature of the Stage IV_p particles, we performed the isopycnic-zonal centrifugation procedure of Lee et al. (Biochem. Biophys. Res. Commun. 36, 748, 1969) which is designed to separate smooth- (SER) from rough-endoplasmic reticulum (RER). We used this technique because particles with a density of 1.18 band in a zone just inboard of the RER but are separated from the less dense SER particles. From liver homogenates they have been characterized enzymatically as lysosomes. Since the much larger Stage III GBG have been characterized as lysosomes, we felt that the ^{67}Ga in the Stage IVp zone might be associated with very small lysosomal-like particles. However, when Lee's ER separation was performed on tumor tissue, the RER zone contained ^{67}Ga and only a very small 260 nm OD peak appeared in the lysosomal zone indistinguishable from the larger RER peak with respect to ^{67}Ga activity. There were incidentally very few particles in those fractions where the SER would normally be observed. The latter results were confirmed by Dr. Lee who has examined a number of different types of tumors and in each instance

1028142

observed that this zonal procedure did not separate RER from SER as it does with liver; indeed, it is doubtful that the SER exist in the tumors examined.

Our molecular anatomy studies have revealed that the amount of ^{67}Ga taken up and bound in hepatoma cells is at least twice that in normal liver cells on an absolute basis, agreeing well with the earlier work on tissue distribution. Further, we have identified the GBG as lysosomes on the basis of their morphology and by their content of lysosomal-specific hydrolytic enzymes. Most recently we have discovered by time-course studies that ^{67}Ga is also bound to much smaller intracellular particles, probably precursors of the larger GBG. However, the true nature of these particles is yet to be determined. Since they appear in the subcellular particle size-range characteristic of endoplasmic reticulum vesicles, and, since ER vesicles probably constitute a variety of organelles (e.g., Golgi, different ER types, and plasma membrane vesicles), it will be necessary to devise means for identifying the class of particles to which they belong.

Characterization of Macromolecular Agent(s) Binding ^{67}Ga. In keeping with the zonal-centrifugation studies, when cold 0.25 M sucrose homogenates of transplanted tumor tissues (2 days after intravenous ^{67}Ga) are centrifuged for 6 x 10^6 g-min, 79 to 95% of the ^{67}Ga activity present in the homogenates is spun down. Previously we showed that if various membrane disruptive treatments are employed (e.g., distilled water) 64 to 88% of the ^{67}Ga activity present in the homogenates is then found in the supernatants after centrifugation. The majority of the ^{67}Ga activity in the supernatants after such treatments is in a bound macromolecular form, since less than 15% can be removed by dialysis or ultrafiltration.

The gallium-macromolecular complex in tissue extracts is decidedly affected by heat and hence studies of its nature must be carried out in the cold. It is also quite sensitive to increases in pH above 7.0. In studies with aqueous extracts of transplanted tumors, over 50% of the ^{67}Ga binding capacity was lost on standing 24 hr at 5°C at pH 9.0; maximum stability occurred at pH ~ 6.0 (~ 5% loss of ^{67}Ga during 24 hr). However, a portion of this apparent stability may be due to the nonfilterability of ^{67}Ga itself (possible polymer formation) although this does not appear to be of major importance, i.e., at pH 6.0 only 60% of $^{67}GaCl_3$ was filterable, but at pH 7.0, where the tumor extracts lost ~10% of their activity, 90% was filterable. In view of these results, the reported existence of lower pH values in tumor tissues may have some bearing on the affinity of tumor tissue for ^{67}Ga.

Approximately 85% of the ^{67}Ga activity in these aqueous tumor extracts is precipitated with proteins in the cold by ammonium sulfate. When the precipitate was redissolved in water, very little ^{67}Ga activity was found to be dialyzable, indicating that this treatment did not change the essential nature of the gallium-macromolecular complex. Rivanol (6, 9-diamino-2-ethoxyacridine lactate), another protein precipitant, carried down ~65% of the ^{67}Ga activity at pH 8.5. Rivanol is most effective as a precipitant at pH 8.5, and the instability of the complex at this pH may account for the lower percentage precipitation.

Our initial attempts at slab-gel electrophoresis (R. L. Hayes and J. E. Carlton) of the gallium-macromolecular complex present in aqueous extracts of tumor were unsuccessful (using a pH 9.0 buffer); the majority of the ^{67}Ga activity migrated far ahead of the protein pattern, undoubtedly due to decomposition of the complex because of the alkalinity of the buffer system. Recently other

attempts at electrophoresis using chilled (5°C) pH 6.5 Mes buffer with a cellulose acetate support (Beckman Microzone) have been successful. Figure 29 shows these results. The effects of administered stable gallium on the ^{67}Ga electrophoretic migration, also shown in Fig. 29, will be discussed in the following section on gel filtration.

EXPERIMENT	PERCENT ^{67}GA IN VARIOUS SEGMENTS										
GALLIUM-67 CHLORIDE	0.03	15.8	5.2	5.6	6.0	11.6	41.9	13.3	0.5	0.1	0.0
TUMOR, CARRIER-FREE	0.30	2.0	14.0	19.0	13.2	47.3	2.8	1.0	1.0	0.3	0.0
TUMOR, 0.1 mg Ga/kg	0.80	14.9	17.7	36.5	25.6	3.1	0.6	0.4	0.2	0.1	0.1
TUMOR, 1.0 mg Ga/kg	0.80	16.4	25.1	44.9	7.9	2.6	1.1	0.5	0.1	0.1	0.2

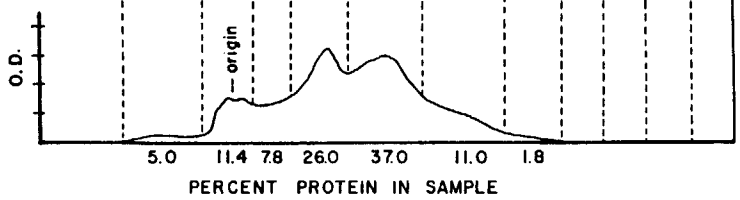


Fig. 29. Electrophoretic pattern and ^{67}Ga content of aqueous extracts of RFT tumors 2 days after administration. Note the effect produced on the electrophoretic pattern by the administration of stable gallium along with ^{67}Ga. The percent ^{67}Ga present in various segments of the electrophoretic pattern is shown in the table for a rat given carrier-free (C.F.) ^{67}Ga, another given 0.1 mg stable Ga/kg, and a third given 1.0 mg stable Ga/kg. Also included is the migration pattern of $^{67}GaCl_3$ alone. (All in Mes buffer, pH 6.5.)

In our initial gel-filtration experiments with tumor extracts, we arbitrarily chose a 0.1 M pH 7.0 phosphate buffer. Later from other experiments on the binding of ^{67}Ga by serum proteins, where the binding is much weaker, we concluded that phosphate interacts with gallium, probably to form a complex anion. It interferes strongly with the binding of ^{67}Ga by plasma proteins and to some extent with the macromolecular ^{67}Ga complex in tumor extracts. An attempt to use Mes at pH 6.3 resulted in an unexplained severely delayed elution of $^{67}GaCl_3$ from the column. We accordingly changed to a 0.1 M Tris-HCl buffer at pH 7.0 and have obtained similar patterns but with a greatly decreased elution in the region of the bed volume. Examples of our recent gel-filtration results with tumor and normal tissues are shown in Fig. 30 and 31. In three different tumor types the ^{67}Ga profiles were similar, with the majority of the ^{67}Ga activity in the tumor extracts (~60%) occurring in two major fractions having approximately molecular weights (if globular) of $1.2\text{-}1.0 \times 10^5$ and $5\text{-}4 \times 10^4$ (Fig. 30). Furthermore, the ^{67}Ga gel-filtration profiles of livers from three normal host animals (Fig. 31) as well as of the spleen from a Fischer 344 rat showed a remarkably lower percentage of ^{67}Ga activity in the region of the $5\text{-}4 \times 10^4$ molecular weight fraction. Table 20 contains a comparison of the percentages of ^{67}Ga found in various regions of the filtration profiles for tumors and normal tissues. Note the high

1028143

percentage of ^{67}Ga in the bed-volume regions for the livers and spleen. Also included are ^{67}Ga percentages for gel-filtration profiles of the tumor extracts used in the electrophoretic studies shown in Fig. 29 where different amounts of stable gallium were administered along with ^{67}Ga. In the ^{67}Ga electrophoretic patterns shown in Fig. 29 it is clear that giving stable gallium with ^{67}Ga decidedly decreases the migration of ^{67}Ga activity. Interestingly enough this was reflected in the gel-filtration profiles by a progressive increase in the percentage of ^{67}Ga activity in the void-volume and bed-volume regions with a simultaneous striking decrease in the percentage of ^{67}Ga in the $1.2\text{-}1.0 \times 10^5$ and $5\text{-}4 \times 10^4$ molecular weight regions, particularly in the $5\text{-}4 \times 10^4$ region. Obviously the material comprising the $5\text{-}4 \times 10^4$ fraction must be minute; the effect of stable gallium on gross tumor uptake of ^{67}Ga was not apparent below a level of 0.05 mg/kg as we have previously shown. We have also observed this same alteration in ^{67}Ga gel-filtration profiles when aqueous tumor extracts have been subjected to heat treatment (60° and 95°C) before filtration (also included in Table 20).

We now believe that the active agent(s) involved in the binding of ^{67}Ga in aqueous tumor extracts is probably protein in nature (precipitation by $(NH_4)_2SO_4$ and Rivanol, electrophoretic behavior, heat instability, and gel-filtration characteristics), although initial attempts at enzymatic hydrolysis, as measured by ultrafilterable ^{67}Ga activity, have been only partially successful. In this latter

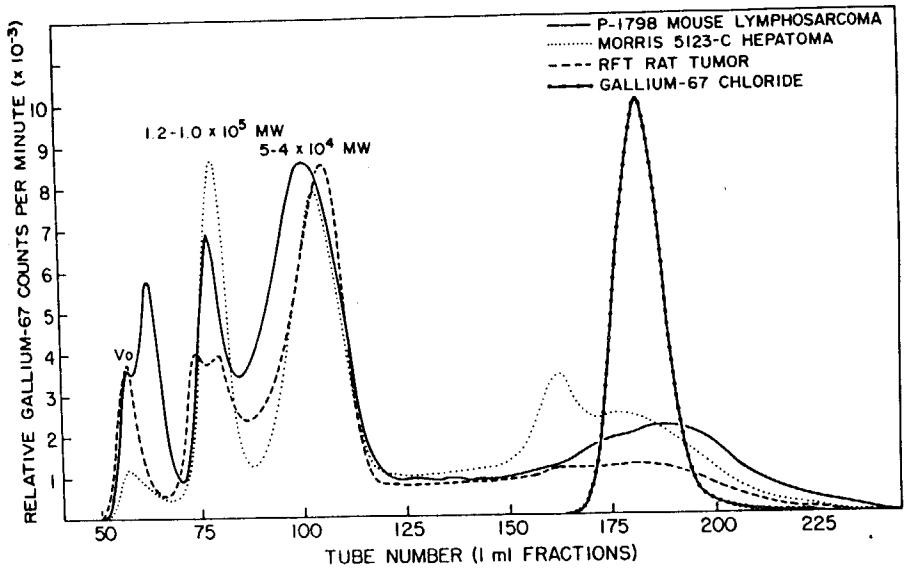


Fig. 30. Gallium-67 upflow-gel-filtration profiles of aqueous extracts of Morris 5123C hepatoma, P-1798 mouse lymphosarcoma, and RFT rat tumor 2 days after administration of ^{67}Ga using Sephadex G-200 superfine (10 to 40 μ dia.) and 0.1 M Tris buffer, pH 7.0. Note the similarity of the ^{67}Ga patterns of all three tumors, particularly for the 5-4 x 10^4 molecular weight region.

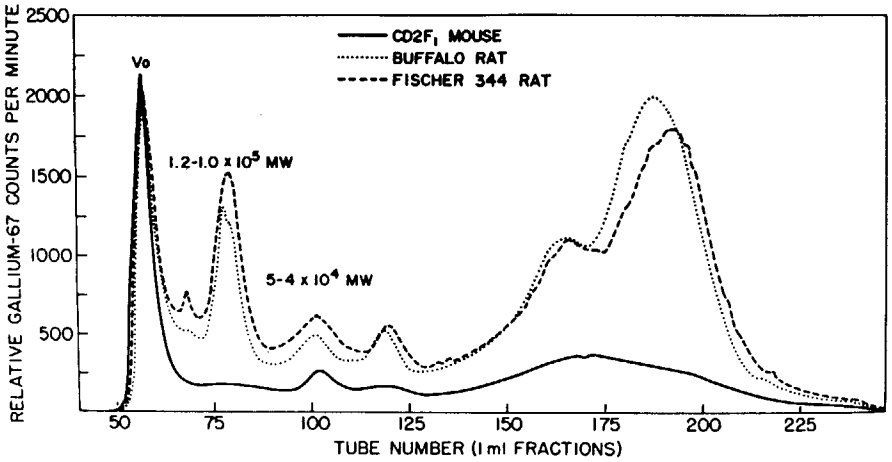


Fig. 31. Gallium-67 upflow-gel-filtration profile of aqueous extracts of livers from a Buffalo rat (host for Morris 5123C hepatoma), Fischer 344 rat (host for RFT tumor), and $CD2F_1$ mouse (host for P-1798 lymphosarcoma) 2 days after administration of ^{67}Ga. The column and conditions were the same as those given in the legend to Fig. 30. The 5-4 x 10^4 molecular weight ^{67}Ga component in the profile is notably lower than that shown in Fig. 30 for tumors.

TABLE 20

PERCENTAGE OF ^{67}Ga IN ZONES of ^{67}Ga GEL-FILTRATION PROFILES FROM AQUEOUS EXTRACTS OF NORMAL AND TUMOR TISSUES

Tissue	Percent ^{67}Ga in various zones of profile*					
	Void + High M. Wt.	1.2-1.0x10^5	5-4x10^4	~2.5x10^4	~5.0x10^3	Bed
Morris 5123C hepatoma	3.0	20.1	40.8	5.9	17.7	12.6
Buffalo rat liver	12.1	10.4	11.2	6.2	15.2	44.9
P-1798 lymphosarcoma	12.7	20.6	40.0	4.4	7.5	15.0
$CD2F_1$ mouse liver	29.1	8.0	13.3	7.7	13.5	28.4
RFT rat tumor	8.4	12.6	52.6	7.1	9.6	9.9
Fischer rat liver	13.3	12.2	13.0	6.6	15.1	39.9
Fischer rat spleen	49.6	11.5	12.5	3.9	6.4	16.1
RFT rat tumor, 0.025 mg Ga/kg	42.3	15.6	8.4	4.3	5.2	24.3
RFT rat tumor, 0.1 mg Ga/kg	39.4	14.3	6.1	3.7	3.0	33.4
RFT rat tumor, 1.0 mg Ga/kg	33.9	9.3	4.6	3.8	2.8	45.6
RFT rat tumor, 60°C (1 hr)	16.7	2.1	8.9	4.7	34.9	32.8
RFT rat tumor, 95°C (1 hr)	6.5	---	5.7	---	---	87.8

* First three tumor studies are average of two experiments; all others are individual runs.

1028144

respect one should note that lysosomal enzymes are quite stable toward proteolysis by other enzymes (Tappel, A. L. In: Lysosomes in Biology and Pathology, Vol. 2, J. T. Dingle and H. B. Fell, eds., American Elsevier, New York, 1969, p. 232) and this may be the reason for our poor hydrolysis results, if the active agent(s) is indeed a lysosomal enzyme(s), as we presently suspect.

Indium, in the same periodic group as gallium, is also known to have an affinity for a wide variety of tumors, although in animal experiments ^{67}Ga was found to be superior to ^{111}In (59). Our rate-zonal SPR procedure, interestingly enough, has indicated that the same type of subcellular organelles that bind ^{67}Ga also appear to bind ^{111}In. Even more interesting is the fact that the 5-4 x 10^4 molecular weight fraction, so prominent in the binding of ^{67}Ga, also appears to be a major component in the ^{111}In gel-filtration profile of aqueous tumor extracts and in turn is a minor component in normal tissue as with ^{67}Ga (Fig. 32). These observations suggest that the mechanism for the binding of ^{111}In is very similar to that for ^{67}Ga, as chemical considerations would suggest.

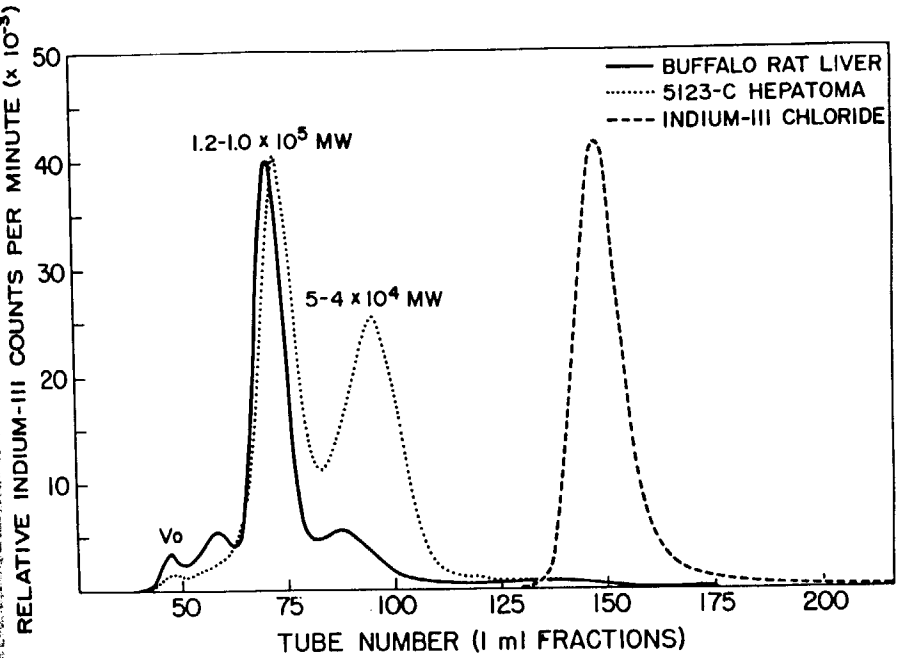


Fig. 32. Indium-111 upflow-gel-filtration profile of aqueous extract of Morris 5123C hepatoma and liver from normal Buffalo rat 3 days after administration of ^{111}In using Sephadex G-200 superfine (10 to 40 μdia.) and 0.1 M phosphate buffer, pH 7.0.

Toxicity of Scandium. We have shown that the element scandium, in small amounts, will produce in experimental animals a rapid clearance of ^{67}Ga from blood and normal soft tissues (except kidney) without affecting the affinity of ^{67}Ga for tumor tissue. This raised the possibility of using this material as an agent for enhancing ^{67}Ga tumor-to-nontumor ratios in scanning. After toxicity studies in the rabbit and dog indicated little likelihood of any adverse effects of scandium in humans at the level found effective in rats and mice (0.5 mg Sc/kg), Phase I studies were commenced in humans. No adverse effects were observed until a level of 0.125 mg Sc/kg was administered. The subject receiving this dose experienced a period of hemolysis sufficient to cause hemoglobinuria. The clinical investigation was immediately halted. (Details of these clinical studies are given in later paragraphs of this section, p. 89.)

We are now attempting to determine the reason for this unexpected reaction (J. J. Rafter, J. E. Carlton, and R. L. Hayes). Preliminary results indicate that man is unique in having a hemolytic reaction to intravenous scandium. No hemolysis was observed in the animals used in our scandium toxicity studies, although we did observe a reversible anemia in rabbits after repeated doses of scandium at a level of 5 mg Sc/kg (10 times the projected effective dose in humans). In vitro studies for autohemolysis using human red blood cells showed definite hemolysis produced by scandium. Rabbit blood tested in this manner did not show this effect (Table 21). No hemolysis occurred in dog blood beyond that occurring in a control test. However, some evidence of hemolysis was detected in the marmoset, a small primate. Further studies have shown that, unlike those of animals, human erythrocytes bind scandium to a remarkable degree when the amount of scandium added to blood samples exceeds a certain level (Table 22). We postulate at this time that this binding occurs only after plasma protein binding sites (possibly transferrin) are saturated by added scandium. After saturation, excess scandium is then free to bind to erythrocytes. Why this should occur only with human red blood cells is unclear; a possible explanation may be that in humans, unlike other animals, one or more of the enzymes responsible for the integrity of the red cell membrane may have a particular affinity for scandium. This affinity could then lead to inactivation of the enzyme(s) with resulting destruction of erythrocytes. We plan to investigate the effect of scandium on these types of enzymes.

Scandium-46 distribution was previously found not to be affected by the addition of stable scandium (0.5 mg Sc/kg) during the first 24 hr after administration. However, in view of the histologically apparent long-term retention of stable scandium in tissues of the rabbit, particularly in those of the reticuloendothelial system, as we noted in last year's Research Report, and also the report by Rosoff et al. (Int. J. Appl. Ratiat. Isotop. 16, 479, 1965) of a biological half time of approximately 1,500 days for ^{46}Sc in man, it was of interest to study the long-term whole-body retention of ^{46}Sc in animals (B. L. Byrd and Evelyn E. Watson). Groups of rats were administered high specific activity ^{46}Sc with and without added stable scandium (0.5 mg Sc/kg) and their whole-body retentions measured by the geometry-independent technique of Gibbs, et al. (J. Nucl. Med. 9, 264, 1968) for 11 months. The results are shown in Fig. 33 together with a computer analysis for

1028145

TABLE 21

IN VITRO HEMOLYTIC EFFECT OF SCANDIUM ON HUMAN AND RABBIT BLOOD

Species	Added scandium (μg Sc/ml blood) 0.0	0.3	0.6	1.3	2.5	5.0	10.0
	Percent hemolysis *						
Man	1.4	4.1	6.6	11.8	12.5	13.4	14.0
Rabbit	2.8	2.9	2.9	3.4	3.6	3.8	3.4

* Average results of tests on blood samples from four humans and four rabbits; samples were incubated at 37°C for 48 hr.

TABLE 22

IN VITRO BINDING OF ^{46}Sc TO RED BLOOD CELLS OF MAN AND VARIOUS ANIMALS

Species	Added stable scandium (μg Sc/ml blood) 0	0.25	0.75	1.25	2.5
			Percent ^{46}Sc binding		
Man*	0.1	0.1	13.4	14.9	---
Rabbit	0.1	0.2	1.5	1.1	1.1
Rat	0.8	0.4	0.5	0.3	0.6
Dog	0.2	0.2	0.7	0.5	0.9
Marmoset	0.5	0.4	0.4	0.3	---

* Average of five subjects.

the various compartments involved in the excretory process. Note that although the whole-body retentions are similar for the two groups at early intervals, the long-term third compartment for the carrier-scandium group had a half time over twice that of the group receiving ^{46}Sc alone, resulting in a considerably greater whole-body retention of scandium by that group. At the termination of this study, tissue distributions of ^{46}Sc were determined for both groups. These results are shown in Table 23. Note that the concentrations of ^{46}Sc in all tissues except the muscle, blood, fat, testes, and brain were considerably higher in the carrier group, particularly in the reticuloendothelial tissues.

Studies of the Radioprotective Drug WR-2721. The radioprotective drug WR-2721 (S-2-[3-aminopropylamino]ethylphosphorothioic acid) has been shown by a number of investigators to have a high protective index against radiation damage in animals (increase of $LD_{50(30)}$ by a factor of 2 to 3) and also to be relatively nontoxic. Yuhas and Storer have shown that it provides higher protection for normal than for malignant tissue (J. Nat. Cancer Inst. 42, 331, 1969). For these reasons consideration is being given to possible chinical use of WR-2721 to enhance the effectiveness of external radiation therapy of malignant disease, i. e., to reduce undesirable effects resulting from injury to normal tissues

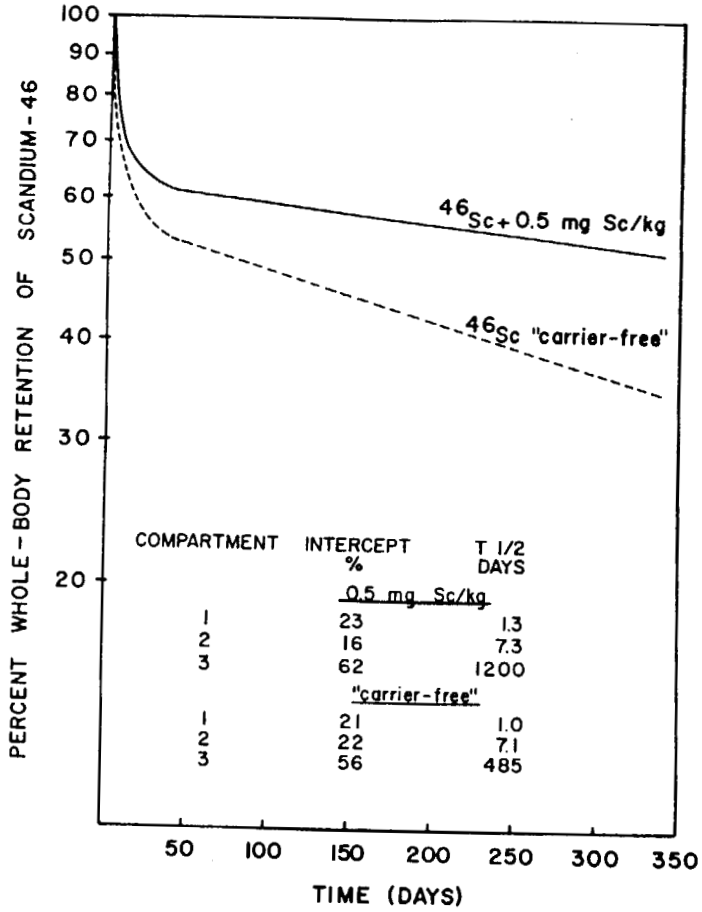


Fig. 33. Long-term (11 months) whole-body retention of ^{46}Sc in the rat. Note that with 0.5 mg Sc/kg carrier the half time of the third compartment is greatly increased accounting for the considerably higher ^{46}Sc retention in this group; with both groups the third compartment is a major component in the retention process.

or possibly to permit higher doses of radiation with greater antitumor effect without increasing the injury to normal tissue. In a cooperative effort, we are now studying the feasibility of this undertaking (Lee C. Washburn*, C. L. Edwards, R. L. Hayes, and J. M. Yuhast). The first phase of the clinical project would involve a study of the metabolism of ^{35}S-labeled WR-2721.

* Presidential Intern
† Oak Ridge National Laboratory.

1028146

The decreased radiation protection afforded to tumor tissue in animals by WR-2721 might simply be the result of decreased uptake of WR-2721 by tumor tissue compared to that occurring in normal tissue. Some preliminary results in tumor-bearing rats and mice suggest this possibility (Table 24); however, other preliminary studies at higher levels of WR-2721 (300 mg/kg) have given lower nontumor-to-tumor ratios.

TABLE 23

EFFECT OF STABLE SCANDIUM ON TISSUE DISTRIBUTION OF ^{46}Sc IN MALE BUFFALO RATS 11 MONTHS AFTER INTRAVENOUS ADMINISTRATION

Tissue	0.5 mg stable Sc/kg	"Carrier-free"
	*Percent administered dose/g**	
Liver	1.26 ± 0.04	0.13 ± 0.01
Spleen	4.61 ± 0.50	2.68 ± 0.14
Kidney	0.46 ± 0.03	0.19 ± 0.04
Lung	0.41 ± 0.02	0.17 ± 0.01
Muscle	0.13 ± 0.01	0.16 ± 0.02
Thymus	1.10 ± 0.10	0.61 ± 0.06
Femur	0.76 ± 0.02	0.51 ± 0.01
Marrow	5.00 ± 0.16	0.77 ± 0.08
Blood	0.0012	0.0030
Plasma	0.0021	0.0038
Small intestine	0.62 ± 0.05	0.080 ± 0.006
Large intestine	0.48 ± 0.05	0.095 ± 0.007
Skin	0.11 ± 0.01	0.070 ± 0.008
Fat	0.021 ± 0.002	0.029 ± 0.010
Testes	0.33 ± 0.02	0.46 ± 0.01
Brain	0.26 ± 0.02	0.071 ± 0.006

* Normalized to body weight of 250 g; five animals per group.

TABLE 24

RELATIVE DISTRIBUTION OF ^{35}S-LABELED WR-2721 BETWEEN TRANSPLANTED TUMORS AND NORMAL TISSUE 30 MIN AFTER INTRAVENOUS ADMINISTRATION*

Tissue	Tumor type			
	7777-hepatoma	RFT	P-1798	CA-755
	Tissue concentration/tumor concentration			
Liver	2.9	5.4	8.7	6.7
Spleen	2.1	3.8	3.1	1.6
Kidney	7.8	13.8	11.9	5.6
Lung	1.5	3.4	2.7	1.5
Marrow	1.8	6.6	---	---
Muscle	0.3	0.8	1.0	0.5
Small intestine	1.3	3.6	3.4	1.9

* WR-2721 dose 1 mg/kg; two animals per group.

Methods for the preparation, purification, storage, dispensing, and quality control of ^{35}S-labeled WR-2721 are presently under development. WR-2721 can hydrolyze to the analogous mercaptan and this compound can in turn oxidize to the analogous symmetrical disulfide. These two substances represent potential impurities in any preparation of WR-2721 and methods for their determination are required in any planned clinical investigation, particularly for the disulfide, since it is approximately three times as toxic as WR-2721 itself. A thin-layer chromatographic procedure has been developed which gives excellent separation of WR-2721 from the mercaptan and disulfide. The method employs silica gel and methanol-chloroform-concentrated ammonium hydroxide system (2:1:1 by volume) and gives an R_F value of 0.20 for WR-2721, compared to 0.42 for the mercaptan and the disulfide.

An alternative, more rapid, colorimetric procedure for detecting trace quantities of mercaptan or disulfide contaminant has also been developed. This method involves the formation of a bright pink color when mercaptans are treated with sodium nitroprusside. Disulfide must first be reduced to the corresponding mercaptan, preferably with sodium sulfite solution. Using this colorimetric method, it is possible to detect as little as 2% of mercaptan or disulfide impurity in 1 mg of WR-2721.

Some minor alterations in the synthesis of WR-2721 have also been explored in order to alleviate problems concerning the quality of the product and the reproducibility of the reaction. The clinical use of WR-2721 has been approved by the ORAU Committee for using Humans as Experimental Subjects and an Investigational New Drug (IND) application is being readied for the U. S. Food and Drug Administration.

Cycloleucine as a Tumor-Scanning Agent. For some time staff members of the ORAU Medical Division and the Isotopes Division at Oak Ridge National Laboratory (ORNL) have planned a joint effort involving the synthesis and clinical testing of various organic and biochemical compounds labeled with ^{11}C ($T_{1/2}$ = 20.4 min). Plans now have been formulated to carry out such a venture. A major advantage of this arrangement will be the proximity of the Medical Division to the ORNL 86-inch cyclotron and the extremely high yields of ^{11}C that this cyclotron is capable of producing (10 to 20 Ci/hr). The short half-life of ^{11}C requires very rapid synthesis and purification of labeled compounds prior to administration, as well as suitable uptake and localization kinetics for the physiological system under investigation. The availability of such high yields of ^{11}C from the 86-inch cyclotron should make it possible to use syntheses requiring more time than is feasible at other cyclotron installations. Alternately, use of methods of synthesis of quite low product yield are also possible, since the quantity of ^{11}C available will be so high that its expendability becomes practical.

It has long been known that, because of the high rate of protein synthesis, tumor tissue in general has the ability to incorporate amino acids circulating in the blood to a greater extent than normal tissue. This has been demonstrated by a massive amount of research involving incorporation of amino acids labeled with ^{14}C, ^{35}S, and ^{15}N. Our survey of the literature dealing with incorporation of amino acids labeled with ^{14}C in normal and cancerous tissues of test animals has revealed that 1-aminocyclopentanecarboxylic acid, commonly called ACPC or cycloleucine, is probably the most promising as a tumor-scanning agent.

1028147

Berlinguet and co-workers (Can. J. Biochem. Phys. 40, 1111, 1962) showed by autoradiography that labeled ACPC is concentrated selectively by the pancreas, bone marrow, and tumor cells in mice. Sterling and co-workers (Biochem. Pharmacol. 11, 135, 1962) found a significant incorporation of ACPC into lymphosarcoma and adenocarcinoma in mice, and the localization was greatest at the earliest period examined (30 min). Unfortunately the format of their data does not permit comparison of the localization in tumors at 30 min with that in other tissues, such as liver, muscle, blood, etc. Hence we undertook a preliminary study examining the incorporation of ACPC labeled with ^{14}C into the various tissues in rats bearing the Morris 5123C hepatoma (Lee C. Washburn and B. L. Byrd). We were able to duplicate Sterling's findings of a maximum tumor incorporation at 30 min, a compatible time scale for work with ^{11}C (Table 25). At this interval the tumor-to-nontumor concentration ratios were approximately 8.

TABLE 25

TISSUE DISTRIBUTION OF ^{14}C-LABELED ACPC IN MALE BUFFALO RATS BEARING MORRIS 5123C HEPATOMAS

Tissue	Percent administered dose/g*			
	10 min	20 min	30 min	60 min
Liver	0.73	0.57	0.43	0.49
Spleen	0.70	0.60	0.46	0.49
Kidney	0.82	0.83	0.71	0.71
Muscle	0.45	0.54	0.47	0.54
Marrow	0.83	0.72	0.53	0.50
Tumor	3.22	2.82	3.55	3.42
Small intestine	1.48	1.1	0.74	0.63
Pancreas	2.84	5.09	2.54	2.01
Blood	0.52	0.58	0.42	0.49
Plasma	0.58	0.57	0.45	0.50

* Normalized to body weight of 250 g.

In further studies, incorporation data in test animals for the corresponding ^{14}C-labeled compounds will serve as guidelines concerning the value of any potential ^{11}C-labeled compound. No procedures will be developed for ^{11}C labeling of a compound until a useful incorporation pattern and time course have been obtained for that agent. Ideas on compounds of interest will be obtained from ^{14}C incorporation data in the literature or in our own studies. A number of such agents have now been identified.

Comparison of ^{67}Ga and ^{111}In in the Citrate and Bleomycin Form. Merrick and co-workers have recently reported at an IAEA symposium in Monaco in October 1972 that, although they obtained much poorer tumor-scanning results in humans with ^{111}In chloride than with ^{67}Ga citrate, when ^{111}In was administered together with bleomycin (a cancer chemotherapy agent with chelating properties) excellent tumor visualization resulted. We have since tested the effect of bleomycin on the tissue distribution of both ^{111}In and ^{67}Ga in tumor-

bearing animals at 4 and 24 hr after nuclide administration (Lee C. Washburn and J. E. Carlton). Table 26 shows the results of this study. No enhancement of ^{67}Ga tumor-to-nontumor ratios was obtained with bleomycin and only slight improvements in ^{111}In tumor affinity were offset by rather massive losses of ^{111}In activity through excretion. These results cannot, of course, be extrapolated directly to humans.

TABLE 26

COMPARISON OF THE TISSUE DISTRIBUTION OF ^{67}Ga AND ^{111}In CITRATE AND BLEOMYCIN IN THE RFT TUMOR RAT*

	Gallium-67				Indium-111			
	Citrate†	Bleomycin‡	Citrate	Bleomycin	Citrate	Bleomycin	Citrate	Bleomycin
	4 hr		*24 hr*		*4 hr*		*24 hr*	
Tumor concentration (%/g) §	1.20	0.42	2.38	1.03	0.34	0.15	0.61	0.20
Ratio tumor concentration to:								
Liver	1.31	0.98	1.82	1.72	0.18	0.07	0.23	0.08
Spleen	1.14	0.58	1.13	0.96	0.15	0.14	0.16	0.17
Kidney	1.57	1.01	3.01	2.10	0.10	0.10	0.14	0.12
Lung	1.63	1.38	8.49	8.79	0.07	0.34	0.23	1.25
Muscle	7.24	7.33	21.80	29.40	2.72	4.61	4.24	7.14
Femur	1.28	1.32	2.22	2.55	0.50	0.78	0.77	0.83
Marrow	0.91	0.94	1.59	2.15	0.15	0.18	0.20	0.19
Blood	0.82	0.81	12.60	13.90	0.12	0.50	0.83	1.74
Percent excretion	8.10	63.10	22.80	67.60	4.00	63.70	6.80	64.80

* Three animals per group.
† 1 mg citrate/kg.
‡ 0.4 mg bleomycin/kg.
§ Percent administered dose/g.

METHODOLOGY IN SUBCELLULAR FRACTIONATIONS

Correction of Sucrose Error in Protein Determinations. For several years we have sought a solution to the inhibition of color development caused by sucrose when the protein content in sucrose gradients is determined by the Lowry procedure. The best approach so far has been to apply a correction factor based on the concentration of sucrose in the fractions collected (Hinton, R. H., et al., Anal. Biochem. 29, 248, 1969). It has two main limitations. First, the sucrose inhibition, although treated as such, is actually not linear with concentration. In addition, utilizing the correction factor requires a time consuming measurement of the sucrose concentration in each sample. Secondly, the protein determination is usually automated since many samples are often run at the same time. If a Technicon Autoanalyzer system is used, the data are displayed on a strip-chart

1028148

recording. Unfortunately, high sucrose concentrations cause the absorption peaks to be quite jagged, and accurate determination of peak heights is difficult.

We have recently developed an automated procedure that effectively eliminates both of these problems (David H. Brown and T. L. Caruthers*). We achieve this by intentionally reducing the sucrose concentration while at the same time increasing the sensitivity of the method. By diluting the zonal sucrose fractions 10-fold, we reduce the concentration of sucrose to a maximum of 6% (assuming the densest part of a gradient to be 60%), eliminating the sucrose noise in the chart data. However, one must then increase the sensitivity of the method at least 10-fold to compensate for loss in dilution by adjusting the reagents and the sample size in the procedure. The precision of the method as determined by the 95% confidence interval of the slope is $\pm 4\,\mu g$/ml of protein/ml with a limit of detectability of $1\,\mu g$/ml.

Semiautomatic Enzyme Analysis. More and more, automated enzymatic assays and other clinical chemistry procedures are needed to meet the analytical demands in both basic research projects and hospitals. But many procedures cannot be conveniently handled by the existing sequential automatic instruments such as the Gilford- and Technicon-type systems. Also the recently developed GeMSAEC system (designed for simultaneous multiple sample analyses) is unsuitable for wet chemistry where precipitate formation is involved. Moreover such systems range in cost from $7,000 to $100,000, expenditures sometimes not feasible.

We have found that it is possible to utilize existing instruments for chemical and colorimetric analysis to perform many analyses simultaneously. Our system employs inexpensive clinical centrifuges, water baths, and reaction vessels (Technicon disposal cups) and operates in a semiautomatic way. The principle is to stack two (or more) cups in an array, the lower cup acting as the reaction vessel and the upper cup as a fluid container. The latter cup has a hole drilled in the bottom small enough to prevent the fluid from draining into the lower cup at g forces of less than 1. The cups are held in a basket-type centrifuge rack. Once the reagent and test samples are metered out to the appropriate cups, the racks are transferred to a centrifuge and spun at approximately 2000 rpm for a short time; this results in a quantitative transfer of the material in the upper cup into the lower cup. The racks are then transferred to the water bath for an appropriate reaction time. Just prior to stopping the reaction a measured amount of "stopper" is placed in each of the upper cups. To stop the reaction the racks are once again centrifuged. If a precipitate, which would interfere with analysis, results from the addition of the "stopper," the racks are spun for an appropriate amount of time for sedimentation. The reaction cups are the conical-bottom Technicon-type which permit the precipitate to collect in a small pellet. This eliminates an additional step since the cups can then be put directly into a Technicon sampling device for sequential analysis of the supernatant part of the reaction mix. If a Gilford-type sequence is to be used the reaction vessel would be a conical-type centrifuge tube, and the centrifuge racks, in this case, would have to accommodate the specific tubes used.

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* Undergraduate Research Student Trainee from Miles College, Birmingham, Alabama.

We have shown that the system works, however, the "delivery cups" are imperfect since once the holes become wetted they tend to leak. A more precise study of dimensional requirements will be made. Presently we use the lower cups for reaction vessels, the starting and stopping reagent being added sequentially. This limited system still has the advantage that precipitates resulting from the addition of the stopping reagent can be centrifuged to the bottom of the cup, after which the cups can be transferred directly to Technicon sequential analyzers. We are presently using this sytem to monitor inorganic phosphate by the Fiske-SubbaRow procedure (without time consuming dialysis) for those enzymes that result in the production of inorganic phosphate.

Density Gradient Generator Development. Commercially available density gradient generators have a number of shortcomings with respect to versatility and maintenance. Various new gradient generator designs of greater versatility have been contemplated during the past 2 years culminating in an instrument array presently being assembled with the cooperation of the Molecular Anatomy (MAN) Program at the Oak Ridge National Laboratory (David H. Brown). It utilizes two positive-displacement syringe pumps (Harvard Apparatus) interfaced with a data tracking device (R. I. Controls Division, Research Incorporated) for generation of any shape gradient. A unique exponential mixer was fabricated for us by the Ross Company; our mixer is scaled down from their industrial-size on-line mixers and has a total holdup volume of only 2 ml. The Ross device mixes two incoming gradient fluids in a 10-stage operation a total of 2×10^6 times and has no moving parts. Since the data tracking device and the pumps can be programmed to operate at a number of speeds, we will have the capability of varying the volume of gradient to be delivered from as small a volume as 50 ml to as large as 8 liters and the latter in less than 1 hr.

CLINICAL INVESTIGATIONS IN NUCLEAR MEDICINE

THE COOPERATIVE GROUP TO STUDY LOCALIZATION OF RADIOPHARMACEUTICALS

The Cooperative Group, formally established in 1970 under ORAU sponsorship, is comprised predominantly of representatives from ORAU schools. At present, 15 institutions are actively participating in the two protocols: In the first protocol with joint support from the United States Atomic Energy Commission and the National Cancer Institute, we are collecting data on lung cancer, lymphoma, and Hodgkin's disease. The second protocol, supported by the American Cancer Society, focuses on other miscellaneous neoplasms. The objective is to provide a sound data base for determining the place of ^{67}Ga, an effective tumor-localizing agent, in the diagnosis and management of various cancers, as well as evaluation of effectiveness of therapy.

During the year, Dr. Thomas P. Haynie, M. D. Anderson Hospital and Tumor Institute, Houston, became an active participant, filling the vacancy created when Dr. Joseph B. Workman resigned from his position at the University of Maryland.

1028149

When the Cooperative Group established itself and embarked on its first project, the organizational and logistic problems were not fully appreciated. What's more some of the original members have been unable to fully participate. We are pleased, however, at the sustained committment and interest of most of the members, and their willingness to contribute generous amounts of time, clearly sacrificing part of their personal time to attend work sessions. We still have not solved the problem of observer differences in interpreting images, a most difficult aspect of nuclear medicine. Yet the work sessions have moved us closer toward defining the nature and extent of these differences. The accumulated experience in scan interpretation has also broadened our understanding of the range of normal and physiologic or pathologic noncancerous processes that produce foci of ^{67}Ga uptake (for example, lacrimal glands on occasion; female breasts, especially related to pregnancy and lactation; kidneys; sites of recent surgical trauma, etc.).

At this writing, answers to the key scientific questions still remain to be developed: 1) How often are unexpected tumors found? 2) How often and under what circumstances does the agent fail to show a known lesion? 3) Does the agent influence decisions on treatment? 4) Does the agent provide new data on the success of therapy or the need for re-treatment?

The Cooperative Group has a number of tangible achievements: 1) surmounted logistical and administrative problems, 2) found a workable way to collect data in a uniform format, 3) broadened the realization of normal ranges and the range of nontumor factors affecting tissue distribution, and 4) improved the criteria used in subjective scan evaluation, a continuing unresolved issue in all radionuclide imaging.

Table 27 details the inventory of accessioned scan studies to 7 December 1972 and an estimated 200 to 300 additional studies are in the encoding stage.

General programs for tabulating in terms of one or more variables have been written and are now being debugged. In addition to information about the patient, institution, and dose, the following variables are being used for selection and comparison. (Pilot tabulations have been produced on those asterisked.)

*CLINICAL STATUS
*MORPHOLOGY
*TOPOGRAPHY
SCAN NUMBER
ANATOMIC SITE
*SIZE OF LESION
*SCAN INTERPRETATION
*EVIDENCE OF TUMOR
KNOWLEDGE OF LESION BEFORE SCAN
LYMPHOGRAPHY
RADIATION THERAPY, DATE
RADIATION THERAPY, CUMULATIVE DOSE
SURGICAL THERAPY, OR BIOPSY
DATE OF SURGERY
HISTORY OF CHEMOTHERAPY
CHEMOTHERAPY INTERVAL
DATE OF CHEMOTHERAPY
DRUGS

Three subcommittees have been formed to analyze the data from Hodgkin's disease, lymphoma, and lung cancer. In work sessions scheduled for late in 1972, they drafted preliminary assessments of numerous detailed tabulations produced by computer programs from the encoding forms.

TABLE 27

INVENTORY OF ACCESSIONED CASES BY DIAGNOSIS AND STATUS OF DISEASE AT TIME OF STUDY (TO 7 DECEMBER 1972)

Diagnosis	Untreated	Early follow-up after initial treatment	Later follow-up with symptoms of recurrence	Later follow-up with no symptoms	Patient in midst of treatment	Other	Total
LYMPHOMA							
Well differentiated lymphocytic	18	1	11	3	4	0	37
Poorly differentiated lymphocytic	39	6	8	5	2	2	62
Mixed-cell type	18	2	8	2	7	0	37
Histiocytic	50	8	23	6	11	3	101
Undifferentiated, pleomorphic type	3	1	0	0	0	0	4
Undifferentiated, Burkitt's	2	0	0	0	1	0	3
Not otherwise specified	22	6	8	2	5	0	43
Subtotal	152	24	58	18	30	55	287
HODGKIN'S DISEASE							
Lymphocytic depletion	5	1	4	0	2	0	12
Nodular sclerosis	58	13	29	7	4	7	118
Mixed cellularity	35	9	28	7	5	2	86
Lymphocytic predominant	20	3	2	7	2	0	34
Not otherwise specified	13	4	41	15	8	1	82
Subtotal	131	30	104	36	21	10	332
LUNG CANCER							
Alveolar adenocarcinoma	27	4	7	0	0	0	38
Squamous, large cell, undifferentiated	50	9	5	0	8	0	72
Squamous, moderately differentiated	49	3	8	2	3	1	66
Squamous, not otherwise specified	4	0	0	0	1	0	5
Small cell	17	2	5	0	0	1	25
Not otherwise specified	29	1	4	1	3	1	39
Subtotal	176	19	29	3	15	3	245
MISCELLANEOUS	132	20	60	8	7	6	233
Total	591	93	251	65	73	24	1097

Interobserver Differences — A Scan-Reading Exercise. We were rudely surprised to learn the extent of differences in interpretation of a given scan by a group of experienced nuclear medicine physicians, all clearly competent. No ways are yet known to eliminate observer differences, and in fact, their true extent has not yet been defined. This difficult task faces the imaging aspect of nuclear medicine, and our scan-reading exercises represent our first step in defining the extent of interobserver differences in tumor localization with radiopharmaceuticals (C. Lowell Edwards and Ralph M. Kniseley).

Each participant submitted randomly chosen scan studies; 42 studies were then interpreted individually without clinical information. The observers recorded each site thought to represent an abnormal concentration as positive, or uncertain, locating the site by means of the subregion code.

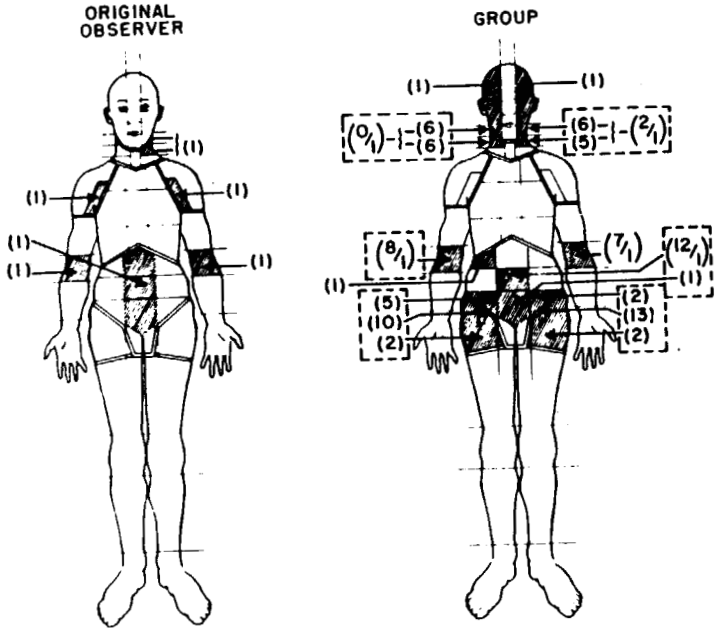
1028150

In almost every case some observers recorded "positive" sites which the majority ignored believing them to be within normal range. Some observers believed that some of the sites recorded by many or the majority of the group to be negative. In 14 of 38 cases, some observers reported a totally "negative"study, where the majority had reported "positive" sites. In 4 cases some observers reported "positive" sites where the majority had scored the study as "negative."

Then we compared the scan interpretations of the participants as a group with those of the original contributor and two other "index observers" from the group, first on the basis of the number of sites called abnormal. The number which were designated in each case as abnormal or equivocal was determined for the group as a whole and the average for the group was then calculated. Similarly in each patient the number of sites interpreted as abnormal or equivocal by the contributor was obtained from the data bank of the Cooperative Group; the number for each of the two index observers was determined from the exercise work sheets. Since there could be no absolute standard for evaluating the results, we chose to compare these numbers with a consensus of the group. The group consensus was defined as the number of specific sites in each case designated as abnormal by at least half of the observers. We tried to compensate for the variations in coding a given anatomic site. For instance, two observers may see the same abnormality, but one may label it as in the supraclavicular space, the other as in the neck or upper chest, since they are working without clinical information or landmarks. Therefore, we have grouped adjacent anatomic sites into clusters in determining the consensus. Thus the standard against which the individual's interpretations were compared included only those sites (or cluster) said to be abnormal by half or more of the observers. We have designated these as "consensus clusters."

Obviously, scan interpretations can differ not only in the number and location of the abnormal sites, but also in the interpretation of the degree of abnormality. We therefore chose an arbitrary formula for scoring the individual interpretations on the extent to which they disagree with the group consensus (Degree of Departure). A scan interpretation agreeing exactly with the consensus would score zero. Each site called positive by an observed but not so designated by the consensus was scored as 2. Likewise each site seen as abnormal by the consensus, but omitted by the individual observer, was scored as 2. If, however, the observer labeled a site uncertain whether it was designated in the consensus, it was scored as 1. Also, if he designated a site as positive and the site was adjacent to the consensus cluster, it received a score of 1. Thus we could score the degree of departure within the entire group, as well as for each individual. Table 28 lists the results for all 42 scans. Figures 34 and 35 illustrate the results from two patients showing the actual distribution of the sites called abnormal by the group and also by the original observer when he entered the case into our data bank. Table 29 shows the number of cases at various levels of departure, both for the group as a whole and also for the original observer's data and for the two index observers. In 9 cases out of 42 the group differed from the consensus by more than 2 points, a difference of more than one positive site or two equivocal sites. The index observers differed from the consensus by scores of more than 2 in only 6 and 7 cases, respectively, while the original observers differed by more than 2 in 18 out of 38 cases.

CASE NO. 17 - LYMPHOMA



	GROUP	ORIGINAL OBSERVER	INDEX OBSERVER NO. 1	INDEX OBSERVER NO. 2
NO. OF LESIONS	6.26	6	7	6
DEGREE DEPARTURE	1.26	12	2	4

Fig. 34. A comparison of the original investigator's interpretation with that of the group of participants. The anatomic diagram on the left indicates the location of the six sites regarded as definitely abnormal (shaded areas) by the original observer. The shaded areas on the right figure indicate sites designated as abnormal by at least one participant. The numbers in parentheses indicate the number of observers calling the site positive (No. 1 in our coding). Where there is a fraction the numerator indicates the number calling the site positive and the denominator the number calling the site uncertain coded 2, 3, or 4. The broken-line boxes group these sites that constitute a concensus cluster (see narrative).

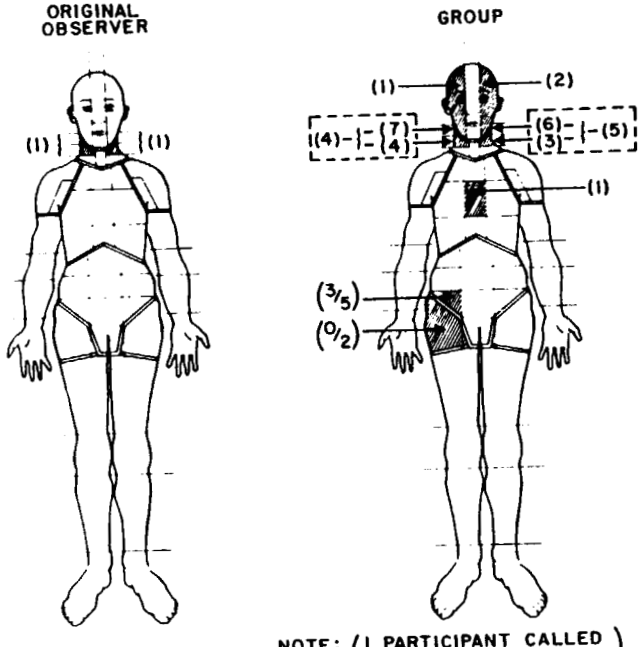
We cannot draw firm conclusions from this exercise. There are uncertain factors, such as the level of motivation for each observer during the exercise. The apparent wide discrepancy between the original investigator and the group consensus stands out, but its significance is uncertain. The original investigator usually interprets the scan with clinical information not available during the exercise, certainly influencing the interpreter toward either a more accurate or

1028151

less accurate interpretation. The individual interpreter in the exercise was not always the one who had first reported on a set of scans submitted by his institution; therefore variability does not necessarily mean inconsistency of a given interpreter.

We believe that the gallium cooperative study has performed a service in calling attention to interobserver variations, a problem that has been recognized in the past but that has had too little attention. The sharing of opinions in these exercises has probably reduced the variability among this group of observers. Broader approaches to the problem may arise from this cooperative effort.

CASE NO. 35 - HODGKIN'S DISEASE



NOTE: (1 PARTICIPANT CALLED ENTIRE SCAN NEGATIVE)

	GROUP	ORIGINAL OBSERVER	INDEX OBSERVER NO. 1	INDEX OBSERVER NO. 2
NO. OF LESIONS	2.66	2	3	3
DEGREE DEPARTURE	1.40	0	2	1

Fig. 35. The comparison of the original investigator's ^{67}Ga scan interpretation with that of the group for case No. 5 (see legend of Fig. 34).

TABLE 28

ANALYSIS OF ^{67}Ga-SCAN-READING EXERCISE DEMONSTRATING INTEROBSERVER DIFFERENCES

Case number	Diagnosis	Number of lesions					Degree of departure			
		Group	Original investigator	Index observer No. 1	Index observer No. 2	Consensus	Group	Original investigator	Index observer No. 1	Index observer No. 2
1	Hodgkin's disease	2.23	3	1	0	1	2.46	4	0	2
2	Lymphoma	2.7	2	2	1	1	0.93	0	0	0
3	Lymphoma	2	1	0	3	–*	2.26	1	0	5
4	Undifferentiated cancer	4.07	6	3	3	3	1.8	4	3	0
5	Hodgkin's disease	2	2	2	1	1	1.6	1	2	0
6	Lymphoma	1.6	2	2	1	1	0.46	0	1	0
7	Lung cancer	1.67	2	2	2	1	1.06	2	1	1
8	Lung cancer	1.2	1	1	1	1	0	0	0	0
9	Hodgkin's disease	1.07	2	1	0	–	1.73	2	1	0
10	Lymphoma	1.67	2	1	1	1	0.60	0	0	0
11	Lymphoma	1.27	3	1	1	1	0.93	4	0	0
12	Adenocarcinoma	0.8	2	2	1	–	1.53	4	2	1
13	Hodgkin's disease	5	0	2	3	4	2.80	8	8	4
14	Hodgkin's disease	1.2	x†	1	1	1	0.27	x	0	0
15	Osteitis	3	x	5	3	3	0.8	x	2	0
16	Hodgkin's disease	2.53	7	3	3	3	1.13	6	0	0
17	Lymphoma	6.26	6	7	6	6	1.26	12	2	4
18	Lymphoma	7.73	6	7	9	6	1.86	4	0	0
19	Lymphoma	1.26	3	1	1	1	0.86	8	0	0
20	Lymphoma	5.66	7	10	5	5	1.4	3	4	4
21	Hodgkin's disease	3.6	3	3	3	3	2.13	0	0	4
22	Lung cancer	0.33	2	0	0	0	0.67	3	0	0
23	Lung cancer	1.06	1	1	1	1	0.33	0	0	0
24	Lung cancer	1.8	2	2	1	2	0.13	0	0	2
25	x	1.06	x	0	1	0	0.6	x	2	0
26	x	0.6	x	0	0	0	1	x	0	0
27	Hodgkin's disease	1.06	2	1	1	1	1.46	2	0	0
28	Adenocarcinoma	1.06	1	1	1	1	1.2	0	0	0
29	Lung cancer	2.66	5	3	1	2	1.26	6	2	2
30	Lung cancer	3.53	6	3	2	4	1.13	5	5	4
31	Lymphoma	0.53	0	1	0	0	0.80	0	1	0
32	Hodgkin's disease	3.93	6	4	3	3	0.86	5	1	0
33	Hypernephroma	1.46	2	1	1	1	0.80	2	0	0
34	Hodgkin's disease	0.2	0	0	0	0	0.28	0	0	0
35	Hodgkin's disease	2.66	2	3	3	2	1.4	0	2	1
36	Hodgkin's disease	2.33	3	0	2	1	2.93	3	2	1
37	Lymphoma	3	3	3	1	1	2.93	2	3	0
38	Hodgkin's disease	2.53	5	4	0	1	3.53	2	6	2
39	Lymphoma	2.33	2	4	1	1	2.33	2	3	1
40	Hodgkin's disease	1.93	2	2	1	1	1.6	6	2	0
41	Lymphoma	4.86	6	6	5	4	1.66	4	2	1
42	Hodgkin's disease	3.6	8	3	1	2	2.4	7	1	2

* – = Not consensus.

† x = Not accessioned in data bank.

1028152

TABLE 29

DEGREE OF DEPARTURE FROM CONSENSUS IN SCAN-READING EXERCISE

Degree of departure	Group average	Original investigator	Index observer No. 1	Index observer No. 2
0 – 0.5	6	11	19	25
0.5 – 1	10	2	6	6
1 – 2	17	7	10	5
2 – 3	8	3	3	0
3 – 4	1	6	1	5
>4		9	3	1

THE EFFECT OF STABLE SCANDIUM ON THE RETENTION AND EXCRETION OF ^{67}GA IN HUMANS

Previous work in rats and mice has shown that when carrier-free ^{67}Ga is infused with 0.5 mg/kg stable scandium (as citrate) the distribution of gallium is changed advantageously for tumor scanning compared to that seen when carrier-free gallium is infused alone. There is earlier excretion of much of the gallium in the urine, more rapid clearance from the blood, and lower concentrations in most tissues, especially the liver and spleen, while the concentration in the tumor is either unaffected or increased as it is in the kidneys and bones. The beneficial implications of these findings toward tumor scanning with ^{67}Ga in humans are obvious (R. L. Hayes and C. L. Edwards).

Our previous extensive toxicity studies in rabbits and dogs indicated a high safety margin for scandium with an effective dose of 0.5 mg Sc/kg body weight. With the approval of our Committee for Using Humans as Experimental Subjects, we filed an application with the U.S. Food and Drug Administration to test the efficacy of scandium in humans. Although Rosoff et al. reported on the metabolism of ^{46}Sc in man (Int. J. Appl. Radiat. Isotop. 16, 479, 1965), they used only~0.007 mg/kg and we were unable to find any reference to the human toxicity of scandium at higher doses; therefore, we had to start with Phase I clinical pharmacology studies looking for evidence of toxicity.

Beginning with a dose (0.005 mg/kg) 1/100 of the anticipated effective dose (0.5 mg/kg) three volunteer patients were then to be given 0.02 mCi ^{67}Ga with scandium citrate by slow intravenous infusion. After the safety of each dose had been demonstrated, other groups of volunteer patients were to receive progressively larger doses of scandium until the effective level or evidence of toxicity was found. After each infusion the blood levels, urinary excretion rates, and whole-body retentions of the tracer dose (0.02 mCi) of ^{67}Ga were determined and compared with a subsequent diagnostic dose (0.035 mCi/kg) of carrier-free ^{67}Ga given a week later without scandium.

Volunteers were recruited from among the patients referred for gallium scans. Each was known to have a tumor and was capable of giving an informed consent after receiving an explanation of the procedure with full disclosure of its experimental nature, the fact that scandium had not previously been given to

people, and that no immediate benefit would accrue to the person participating in the study. Despite these rather stringent conditions a high percentage of candidates volunteered to participate.

Scandium citrate was given to eight volunteer patients. The first three received only 0.005 mg Sc/kg and none displayed any adverse or beneficial effects of the scandium. Four volunteer patients then received 0.05 mg/kg without incident. Some of them complained of a peculiar taste during infusion, but otherwise experienced no symptoms, and no adverse effects were noted. The data on these patients do indicate a slight increase in the urinary excretion rate of ^{67}Ga, and a more rapid disappearance from the blood than was seen when the 0.005 mg/kg doses of scandium were given.

The eighth patient, a 40-year-old man with multiple myeloma, was given 0.125 mg/kg (8.35 mg) scandium. Figure 36 shows the rapid disappearance of the

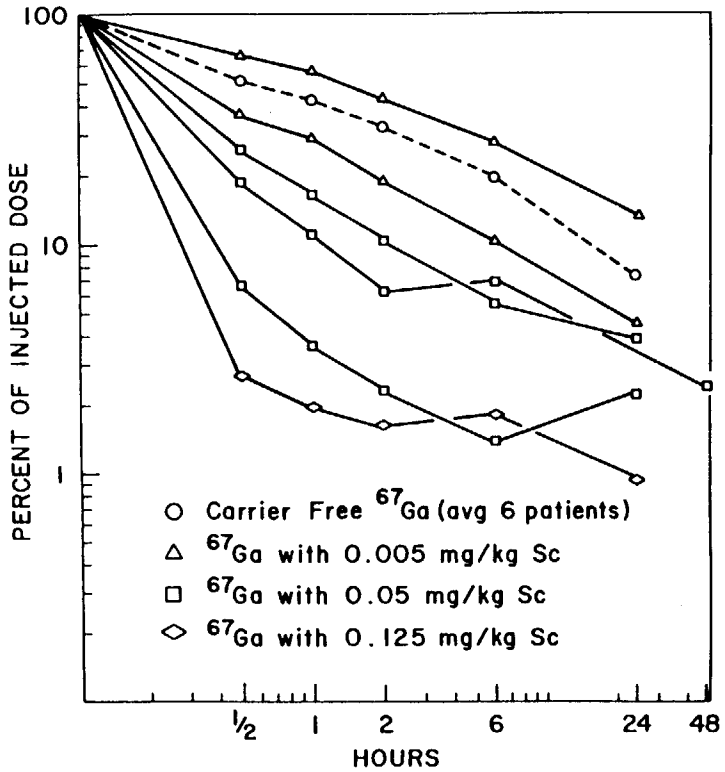


Fig. 36. The effect of the blood levels of ^{67}Ga after intravenous injection of different doses of stable scandium citrate given with 0.02 mCi ^{67}Ga. The blood values are expressed as the percent of the injected dose in the whole blood volume (the whole blood volume in liters is assumed to be equal to 0.07 times the body weight in kilograms). The values given for carrier-free ^{67}Ga without scandium are average values in the same six patients at least 1 week after receiving the scandium.

1028153

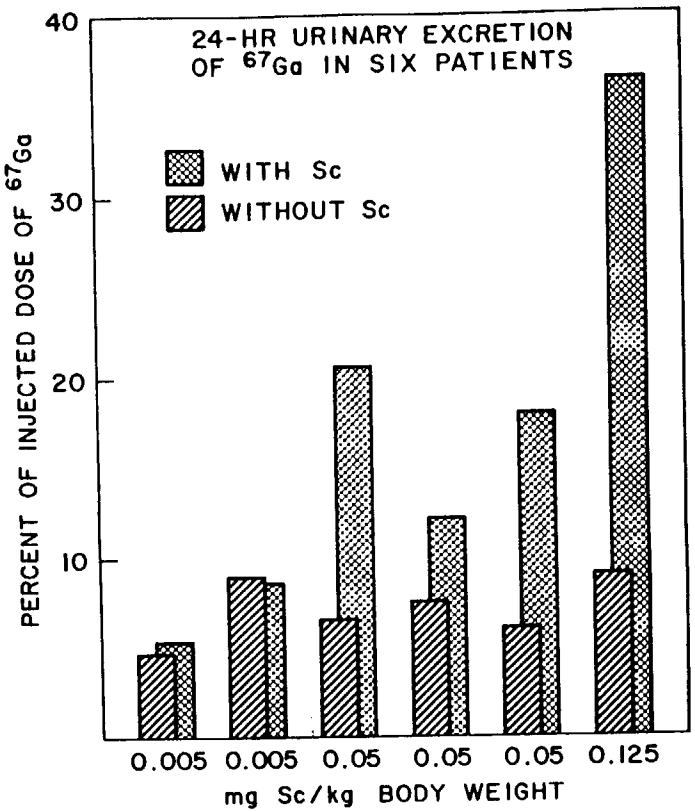


Fig. 37. The urinary excretion of ^{67}Ga in six patients during the first 24 hr after the infusion of different doses of scandium citrate with 0.02 mCi ^{67}Ga compared with subsequent scanning doses of carrier-free ^{67}Ga without scandium.

gallium from his blood. At 30 and 60 min after the injection of the tracer dose of ^{67}Ga only 2.7 and 2.0% of the dose remained in the blood, compared to 50 and 43.5% of the subsequent diagnostic dose. This figure also shows the more rapid blood clearance after the 0.05 mg/kg doses of scandium. Figure 37 shows the increased 24-hr urinary excretion after the 0.05 and 0.125 mg/kg doses of scandium. Likewise, Fig. 38 shows the whole-body retention to be correspondingly less at 24 and 48 hr.

Other than the peculiar taste noted by the patient receiving the 0.125 mg/kg dose, no symptoms appeared during or for nearly 6 hr after the infusion. He then complained of severe nausea, and for the first time noted his urine was dark red (his urine at 4 hr had been clear). It contained many hemoglobin casts and gave a strongly positive reaction for blood. The hemoglobulinuria was painless and cleared by 24 hr. By then, however, his hematocrit had fallen from 37 to 28.5%,

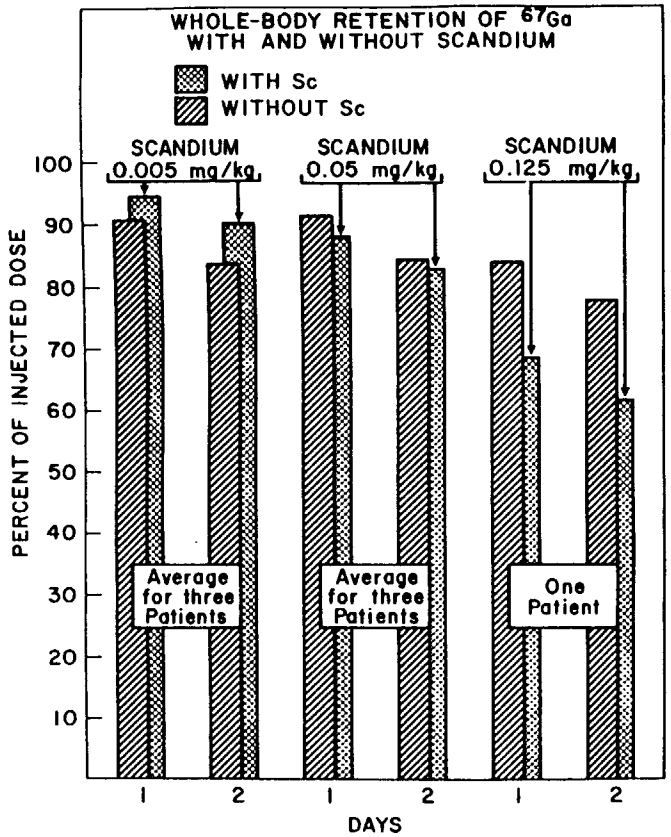


Fig. 38. The whole-body retention of a tracer dose of ^{67}Ga (0.02 mCi) injected intravenously with various doses of scandium citrate compared with the whole-body retention of subsequent scanning doses (0.035 mCi/kg) of carrier-free ^{67}Ga without scandium given 1 week later.

hemoglobin from 12.0 to 10.1 g/100 ml, and serum bilirubin had increased to 2.5 mg/100 ml with only 0.4 mg direct reacting. His lactic dehydrogenase had risen from 160 to 1,290 units and serum iron had increased from 62 to 154 μg/100ml. His hemoglobin decreased further, reaching a nadir of 8.7 g/100 ml with a hematocrit of 25% on the third day, followed by a reticulocytosis and a return of his blood count and chemistries to previous levels or better. Seven months later there was no evidence of delayed adverse effect either on his kidneys or marrow.

This brisk hemolytic reaction prompted a review of the laboratory data on the previous volunteers given lower doses of scandium. One patient, a 32-year-old woman with Hodgkin's disease who had received 0.05 mg/kg (3.25 mg), also

1028154

showed evidence for a mild hemolytic reaction. On the day after the scandium infusion her bilirubin was 1.8 mg/100 ml total (0.2 mg direct), her lactic dehydrogenase had increased from 160 to 545 units, and her serum iron from 59 to 122 μg/100 ml. In the meantime her hematocrit had dropped from 36 to 32% on the third day and was followed by a reticulocytosis of 2.4% by day 7. No change in the urine was noted. A comparison of these patients disclosed only one common feature; both had hyperglobulinemia but neither had a positive Coombs' test, nor evidence of spontaneous hemolysis. Futher testing of scandium in humans has been suspended until the mechanism of the hemolysis can be determined and assurance has been obtained that further trials can be done without undesirable effects.

COMPUTER-INTERFACE FOUR-PROBE COUNTER: INITIAL CLINICAL APPLICATIONS

In preceding Research Reports we mentioned development of an electronic interface from a four-probe counter to an IBM 1800 computer. Such work represented a joint effort among members of the instrumentation, clinical, and computer groups (F. A. Goswitz, A. C. Morris, Jr., K. H. Kim,* Paulette A. Aaron, and A. E. McDow, Jr.). During the past year we used the four-probe counter in ferrokinetic, blood volume, and red cell survival studies, and also used the system in determining the behavior of radiocolloids after intravenous injection. The external-counting system has advantages for many clinical studies, among them the ability to measure radioactivity continuously and the avoidance of multiple blood-sample withdrawals. Here we will discuss one application of the system involving the dynamic study of the plasma clearance of a radiocolloid and its uptake by reticuloendothelial organs in various hematologic diseases.

The radiocolloid (0.2 mCi/m² ^{198}Au colloid or 0.25 mCi/m² ^{99m}TcS colloid or both) is injected in an antecubital vein. In 10 to 30 sec the radiocolloid will pass from the arm vein to the left ventricle. A rapid-disappearance curve of the radiocolloid becomes visible on the heart tracing and is followed by slower components. At approximately 12 to 14 min after the injection the heart counts reach a plateau and continue at this count rate during the remainder of the study. Meanwhile, within 3 min after injection the liver count-rate rises to exceed the falling heart-curve. The liver gradually phagocytizes the colloid particles until the activity plateaus at about 15 min after injection. We have been impressed by the early, rapid rise of count rates for the spleen and sacrum which usually peak within 1 min after intravenous injection. The spleen-to-heart cross-over time should occur at approximately 5 min.

Table 30 presents some initial results in the study of cancer patients with and without hematologic disease using both ^{198}Au and ^{99m}TcS colloids. The ^{99m}TcS colloid prepared by the sodium sulfate method had larger particles, thereby accounting for a quicker uptake by the liver and an earlier plateau in its activity. Disappearance of radioactivity over the heart is faster because the sulfur colloid is phagocytized more rapidly by the liver. We usually find more radioactivity concentrated in the spleen with ^{99m}TcS colloid than with ^{198}Au colloid.

* North Carolina Central University, Durham.

In polycythemia rubra vera the peak sacral activity is attained very quickly; the liver-sacrum ratio is less than normal because patients with polycythemia rubra vera concentrate more radiocolloid in their marrow. In acute granulocytic leukemia the initial clearance from the blood (heart) is rapid, since the liver, spleen, and marrow are phagocytizing the radiocolloid at an increased rate; the ratios of liver to sacrum and spleen to heart at plateau of radioactivity are increased. Patients with chronic granulocytic leukemia are practically the same as patients with no hematologic or lymphatic disorder except that in chronic granulocytic leukemia the spleen-heart ratios at 25 min after injection are larger. Particularly striking is the spleen's slow rate of ^{99m}TcS uptake in chronic granulocytic leukemia.

Patients with chronic lymphocytic leukemia and lymphocytic lymphoma show the same results as those with no hematologic malignancy, except for a higher, but not necessarily faster, splenic uptake (Table 31). Although patients

TABLE 30

RESULTS OF MONITORING THE DISTRIBUTION OF ^{198}Au AND ^{99m}TcS COLLOIDS IN PATIENTS WITH AND WITHOUT HEMATOLOGIC DISEASE*

No. of patient	Heart: Peak activity	Heart: Initial clearance	Heart: Plateau	Liver: Uptake to pass heart	Liver: Peak activity	Spleen: Peak activity	Sacrum: Peak activity	Ratio at plateau: Liver-sacrum	Ratio at plateau: Spleen-heart
	Sec	Sec	Min	Sec	Min	Sec	Sec		
				"Controls" (no hematologic or lymphatic tumor)					
1	20	30	14	90	12	35	70	4:1	1:2
2	30	50	13	150	12	40	60	9:1	1:2
3	30	20	13	80	18	50	90	6:1	1:1
4	14	10	12	70	12	30	50	6:1	1:3
5	15	75	12	120	15	60	60	4:1	1:2
6	*10*	*10*	*9*	*180*	*10*	*30*	*40*	*4:1*	*1:1*
				Polycythemia rubra vera					
1	15 *17*	25 *20*	15 *18*	40 *25*	11 *12*	35 *30*	30 *50*	3:1 *1:1*	1:2 *1:3*
2	12 *12*	43 *15*	15 *12*	25 *20*	15 *10*	40 *25*	40 *80*	1:1 *2:1*	1:1 *1:2*
3†	13	12	12	180	7	30	20	2:1	1:3
4	10 *15*	160 *35*	9 *7*	140 *40*	12 *9*	60 *80*	12 *40*	1:2 *2:1*	1:1 *2:1*
5	16	16	5	120	18	40	25	2:1	1:1
				Acute granulocytic leukemia					
1	20 *15*	70 *5*	11 *14*	60 *36*	12 *7*	30 *20*	45 *24*	5:1 *12:1*	2:1 *3:1*
2	20 *14*	30 *10*	12 *10*	55 *42*	12 *12*	25 *15*	30 *42*	3:1 *14:1*	3:1 *4:1*
3	20	12	8	50	9	30	40	4:1	2:1
4	*10*	*8*	*22*	*40*	*18*	*30*	*40*	*4:1*	*1:1*
5	17	23	7	55	10	25	40	13:1	2:1
				Chronic granulocytic leukemia					
1	24 *13*	20 *10*	9 *13*	50 *60*	18 *15*	30 *180*	60 *60*	7:1 *13:1*	2:1 *3:1*
2†	26 *22*	44 *27*	17 *10*	76 *60*	13 *8*	40 *240*	60 *55*	10:1 *5:2*	3:1 *4:1*
3	23 *10*	14 *25*	2 *3*	90 *70*	10 *9*	55 *480*	50 *45*	5:1 *3:1*	2:1 *2:1*
4	14 *12*	20 *11*	10 *8*	160 *45*	10 *6*	140 *200*	40 *45*	2:1 *3:1*	2:1 *6:1*
5	15 *15*	10 *8*	12 *10*	40 *60*	12 *18*	360 *540*	30 *40*	9:1 *2:2*	4:1 *7:1*

* Numbers in italics denote ^{99m}TcS values.
† These patients also have myelofibrosis (proven by marrow biopsy).

1028155

TABLE 31

RESULTS OF MONITORING THE DISTRIBUTION OF ^{198}Au AND ^{99m}TcS COLLOID IN PATIENTS WITH LYMPHOMAS OR CHRONIC LYMPHOCYTIC LEUKEMIA*

No. of patient	Heart: Peak activity	Heart: Initial clearance	Heart: Plateau	Liver: Uptake to pass heart	Liver: Peak activity	Spleen: Peak activity	Sacrum: Peak activity	Ratio at plateau: Liver-sacrum	Ratio at plateau: Spleen-heart
	Sec	Sec	Min	Sec	Min	Sec	Sec		
				Hodgkin's lymphoma					
1	10 *10*	10 *20*	15 *13*	80 *60*	8 *13*	30 *540*	40 *60*	2:1 *1:2*	1:1 *2:1*
2	10 *15*	8 *10*	9 *12*	70 *60*	11 *15*	30 *720*	55 *15*	4:1 *5:1*	1:1 *2:1*
3	17	40	8	60	9	35	40	7:1	1:3
4	15	8	9	40	9	120	50	3:1	1:1
				Non-Hodgkin's lymphoma					
1†	15 *17*	80 *16*	5 *18*	110 *40*	9 *12*	25 *30*	40 *53*	3:1 *3:1*	1:4 *2:1*
2†	12	10	10	50	10	25	30	2:1	2:1
3‡	20	10	12	70	9	35	35	5:1	1:1
4‡	*18*	*22*	*8*	*220*	*9*	*35*	*60*	*4:1*	*1:5*
5§	25	10	15	90	17	30	60	2:1	1:1
6§	20	10	15	80	15	30	35	2:1	2:1
				Chronic lymphocytic leukemia					
1	17 *18*	17 *180*	7 *12*	90 *50*	12 *9*	30 *120*	40 *90*	8:1 *5:1*	2:1 *2:1*
2	18 *12*	26 *10*	18 *12*	90 *40*	19 *15*	600 *540*	90 *40*	4:1 *4:1*	3:1 *3:1*
3	*20*	*12*	*12*	*70*	*12*	*180*	*40*	*2:1*	*2:1*
4	20	15	6	90	12	90	35	2:1	1:1
5	10	15	13	30	12	30	40	3:1	1:1

* Numbers in italics denote ^{99m}TcS values.
† Lymphocytic lymphoma.
‡ Reticulum-cell sarcoma.
§ Malignant lymphoma, mixed-cell type.

with Hodgkin's lymphoma and reticulum-cell sarcoma have lower splenic uptakes than those with acute granulocytic leukemia, they have similar increased rates of uptake by the liver with rapid blood-clearance.

We are observing altered reticuloendothelial uptake of radiocolloid in different hematologic disorders and note differences in distribution of ^{198}Au and ^{99m}TcS colloids. In addition we are recording temporal patterns in uptake of colloid by liver, spleen, and marrow for selected disorders and note differences related to disease states.

INSTRUMENTATION AND COMPUTER METHODS

A UNIVERSAL, SOLID-STATE ATTACHMENT FOR THE COMPUTER RECORDING OF RECTILINEAR SCANNING DATA

Early in 1972 we began developing an instrumentation system to transmit the counting-rate data from a dual-head, whole-body scanner to our computer (T. R. Barclay, A. C. Morris, Jr., and A. E. McDow, Jr.). Initially, this development was aimed at interfacing the information from just one kind of scanner; how-

ever, as the designs progressed we noticed improvements or modifications to our circuits permitting us to connect *any* rectilinear scanner to an on-line computer.

Two types of information must be transferred to encode the data matrix from a scan. First, the computer must be given data accurately locating the position over the patient where the detector head(s) are receiving counts, this position information continously being generated as the scan progresses. Second, pulse-counting-rate data are also required continuously from the scanner, in an electronic form that is positively accepted by the computer.

We answered the first of these needs by developing a new encoder that 1) generates an electronic pulse for precisely every 1/4 inch of scanner sweep motion, and 2) produces another, separate, pulse signal every time the detector sweep passes over the center line of the patient (Fig. 39). Our element encoder is

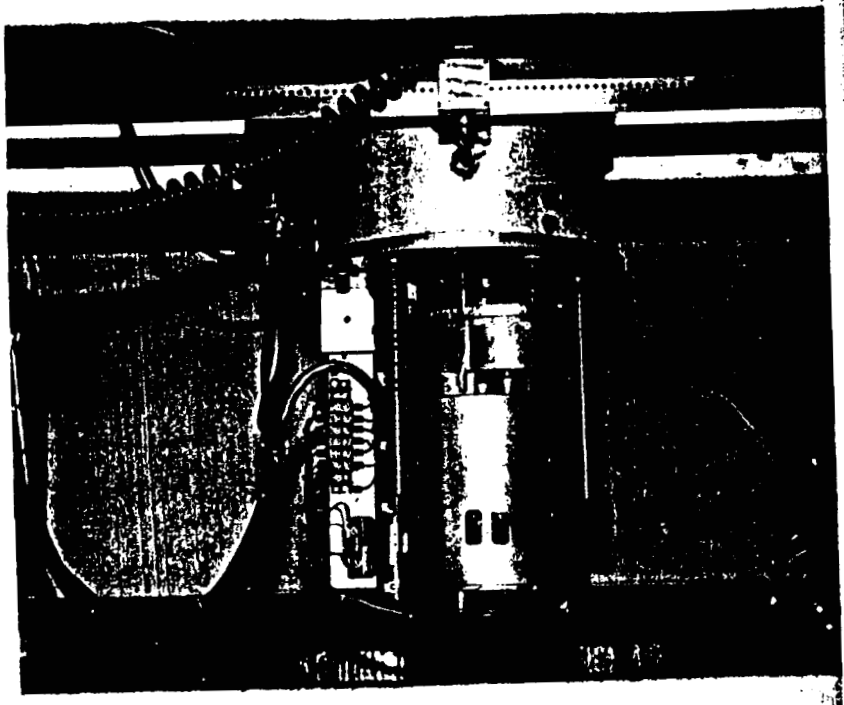
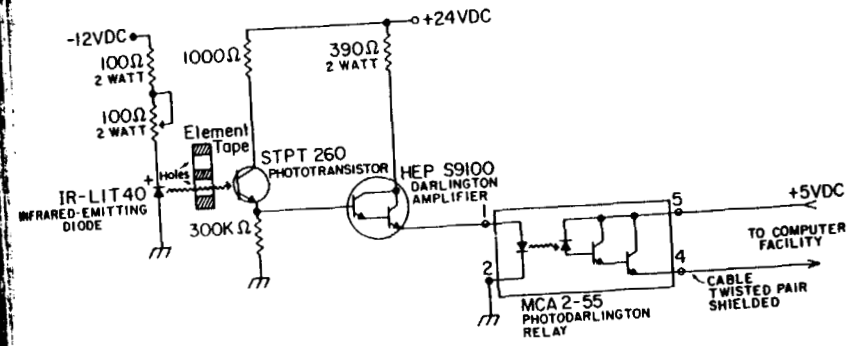


Fig. 39. Under view of scanner drive with attachment of encoding mechanism. The 1-inch steel element tape and its row of 1/8-inch holes spaced every 1/4 inch crosses the top. Our photodetector housing, mounted at top center with a coiled electrical cord connected, is fastened to the detector head and as the head moves to and fro the element tape must pass through it thereby generating our "element-step" and "line-center" signals.

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Unless otherwise noted, all resistors ¼ watt, ±5%, molded carbon

Fig. 40. Electronic circuit for generating our "element-step" and "line-center" signals. The element tape moves between an infrared-emitting diode and a silicon phototransistor. Pulses amplified through the Darlington amplifier pass on to the photodarlington relay, which has an "on" resistance of about 6 ohms and will transmit a closure of either electrical polarity to the computer.

manufactured from a 1-inch-wide tool-steel strip into which an upper row of 1/8-inch-diameter holes is accurately machined on 1/4-inch spacings (running along top of Fig. 39). A single hole of the same size is drilled through the lower part of the tape to signal the patient's center line. As the scanner head sweeps back and forth, this tape passes through the housing of a photoelectric detector, seen at the top center of this figure. The photodetector housing contains two solid-state infrared sources mounted on one side of the tape, with a pair of silicon photo-transistors positioned on the opposite side. These elements detect the presence or absence of the tape holes as the tape moves between them, and produce electronic pulses accordingly. One of our electronic circuits for amplifying the "element-step" and "line-center" pulses from this photodetector housing is given in Fig. 40; also shown is one of the photodarlington relays supplying on every pulse an "electronically hard" contact closure to the distant computer. These "element-step" and "line-center" signals, plus a "direction" signal we obtain from relay contacts in the scanner drive, is the only information required by the computer to accurately form a scan-element matrix.

The second type of information for the computer (counting-rate pulses) is the output of the scanner's spectrometers. Pulses from ordinary spectrometers are too weak to drive our long (400 ft) transmission lines; so we boost these signals through Burr-Brown No. 3341/15C inverting operational amplifiers.

Owing to the large number of interconnections between the scanner, spectrometers, photodetector housing, computer, and a future remote computer terminal, we have incorporated all of our circuitry in NIM-Bin modules, facilitating our construction of the interface, initial testing, modification, and eventual maintenance work.

Our design has the major advantage of being operable with most commercial on-line computers. Our key to this flexibility are the optically isolated

photodarlington relays (Fig. 39) which effectively isolate the circuits of the scanner (and its interface modules) from those of the computer, an innovation precluding many problems of interaction and interference. Thus, the computer senses only a remote closure of solid-state contacts without other extraneous signals. The complete instrumentation for our counting pulses has a pulse-pair resolution time of 10 μsec, so that theoretical counting rates of up to 100,000/sec can be accommodated, many times faster than rates during the most active scanning procedures. A block diagram of the integrated scanning connection is shown in Fig. 41.

THE NEW HIGH-LEVEL WHOLE-BODY COUNTER

Nuclear medicine has sometimes been handicapped by insufficient data on large test doses or therapeutic doses of radioisotopes, particularly whole-body-retention data. This has come about because most instruments are designed for small doses and are so overloaded by large doses that they do not function accurately. It is relatively easy and inexpensive to develop instruments suitable for these larger doses, and long periods of observation are possible when such an instrument is supplemented by a more sensitive one when decay and excretion have lowered the count rate.

In developing a new high-level whole-body counter we (Harold D. Hodges, W. D. Gibbs, and Charles Coffey*) considered as its primary function measuring 1.0 to 100 mCi amounts of gamma radiation. We thereby can quantitate the whole-body retention of a gamma-emitting radioisotope at any given time after it is given.

The counter consists of two 2-inch sodium iodide detectors, two 7.5 × 10.5-lead box shields with attenuators, one 71.5-inch cot with pad, and the necessary electronic recording and readout equipment. At present, the counting rate is recorded by a multichannel analyzer, calibrated from 0 to 2.0 MeV at 200 channels. Data are read out on paper tape and transferred to our computer for analysis.

Each detector is connected to separate high-quality power supplies and the signals returned to the analyzer through a single preamplifier. With this system we can calibrate each detector independently thus improving the efficiency of the instrument.

Located in the corridor adjacent to the low-level whole-body counting room, the cot is on a hinged mount to the wall so that it can be raised or lowered as needed. The two detector shields are permanently mounted on an I-beam above the cot. Each detector within its shield approximately 51 inches from the center and 86 inches above the cot's surface, has an overlapping view of the total surface area of the cot. The lead attenuators can be moved to cover the detectors whenever a subject contains more than 3 mCi; with lower activity they are not needed (Fig. 42).

With point sources the count-rate variation from the head to the foot of the cot is less than 5% at either end, all counts normalized to the count rate at the center

* United States Atomic Energy Commission special fellow in Radiation Science and Protection, from the University of Kentucky at Lexington.

1028157

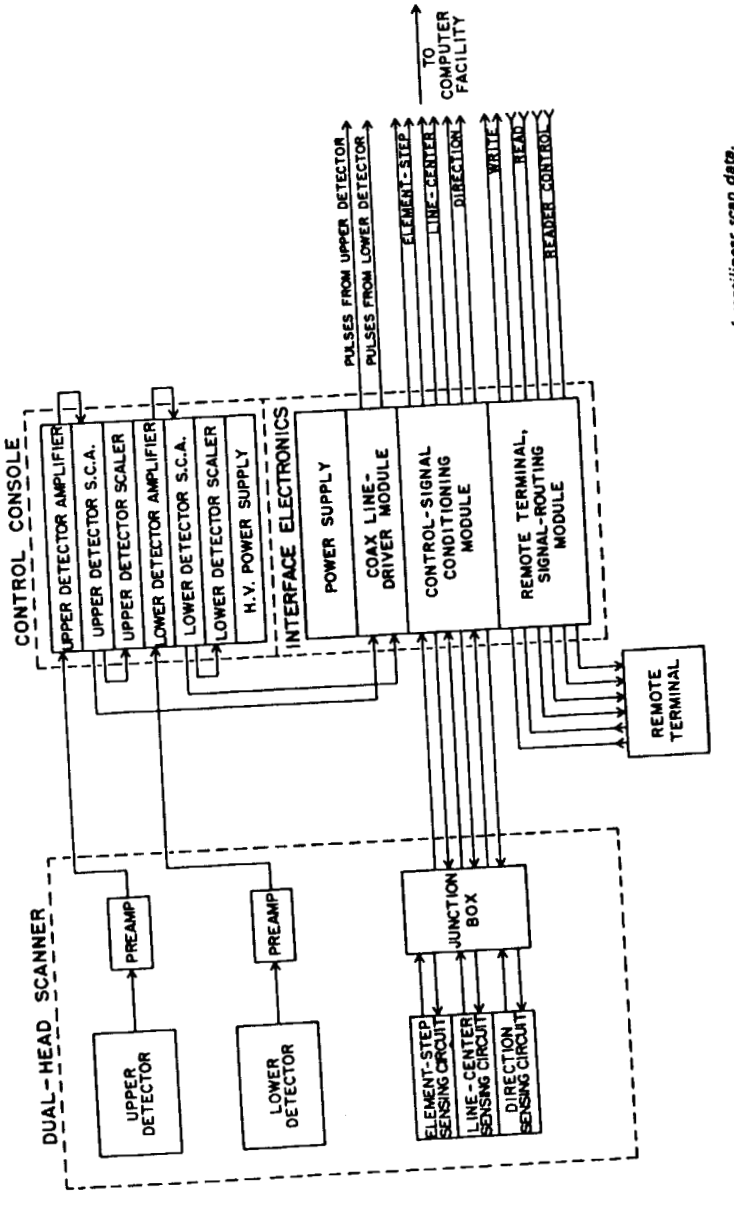


Fig. 41. Block diagram of the interconnected system for computer recording of rectilinear scan data.

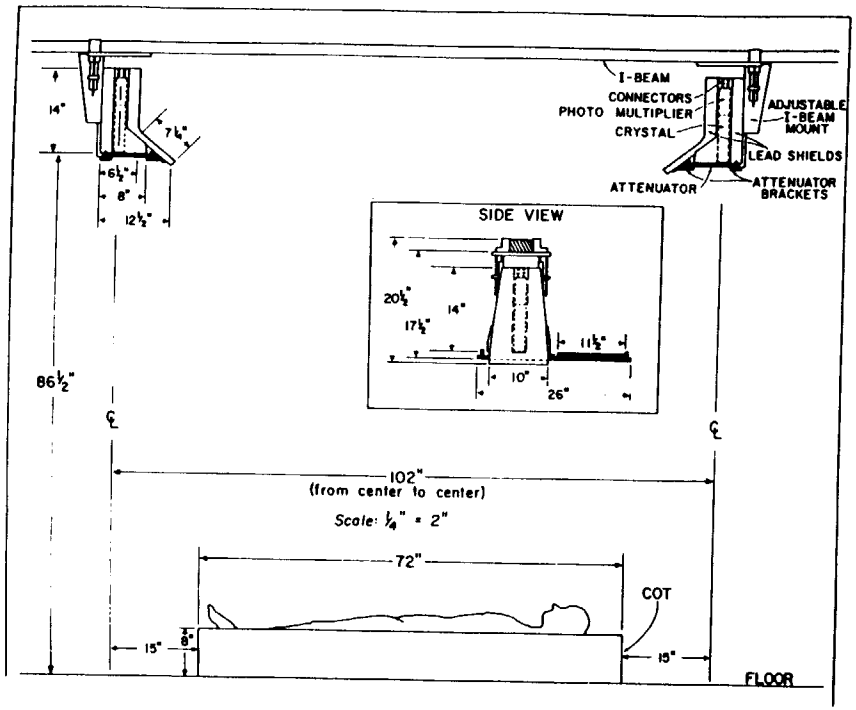


Fig. 42. Geometry and components of the high-level whole-body counter.

position. This percent was derived by a series of measurements along the length of the cot. The results are given in Fig. 43 and 44, along with the values expected on the basis of the inverse-square law alone. All measurements are taken from a point source of 2.0 mCi ^{131}I placed first at the center of the cot, then moved in increments of 10 cm to cover a total distance of 160 cm. Measurements with and without the attenuators and as indicated by the response curves, practically coincide with the expected inverse-square variation. We think the slight deviation seen near the ends of the cot is caused by the increased slant distance through the lead attenuator compared with radiation coming from the cot's center.

To date, we have measured retentions of patients given either ^{67}Ga or ^{131}I. The ^{67}Ga patients received on the average about 2.5 mCi of radioactivity while the ^{131}I patients ranged from 1 to 100 mCi. All ^{67}Ga patients were counted without the use of the attenuators. The ^{131}I patients given 2.0 mCi or greater were counted with attenuators. A 10-liter bottle containing a fraction of the dose is counted under the same conditions and used as a reference standard. Two 1-min counts are taken with the patient first supine then prone. A routine study on any patient usually involves daily counting from 3 to 5 days.

1028158

Because of its reliability and accuracy, this instrument is most useful for measuring whole-body retention in the diagnostic and therapeutic dose range. Considering the short counting time required, along with the simpler computer analysis of the data, its advantage to both the patient and to the technician is appreciated. In addition, the simplicity of design allows construction for a modest sum (approximately $2,000 plus electronic equipment).

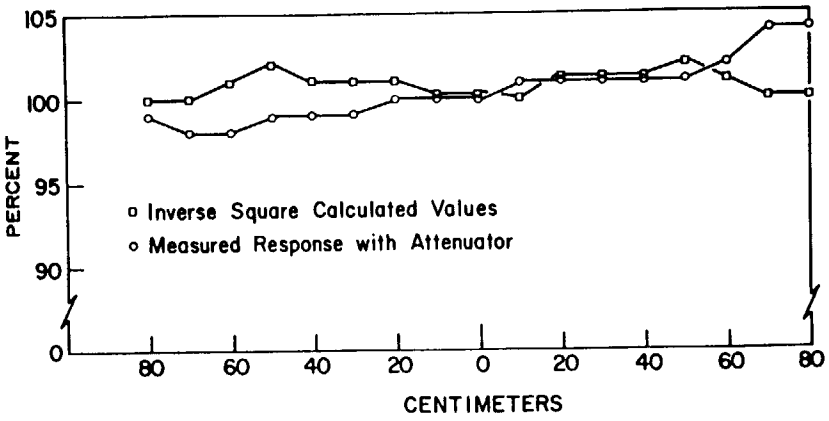


Fig. 43. The ^{131}I point-source measurements taken without attenuation as compared with the expected inverse-square variation.

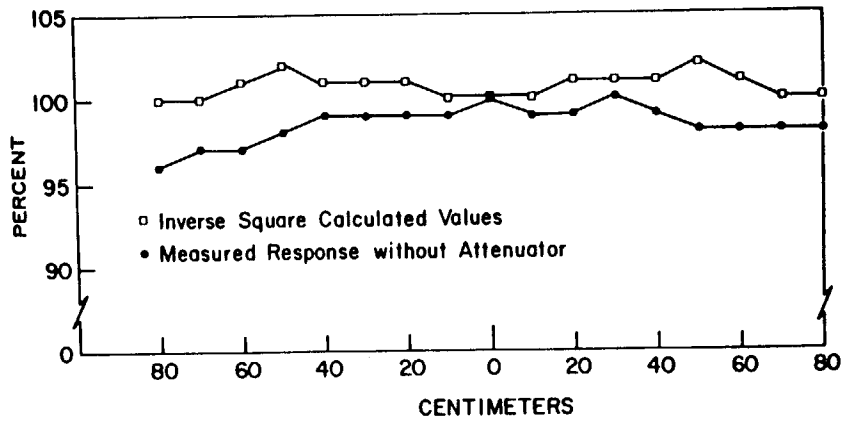


Fig. 44. The ^{131}I point-source measurements taken with attenuation as compared with the expected inverse-square variation.

APPLICATION OF COMPUTER METHODS TO RESEARCH

The ORAU Medical Division computer facility is organized mainly for service to research efforts. Through carefully controlled priorities and well-defined objectives, we undertake to provide top quality support to our investigators. Special effort is directed to those who need laboratory-associated computing systems for on-line and time-shared acquisition and analysis of data. Our computer facility moreover is an ORAU-wide resource, providing service to the Training and Technology project and the University Programs Office in their data banks and analyses.

Budget limitations have forced us to move slowly in developing computer methods, and we have concentrated our efforts on only the highest priority systems; we have, however, systematically projected our long-range applications to avoid short-term tangential systems that might become limiting in scope. At this point it appears that we have successfully developed open-ended hardware and software systems (A. E. McDow, Jr. and Martha Hansard).

Three recent additions have greatly increased our capability and efficiency. The speed of the IBM 1800 core was increased from 4 μsec to 2 μsec and the size of the core was increased to 24 K, permitting us to convert from the Time Sharing Executive (TSX) to the more efficient IBM Multiple Programming Executive (MPX). We also acquired and interfaced a Digital Equipment Corporation PDP-11 minicomputer as a data-acquisition-buffer system for the existing IBM 1800; this gave us a more powerful and flexible tool for expanding our computer methods.

The PDP-11 brought another significant development; in addition to the processor functions, it supports a multiuser time-sharing system using BASIC, which provides individual investigators access to a powerful resource for individual data calculations.

The MPX operating system is in operation but the limited size of the available core has hampered the total conversion from TSX to MPX, and we are currently operating both systems while completing the conversion of the programs designed for operation under TSX. The low-exposure-rate total-body irradiator data acquisition and analysis programs are the last major ones to be converted. Because of the size of this programming system and the limited core storage, these programs will require considerable modifications to fit the available space. As mentioned last year, the carbon dioxide data acquisition and presentation systems for physiological monitoring of irradiated patients had already been established under the TSX operating system.

The development of an interactive clinical data bank is one of our primary objectives. For both hematology and clinical chemistry tests, data banks stored on magnetic three-tape systems are updated daily. We have working programs that allow retrieval of this information with three options: date, patient, and name of test. A physician can obtain all test results on a particular patient on a given day, one particular result, or all results acquired at ORAU on that patient. Also, the results of all tests of a particular type could be retrieved for all patients in a study group, for example, to assess response to therapy. In addition, daily and weekly lists are printed for each inpatient giving the physician the hematology and clinical chemistry values for tests performed during that day or week.

1028159

The data bank for the Gallium Cooperative Study has been completely implemented and we now have stored on magnetic tape pertinent information on scans of over 1000 patients. There are two three-tape systems, providing an order system for patient and date. To maintain this effort we add to the tape new information received from the participating institutions, and print a monthly inventory which is sent to the participants.

A generalized frequency program has been written to produce one-, two-, or n-dimensional frequency tables over any values in the gallium data bank. The program also enables the user to select subsets of the total data bank on which to run the frequency counts. With this bank now fully implemented and with a sufficient quantity of data, more statistical methods may be used in analyzing the usefulness of gallium for managing the treatment of cancer patients.

BASIC STUDIES RELATED TO RADIATION, NEOPLASIA, AND METABOLISM

Most of these investigations have arisen from our primary missions in radiation effects and cancer; they tend to be more basic than much of the research reported in the preceding part of this book. Some of these studies are to pursue leads developed earlier, and some are designed to obtain specific information needed for applied portions of our work; they generally take advantage of the special resources of the Division, especially the clinical unit, and also are aided by the scientific community in Oak Ridge.

SUMMARY OF ACCOMPLISHMENTS

The biochemistry program continued work on radiation effects and cancer. New data confirmed a decided reduction in free fatty acids of plasma as a result of total-body irradiation in the rat; this change was correlated with a decrease in the rate of release of these substances from isolated fat pads. Further information was obtained on the biosynthesis of ether-linked lipids and on the enzyme system for breaking down these compounds. The former enzymes are prominent and the latter enzymes are generally lacking in cancer cells. Studies of cells growing in vitro yielded information on the factors that regulate the biosynthesis of ether and ester bonds in glycerolipids; cells in suspension cultures synthesized much more of them than did those grown in monolayer cultures. An investigation was done on cell membrances of liver cells. A new lipid class, hydroxyalkylglycerolipids, was discovered in the harderian glands of rabbits. Pulmonary adenomas produced in mice by feeding urethan were shown to have a great capacity to synthesize a material that appears to be pulmonary surfactant. Further progress was made on understanding lipid metabolism in reproductive and nervous tissues.

In the marmoset research program additional information about red cell types has been developed through the production of immune reagents; this has resulted in new ways of identifying and studying the chimerism that is almost universal in these animals. An interesting series of observations was made on marmoset marrow growing in culture. The mixed-leukocyte-culture technique was developed and shown to be useful for demonstrating histoincompatibility. The intestinal neoplasms previously discovered in the marmoset colony were shown to have arisen in the colon rather than the small intestine as previously thought. An extensive, inflammatory, hyperplastic, adenomatous disease of the intestines, not clearly related to the malignancy, was also discovered.

Studies done as a part of the ultrastructural anatomy program showed that much of the enhanced uptake of ^{67}Ga in leukemic AKR mice was accounted for by concentration of the nuclide in macrophages. An investigation of the total retention of ^{67}Ga in normal mice revealed that total-body irradiation causes a distinct dose-related decrease in retention of this radionuclide.

The cytogenetics team reports the results of using a technique for identifying a fluorescing structure, the Y body found in the male cells, as a means of determining the number of male cells in a woman given a marrow graft from a male donor. Further studies in normal patients led to some general conclusions about fluorescence of interphase cells. Progress was made in developing a technique for differential staining of chromosomes. Methods were developed for a statistical analysis of the extensive data on chromosomal effects of oral contraceptives; this study also contains invaluable data on the control group not taking contraceptives. Preliminary results are available on a study of fibroblasts as affected by contraceptive medications. The normal incidence of aberrations in fibroblasts of control subjects seems similar to that seen in lymphocytes. Certain sex hormones added to cultures appeared to increase the incidence of chromatid lesions.

Reports from the medical physics group included a study of the retention of radioactive gold thioglucose in mice and a review of factors related to whole-body retention of ^{67}Ga in patients.

1028160

BIOLOGICAL CHEMISTRY

The research program in Biological Chemistry at the Medical Division is concerned with two major problems: 1) effect of irradiation on biochemical properties of mammalian cells, and 2) basic studies on metabolic pathways and membranes in cancer cells.

Current irradiation projects deal with the effect of ionizing irradiation on lipid metabolism in adipose tissue, surface and intracellular membranes, bone marrow, and human blood cells and the possible therapeutic application of tritium localization in tumors, based on selective metabolic utilization of 1-^{3}H-fatty alcohols by radiosensitive tumor cells.

The experiments on the elucidation of metabolic pathways involving glycerolipids in cancer cells are being explored with human leukemic cells, transplantable animal tumors, and a number of primary animal tumors induced by chemicals and viruses. The main emphasis in these studies is on the metabolism and function of ether-linked lipids and related precursors.

IRRADIATION EFFECTS: ADIPOSE TISSUE

Lipids are a major source of metabolic energy when fatty acids from adipose tissue are mobilized to the liver and other organs under certain nutritional and stress conditions. It has generally been known that the exposure of various animal species to lethal and sublethal total-body X irradiation significantly alters plasma lipids and lipoproteins. Earlier work on the etiology of radiation-induced hypertriglyceridemia in rabbits indicated that the lipoprotein lipase activity in adipose tissue was inhibited by X rays. This enzyme controls the release of fatty acids from lipoprotein triglycerides of plasma into adipose tissue and its decreased activity is believed to play an important role in the radiation-induced hypertriglyceridemia.

About 1 year ago we obtained preliminary results indicating that the level of free fatty acids in rat plasma is drastically decreased by X irradiation. The level of free fatty acids circulating in the plasma is influenced by metabolic activity in adipose cells. The mature adipose cell contains approximately 95% of the lipid as triglycerides, which are continually undergoing lipolysis and reesterification, processes regulated by delicate nervous and hormonal controls in addition to nutritional factors.

We (Ten-ching Lee, Nelson Stephens, Anita Moehl, and Fred Snyder) have recently completed our experiments on the effect of X irradiation on the release of free fatty acids from adipose tissue. Data were obtained in vivo on the content of free fatty acids in plasma and in vitro on the rate of lipolysis in both male and female rats (Tables 32 and 33). We confirmed our preliminary findings on the reduction of plasma free fatty acids in irradiated animals (up to 33%). This alteration is highly significant ($P < 0.001$) when compared to controls and points out that the metabolism of lipid in fat depots is severely inhibited by X rays. In addition, our results demonstrate that the effect of irradiation is seen as a specific response in the isolated fat pad. For example, results obtained in vitro on the rate of free fatty acids released from epididymal and genital fat pads from irradiated animals was 32 to 46% less than that found for the controls. These data clearly

TABLE 32

EFFECT OF TOTAL-BODY X-IRRADIATION (600 R) ON PLASMA FREE FATTY ACID CONTENTS OF FASTED RATS*

Animals	Control	X-Irradiated
	mg/100 ml	
Female	18.44 ± 1.12 (12)	12.47 ± 0.77 (12)
Male	18.26 ± 1.36 (5)	12.18 ± 1.56 (5)

* The values in parentheses indicate the number of experiments. The figures preceded by ± indicate the standard error of means. The rats were fasted for 16 hr before sham and X-irradiation treatment, and they were killed 48 hr later.

TABLE 33

EFFECT OF TOTAL-BODY X-IRRADIATION (600 R) ON LIPOLYSIS IN ADIPOSE TISSUE OF FASTED RATS*

Animals	Free fatty acids released (μmoles/g/10 min)	
	Control	X-Irradiated
Female	1.20 ± 0.02 (4)	0.65 ± 0.03 (3)
Male	1.01 ± 0.07 (6)	0.69 ± 0.04 (3)

* The values in parentheses indicate the number of experiments. The figures preceded by ± indicate the standard error of means. The fat pads isolated from control and X-irradiated rats were placed in vials containing 5 ml Krebs-Ringer-bicarbonate buffer with 5% albumin (essentially free of fatty acids) and incubated 1 to 3 hr in a Dubnoff metabolic shaker under 95% O_2-5% CO_2. All other experimental conditions are the same as those described in Table 32.

1028161

demonstrate that X irradiation prevents adipose tissue from being properly utilized as an energy source. The exact mechanism remains unclear but is under investigation.

In a separate experiment we examined the type and quantity of phospholipids in adipose tissue. These polar lipids are often ignored when adipose cells are investigated, since they are present in only minute quantities. It has been known for some time that adipose tissue contains small but significant quantities of alk-1-enyldiacylglycerols which are often referred to as "neutral plasmalogens." In a recent report (9) M. R. Grigor,* Anita Moehl, and Fred Snyder described the plasmalogen content of the phospholipids and the nature of the acyl- and alk-1-enyl moieties in the ethanolamine and choline phospholipid fractions from porcine, bovine, and rat adipose tissue. About 50% of the ethanolamine phospholipids in the pig and beef samples consisted of alk-1-enylacylglycerophosphorylethanolamine and the corresponding value for the rat sample was near 35%. In the ethanolamine and choline phospholipid fractions, the *O*-alk-1-enyl moieties were almost exclusively 16:0, 18:0, and 18:1, whereas the acyl moieties had chain lengths ranging from 16 to 22 carbon atoms with a high degree of unsaturation. Our results indicate that the phospholipids from adipose tissue like those from nervous tissues, cardiac and skeletal muscle, and kidney are particularly rich in the ethanolamine plasmalogens.

LIPID METABOLISM IN CANCER CELLS: BIOSYNTHESIS AND DEGRADATION

Glycerolipids can contain their aliphatic moieties in either ester or ether linkages with the glycerol moiety. In cancer cells, unlike most normal cells, a significant quantity of the ether-linked lipids (*O*-alkyl and *O*-alk-1-enyl) is found. Previously we have described enzyme systems in the tumor preparations that can synthesize both types of ether lipids. We have continued to elucidate these pathways; brief descriptions of our recent published work follow.

Biosynthesis of Alkyl Glycerolipids. The role of acyldihydroxyacetone phosphate, NADH, and NADPH in the biosynthesis of alkyl glycerolipids by microsomal enzymes of Ehrlich ascites tumor was investigated by Robert L. Wykle, Claude Piantadosi,† and Fred Snyder (21). Palmitoyl dihydroxyacetone phosphate and [1-^{14}C]hexadecanol were found to serve as precursors of *O*-alkyl lipids in a microsomal system from Ehrlich ascites tumor (Fig. 45). Both acyldihydroxyacetone phosphate and dihydroxyacetone phosphate were tested (Table 34). The system required no CoA when acyldihydroxyacetone phosphate was used as the glycerol source, and significantly lower concentrations of acyldihydroxyacetone phosphate than dihydroxyacetone phosphate were required for the optimal biosynthesis of *O*-alkyl lipids; a similar reaction was catalyzed by microsomal preparations from mouse preputial-gland tumors. These findings indicated that acyldihydroxyacetone phosphate is a more efficient precursor of *O*-alkyl lipids than dihydroxyacetone phosphate. The CoA, ATP, and mg^{++} that are required when dihydroxyacetone phosphate is the glycerol source are

* Current location: Medical Research Council of New Zealand.
† School of Pharmacy, University of North Carolina, Chapel Hill.

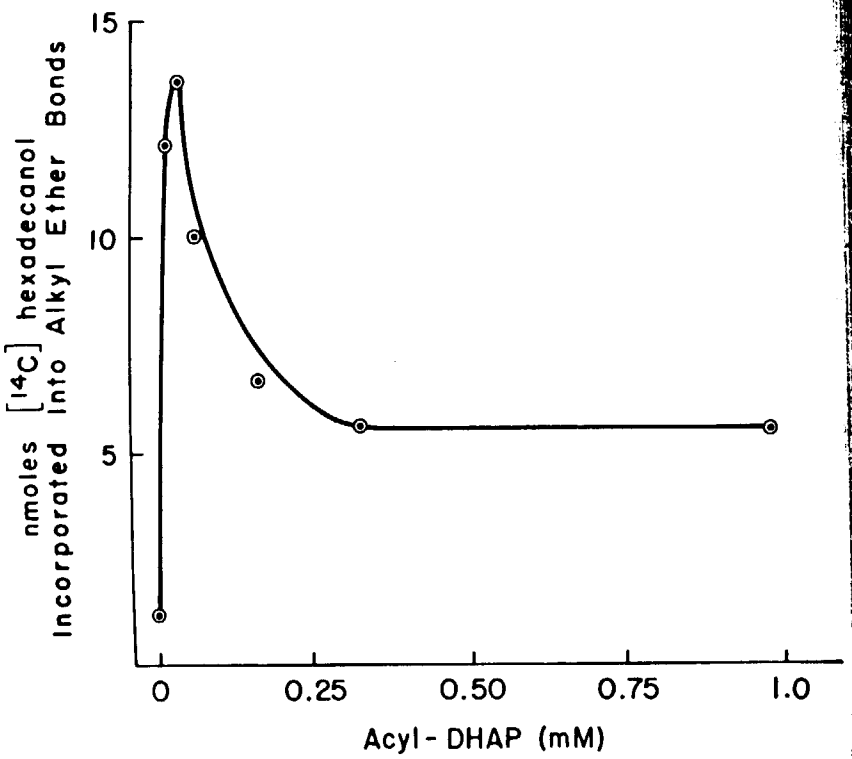


Fig. 45. The effect of concentration of acyldihydroxyacetone phosphate on the biosynthesis of O-alkyl lipids from [1-^{14}C]hexadecanol. The complete system and conditions were the same as those listed for Table 3. CoA and dihydroxyacetone phosphate were not present. The incorporation of [1-^{14}C]hexadecanol into O-alkyl lipids was linear with the time under these conditions at all concentrations of acyldihydroxyacetone phosphate shown.

necessary for the formation of the acyldihydroxyacetone phosphate; the synthesis of acyldihydroxyacetone phosphate from [^{14}C]dihydroxyacetone phosphate and palmitic acid was demonstrated in the microsomal system. The *O*-alkyldihydroxyacetone phosphate formed in the system was reduced by either NADPH or NADH to 1-*O*-alkylglycerophosphate and subsequently acylated to form 1-*O*-alkyl-2-acylglycerophosphate (Table 35). The data also indicated that acyldihydroxyacetone phosphate was reduced to acylglycerophosphate by both NADH and NADPH.

Degradation of Alkyl Glycerolipids. Preparations from liver and intestine possess enzymes which oxidatively cleave the *O*-alkyl bond in glycerolipids. In contrast, neoplastic cells, rich in ether-linked lipids, generally exhibit a

1028162

very low level of cleavage activity. However, two poorly differentiated rat hepatomas that closely resemble normal liver and that are relatively poor in ether-linked lipids did possess cleavage activity, the activity of the slowest growing hepatoma being nearly equivalent to that of rat liver.

TABLE 34

SYNTHESIS OF *O*-ALKYL LIPIDS FROM DHAP* AND ACYL-DHAP BY MICROSOMES OF EHRLICH ASCITES CELLS

	[1-^{14}C] Hexadecanol incorporated into *O*-alkyl lipids†			
	Additions			
System	DHAP	Acyl-DHAP	DHAP + acyl-DHAP	None
	nmoles			
Complete‡	8.6; 8.6	14; 13	15; 14	0.3; 0.2
Complete minus Mg++	1.7; 1.7	11; 9.6		
Complete minus CoA	0.2; 0.3	9.6; 9.6		
Complete minus ATP	0.3; 0.4	3.2; 3.5		

* Dihydroxyacetone phosphate.

† *O*-Alkyl lipids were measured after reduction of the lipids by Vitride and separation of the products by thin-layer chromatography.

‡ The complete system in 3 ml contained ATP (10 mM), CoA (0.1 mM), $MgCl_2$ (4.33 mM), potassium phosphate buffer (0.1 M, pH 7.1), [1-^{14}C] hexadecanol (16 μ M, 46 mCi/nmole), and microsomes from Ehrlich ascites cells (≃2 mg protein); DHAP (1.66 mM) and acyl-DHAP (67 μM) were added as indicated. The incubation was carried out by shaking at 150 oscillations/min at 37°C for 1 hr.

TABLE 35

EFFECT OF NADH AND NADPH ON SYNTHESIS OF 1-*O*-ALKYL-2-ACYLGLYCEROPHOSPHATE FROM ALKYL-DHAP* BY MICROSOMES OF EHRLICH ASCITES CELLS

	[1-^{14}C] Hexadecanol in products from incubation	
Additions†	*O*-Alkyl-DHAP	1-*O*-Alkyl-2-acylglycerophosphate
	nmoles	
None	10; 11	0.1; 0.1
Plus NADPH	1.5; 1.7	9.1; 9.4
Plus NADH	1.4; 1.6	9.6; 9.1

* Dihydroxyacetone phosphate.

† The NADH (1.66 mM) and NADPH (1.66 mM) were added after the incubation had been in progress for 1 hr and the incubation was then continued for 30 min at 37°C. All other components were added at the beginning of the incubation. The system contained NaF (20 mM), DHAP (1.66 mM), and no acyl-DHAP. All other components and conditions were the same as listed for Table 34.

Initial studies by Tietz et al. (J. Biol. Chem. 239, 4081, 1964) indicated that oxidative cleavage of the alkyl ether bond by rat liver microsomes occurs according to the following reaction.

$$\text{1-}O\text{-hexadecylglycerol} + O_2 + \text{Pte}\cdot H_4 \longrightarrow \text{hexadecanal} + \text{glycerol} + \text{Pte}\cdot H_2 + H_2O$$

They proposed hydroxylation of the first carbon of the alkyl chain as the initial step in the cleavage of glyceryl ethers and spontaneous breakdown of the resulting hemiacetal to yield glycerol and fatty aldehydes. The role of the reduced pteridine is thought to be analogous to that in the hydroxylation of phenylalanine by phenylalanine hydroxylase. Thus, the reduced pteridine participates as a cosubstrate and reduces one atom of the oxygen molecule to water while the other atom hydroxylates the substrate.

The effect of the oxidized and reduced pyridine nucleotides on the activity of the cleavage system has not been studied in detail. To some extent, attention has centered on the effect of the nucleotides on the auxiliary enzyme systems which include the pteridine reductase and the enzymes that oxidize or reduce the initial product of the reaction, the fatty aldehyde. Since the quantity and type of products formed are dependent on the nucleotides present, the velocity of the cleavage reaction can be determined under various conditions only when substrate disappearance is measured. Previous workers have usually used fatty acid or aldehyde production as an index of cleavage activity.

Because of the apparent role of the cleavage system in regulating cellular levels of alkyl glycerolipids, we wanted to establish the optimal assay conditions for evaluating the existence and activity of the enzyme. This approach led us to reexamine the subcellular distribution and cofactor requirement of the *O*-alkyl cleavage enzyme of rat liver and to study some of the other catalytic properties of the enzyme.

James F. Soodsma,* Claude Piantadosi, and Fred Snyder carried out studies on the partial characterization of the alkylglycerol cleavage enzyme system of rat liver (18). The bulk of the enzyme system could not be removed from the microsomal fraction by washing. Reduced glutathione (10mM) increased the extent of the reaction 5-fold (Fig. 46). Ammonium ions also increased the extent of the reaction and, in addition, appeared to activate the enzyme; the optimal concentration of ammonium sulfate was 4.8 mM (Fig. 47). Several other cations and anions tried did not stimulate the enzyme. Reduced pteridine was absolutely required for enzyme activity and the optimal concentration was 1.25 mM. The cleavage activity was stimulated 1.2-fold by NAD^+ or NADH; $NADP^+$ and NADPH had no effect. In the presence of near optimal concentrations of all known cofactors, the rat liver-soluble fraction stimulated the cleavage activity 1.4- to 1.5-fold. The stimulation was produced by a heat-labile, nondialyzable component. The optimum pH for the reaction is approximately 9.0. In the absence of added pyridine nucleotides, the long-chain products of the cleavage reactions are aldehydes. In this crude system fatty acids were formed in the presence of the reduced as well as the oxidized nucleotides, and fatty alcohols were isolated only in the presence of NADH (Table 36).

* William K. Warren Medical Research Center, Tulsa, Oklahoma.

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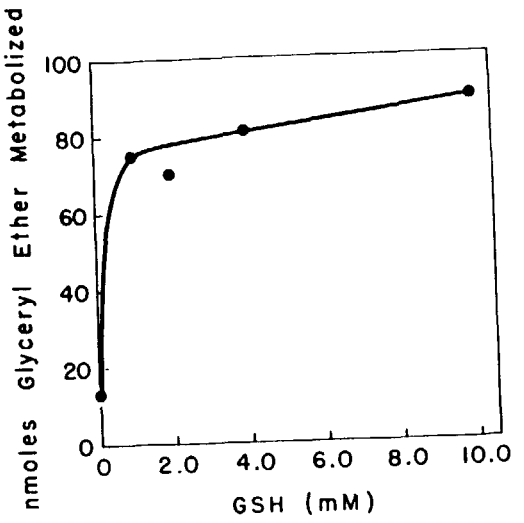


Fig. 46. Effect of GSH on the cleavage of alkylglycerols by microsomes of rat liver. The concentration of GSH was varied from 1.0 to 10.0 mM in the standard reaction mixture.

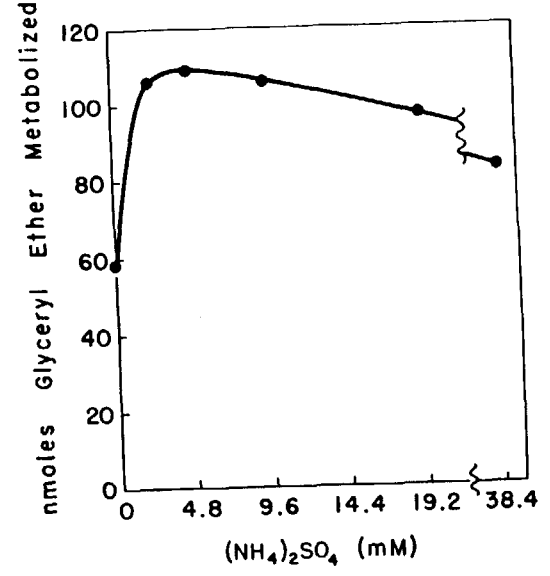


Fig. 47. Effect of ammonium sulfate on the cleavage of alkylglycerols by microsomes of rat liver. The concentration of ammonium sulfate was varied from 2.40 to 38.4 mM in the standard reaction mixture.

TABLE 36

EFFECT OF PYRIDINE NUCLEOTIDES AND RAT LIVER-SOLUBLE FRACTION ON FORMATION OF LIPID PRODUCTS OF ALKYLGLYCEROL CLEAVAGE REACTION*

Addition	Products formed			
	Fatty alcohols	Fatty acids	Fatty aldehydes	Total products
		nmoles		
None	1.39	1.39	83.1	85.9
NAD+	0.71	102.0	3.10	106.0
NADH	12.9	31.6	59.1	104.0
NADP+	1.92	31.0	54.1	87.0
NADPH	1.48	17.3	62.3	81.1
Soluble fraction	1.24	5.68	131.0	138.0

* The standard reaction mixture was supplemented with each of the cofactors listed or the soluble fraction. The concentration of the nucleotides was 1.0 mM. Soluble fraction protein (2 mg) was used. The reaction mixtures were incubated for 10 min at 37°C.

The role of the ammonium ions in the *O*-alkyl cleavage system is not known. Either they are simple cationic activators of the enzyme or they could serve as a substrate in the reaction and become part of the lipid molecule to form a reactive intermediate. A more detailed understanding of the cleavage system will depend not only on determining the function of the ammonium ions but also on the role of the soluble fraction component and on resolution of the microsomes into the components of the hydroxylase system which catalyze this complex reaction. Experiments for these purposes can now be attempted. Also, further experiments can be contemplated for determining the role of this enzyme system in regulating tissue levels of alkylglycerols.

Biosynthesis of Alk-1-enyl Glycerolipids. Robert L. Wykle, M. L. Blank, Boyd Malone, and Fred Snyder recently obtained evidence for a mixed-function oxidase in the biosynthesis of ethanolamine plasmalogens from 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine (20). Our earlier results had demonstrated that the postmitochondrial fraction of Ehrlich ascites cells and preputial-gland tumors can synthesize ethanolamine plasmalogens from long-chain fatty alcohols and dihydroxyacetone phosphate or 1-alkyl-2-acyl-*sn*-glycero-3-phosphate when incubations are carried out in the presence of $NADP^+$. The fact that almost all the *O*-alk-1-enyl lipids synthesized were in the ethanolamine fraction suggested to us that the conversion of an *O*-alkyl moiety to an *O*-alk-1-enyl moiety occurred on intact 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine (alkylacyl-GPE). Furthermore, the stimulatory effect of CDP-ethanolamine on the formation of plasmalogens in these systems also implied alkylacyl-GPE as the precursor. Our current studies with alkylacyl-GPE and 1-alkyl-*sn*-glycero-3-phosphorylethanolamine, both labeled with ^{14}C in the alkyl moiety, have shown that alkylacyl-GPE is converted to 1-alk-1-enyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine (plasmalogen) by enzyme preparations from tumors (Fig. 48). Tables 37 and 38 demonstrate that the conversion of the *O*-alkyl to *O*-alk-1-enyl linkage requires molecular oxygen and a reduced pyridine nucleo-

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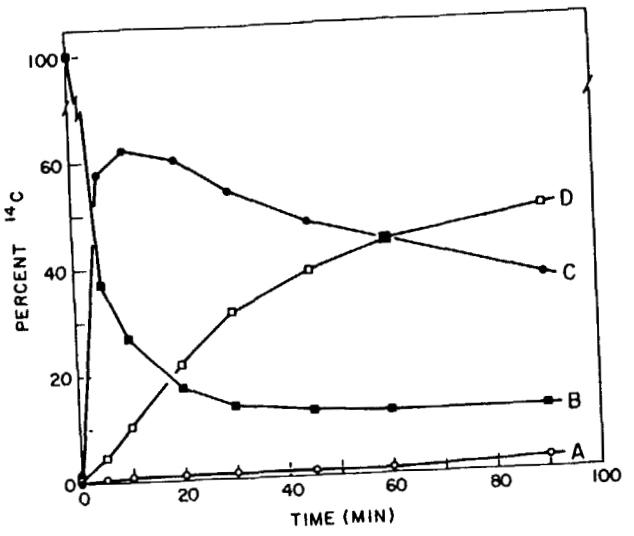


Fig. 48. Time course of reaction for the synthesis of ethanolamine plasmalogens by the postmitochondrial fraction of the Fischer sarcoma. Incubations contained ATP (10 mM), Mg^{++} (4 mM), NADP+ (2 mM), CoA (0.1 mM), and 6 nmoles of [^{14}C] alkyl-GPE in a total volume of 3 ml of Tris-HCl buffer (0.1 M, pH 7.1). Recoveries of total ^{14}C from the incubations were 101 ± 4.5% standard deviation. All values on the graph show only the polar glycerolipids containing either O-alkyl or O-alk-1-enyl moieties. A, alk-1-enyl-GPE; B, alkyl-GPE; C, alkylacyl-GPE; and D, alk-1-enylacyl-GPE.

TABLE 37

EFFECT OF OXYGEN, CYANIDE, AND CARBON MONOXIDE ON CONVERSION OF [1-^{14}C] ALKYL-GPE INTO ETHANOLAMINE PLASMALOGENS BY MITOCHONDRIAL SUPERNATANTS FROM FISCHER SARCOMAS*

Sample	Gas phase	KCN (1 mM)	Percentage of [^{14}C] ethanolamine phospholipids as plasmalogens†
1	O_2	–	30, 28
2	O_2	+	10, 6
3	O_2-CO (10:90, v/v)	–	35, 31
4	Argon‡	–	1.4, 0.91
5	Air‡	–	40, 35

* Each vial contained [1-^{14}C] alkyl-GPE (≃6 nmoles), ATP (10 mM), $MgCl_2$ (4 mM), NADP (2 mM), CoA (0.1 mM), D-glucose-6-P (6 mM), glucose-6-P dehydrogenase (5 units), and postmitochondrial supernatant (40 mg protein) in a final volume of 3 ml of 0.1 M Tris-HCl buffer, pH 7.1. All vials were tightly capped, except as noted for Samples 4 and 5, and were incubated at 37°C for 60 min.

† Of the [1-^{14}C] alkyl-GPE added, 80 to 90% was acylated in all the samples.

‡ Bubbled continuously through vials containing a needle inlet and outlet.

TABLE 38

EFFECTS OF OXIDIZED AND REDUCED PYRIDINE NUCLEOTIDES ON BIOSYNTHESIS OF ETHANOLAMINE PLASMALOGENS BY MICROSOMES FROM FISCHER TUMORS*

Additions	Percentage of [^{14}C] ethanolamine phospholipids as plasmalogens†
None	1.3, 1.3
$NADP^+$ (2 mM)	2.0, 1.6
NAD^+ (2 mM)	2.8, 2.3
NADPH‡	10.3, 10.8
NADH (2 mM)	13.5, 12.0

* The complete system contained microsomes (washed once) from Fischer tumors (6.7 mg protein), ATP (10 mM), CoA (0.1 mM), Mg^{++} (4 mM), and [^{14}C] alkyl-GPE ($\simeq$ 20 nmoles) in a total volume of 3 ml of Tris-HCl buffer (0.1 M, pH 7.1). Incubations were carried out for 90 min at 37°C.

† 50 to 60% of the alkyl-GPE was acylated.

‡ Contained $NADP^+$ (2 mM), D-glucose-6-P (6.7 mM), and 5 units of glucose-6-P dehydrogenase.

TABLE 39

EFFECT OF ATP, Mg^{++}, $NADP^+$, AND NAD^+ ON BIOSYNTHESIS OF [^{14}C] ALK-1-ENYLACYL-GPE FROM [^{14}C] ALKYLACYL-GPE BY MITOCHONDRIAL SUPERNATANTS FROM FISCHER SARCOMAS

System	Percentage of [^{14}C] ethanolamine phospholipids as plasmalogens*	
	Experiment 1	Experiment 2
Complete† + $NADP^+$ (2 mM)	13.4	
Complete† + NAD^+ (2 mM)	13.4	8.4
No. 1 minus ATP	3.1	
No. 1 minus Mg^{++}	7.6	
No. 2 minus ATP	3.4	1.8
No. 2 minus Mg^{++}		5.2
No. 2 minus NAD^+	1.0	1.0

* Average of duplicate samples.

† Complete system contained ATP (10 mM), Mg^{++} (4 mM), [1-^{14}C] alkylacyl-GPE (12 nmoles), and 1.5 ml of the mitochondrial supernatant from Fischer tumors ($\simeq$ 30 mg protein) in a final volume of 3 ml of Tris-HCl buffer (0.1 M, pH 7.1). All incubations were carried out at 37°C for 90 min.

tide (either NADH or NADPH) and is inhibited by cyanide, but not by carbon monoxide. The data implicate the involvement of a microsomal electron transport system that requires cytochrome b_5 instead of P-450 in the biosynthesis of plasmalogens. These findings indicate that a microsomal mixed-function oxidase, similar to the one required in the biosynthesis of monoenoic fatty acids, is involved in the biosynthesis of ethanolamine plasmalogens; ATP and Mg^{++} stimulated the synthesis of plasmalogens (Table 39).

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REGULATORY MECHANISMS IN THE BIOSYNTHESIS OF ETHER AND ESTER BONDS IN GLYCEROLIPIDS

In earlier experiments, based on compositional analysis and ^{14}C tracers, we found that tissue culture cells in suspension culture synthesize large quantities of ether-linked lipids, whereas the same cells grown as monolayers do not. We are currently carrying out more detailed experiments on the various factors involved in this phenomenon, since these tissue culture systems appear to be ideal for investigating the mechanisms that regulate the metabolism of ether and ester bonds in glycerolipids. In these studies, we (M. L. Blank and Fred Snyder) compared the biosynthesis of alkyldiacylglycerols from [1-^{14}C]hexadecanol in L-M cells in suspension and monolayer cultures. Cells in suspension cultures synthesized relatively large amounts of alkyldiacylglycerols (5 to 10% of the ^{14}C label) compared to the cells grown as monolayers containing < 1% of the ^{14}C label incorporated into ether lipids. The maximum ^{14}C incorporated into alkyldiacylglycerol fraction, as well as most other lipid classes, occurred about 3 hr after incubating the [1-^{14}C]hexadecanol in the suspension cultures; the optimum cell concentration was approximately 1×10^6 per ml. Analysis of the labeled alkyldiacylglycerols, isolated from the lipids of cells in suspension cultures, verified its structure and showed that little, if any, modification of the chain length of the hexadecanol had occurred during the incubations. In some experiments, the *O*-alkyl moieties of glycerolipids in cells grown in suspension cultures were prelabeled with ^{14}C so that the turnover of the *O*-alkyl moieties could be traced when these cells were transferred to monolayer cultures. After the prelabeled cells grew overnight as monolayers, they contained essentially no ^{14}C label in the *O*-alkyl moieties although substantial amounts of labeled acyl groups were still present. Further work is warranted with the two different culture methods since these systems should serve as a valuable model for investigating regulatory controls and the function of lipids.

BIOCHEMICAL STUDIES OF INTRACELLULAR AND SURFACE MEMBRANES IN MAMMALS

It is recognized that lipid, together with protein, forms an essential part of cellular membranes. Several investigators (Arias et al., J. Biol. Chem. 244, 3303, 1969; Widnell and Siekevitz, J. Cell Biol. 35, 142A, 1967), based mainly on the results from the turnover studies of different membrane proteins, have suggested that all membranes within hepatocytes are in a dynamic state with a similar overall rate of renewal. Ten-ching Lee, Nelson Stephens, Anita Moehl, and Fred Snyder (30) have tested this concept by investigating the turnover rates of various phospholipids in the plasma membranes of normal rat liver, determined by a single-isotope administration method and double-labeling technique using acetate and glycerol as precursors; we have compared these results with those obtained for microsomal membranes. We isolated the plasma membranes using a combination of the methods of Berman et al. (Biochim. Biophys. Acta 183, 10, 1969) and Neville (Biochim. Biophys. Acta 154, 540, 1968); the microsomes were prepared in a conventional manner.

The following enzyme markers were used to check the purity of the membrane fractions: 5′-nucleotidase (EC 3.1.3.5), characteristic of plasma

membranes; cytochrome oxidase (EC 1.9.3.1), a mitochondrial marker; and glucose-6-phosphatase (EC 3.1.3.9), a microsomal marker.

Individual phospholipid classes of plasma membranes had similar half-lives to those of microsomal membranes, except that the sphingomyelin of the plasma membranes turned over more slowly than that of microsomal membranes. Within a given membrane fraction, the various phospholipid classes are degraded at different rates (Fig. 49, Tables 40 and 41).

The exact mechanism(s) for the degradation of membrane phospholipids is still unclear, but apparently one or more of the phospholipases present in microsomes, plasma membranes, and lysosomes is involved. Since the half-lives of certain plasma membrane phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol + phosphatidylserine) approach that of microsomal phospholipids, it would seem there is a dynamic relationship between these two membrane systems even though there are pronounced differences in their biosynthetic enzymes. For example, plasma membranes do not possess the enzyme(s) required for the de novo synthesis of nitrogen-containing phospholipids such as phosphatidylcholine; the de novo synthesis of these

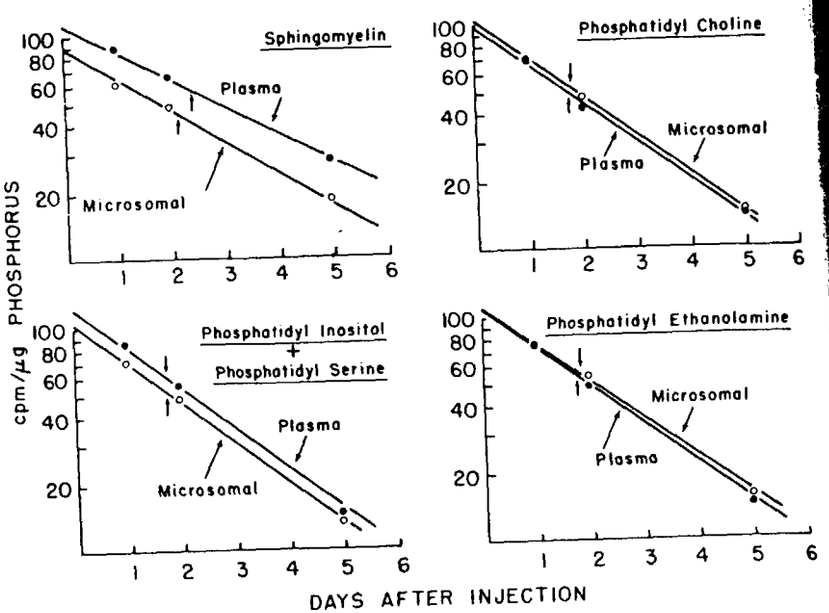


Fig. 49. Decay of specific radioactivity of individual phospholipids in plasma membranes and microsomes after injecting sodium-[2-^{14}C]acetate. Rats weighing 150 to 165 g each were injected intraperitoneally with sodium-[2-^{14}C]acetate (26 μ moles and 100 μ Ci/100 g body weight); the animals were killed at the time indicated. Arrows denote half-life times based on graphical extrapolation.

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TABLE 40

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN PLASMA AND MICROSOMAL MEMBRANES OF RAT LIVERS AFTER THE ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE*

Phospholipid	Ratio ^{3}H/^{14}C	
	Plasma membrane	Microsomal membrane
Sphingomyelin	0.4	0.7
Phosphatidylcholine	2.2	2.3
Phosphatidylinositol	1.2	1.1
Phosphatidylserine	1.6	1.5
Phosphatidylethanolamine	1.9	2.1

* Each value is the mean of duplicate samples. Rats were first injected with the ^{14}C compound and then 60 hr later were injected with the ^{3}H compound; animals were killed 4 hr after receiving the ^{3}H.

TABLE 41

RELATIVE TURNOVER OF PHOSPHOLIPID CLASSES IN PLASMA AND MICROSOMAL MEMBRANES OF RAT LIVERS AFTER THE ADMINISTRATION OF ^{14}C- AND ^{3}H-LABELED ACETATE*

Phospholipid	Ratio ^{3}H/^{14}C	
	Plasma membrane	Microsomal membrane
Sphingomyelin	0.5	1.1
Phosphatidylcholine	5.7	5.9
Phosphatidylinositol + phosphatidylserine	2.5	2.3
Phosphatidylethanolamine	5.8	6.9

* Each value is the mean of duplicate samples. Rats were first injected with the ^{14}C compound and then 60 hr later were injected with the ^{3}H compound; animals were killed 4 hr after receiving the ^{3}H.

phospholipids apparently occurs exclusively in the endoplasmic reticulum. Thus, the supply of phospholipid components of the plasma membrane appears to depend totally on the endoplasmic reticulum. The exchange of intact phospholipid molecules between membrane organelles might play an important role in such processes.

CHEMICAL IDENTIFICATION AND ENZYMIC SYNTHESIS OF A NEWLY DISCOVERED LIPID CLASS — HYDROXYALKYLGLYCEROLIPIDS

The harderian gland, deep within the orbit of the eye in animals that possess a nictitating membrane, is thought to be an accessory lacrimal gland. It secretes an oily substance that facilitates movement of the third eyelid. Previous studies of the lipids from normal harderian glands (K. Kasama, N. Uezumi, and S. Hasegawa, 1971, unpublished data) and their tumors (Kasama et al., Biochim. Biophys. Acta 202, 56, 1970; Itoh and Kasama, Gann 61, 271, 1970) in mice have demonstrated that these glands are extremely rich (60 to 70 wt%) in glycerolipids that contain O-alkyl moieties.

During a study of the *O*-alkyl lipids in the harderian glands, K. Kasama,* W. T. Rainey, Jr.,† and Fred Snyder (27) isolated and identified a new class of alkyl glycerolipids in lipid extracts from the pink portion of the harderian glands of rabbits. After $LiAlH_4$ reduction, the esterified lipid yields hydroxyalkylglycerols. Identification was based on chromatography, infrared spectroscopy, and mass spectra of various derivatives of this lipid and of standards prepared chemically (Fig. 50). The isomers of the hydroxyalkylglycerolipids (HAG) isolated consisted of *O*-hexadecyl moieties with hydroxyl groups at C-10 and C-11 and of *O*-octadecyl moieties with hydroxyl groups at C-11 and C-12. In the natural state, all the hydroxy groups are thought to be esterified with aliphatic moieties, and this lipid class accounts for approximately 80% of the total lipids of the pink harderian gland of rabbits. Enzymic experiments demonstrated that [1-^{3}H]octadecane-1,12-diol was readily incorporated into the HAG when

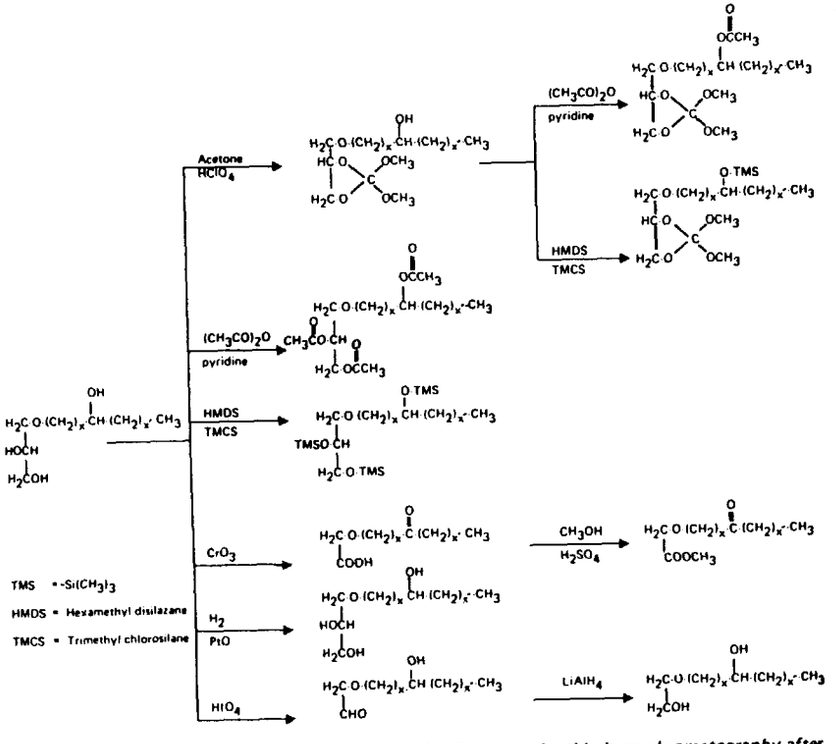


Fig. 50. Chemical reactions used to identify the HAG isolated by thin-layer chromatography after $LiAlH_4$ reduction, Vitride reduction, or saponification of the purified unknown ether lipid (U) isolated from lipid extract of the pink harderian glands of rabbits.

* Present location: Mie Prefectural University School of Medicine, Tsu, Japan.
† Oak Ridge National Laboratory.

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TABLE 42

EFFECT OF DIHYDROXYACETONE PHOSPHATE AND COFACTORS ON THE BIOSYNTHESIS OF HYDROXY-SUBSTITUTED ALKYL GLYCEROLIPIDS BY A MICROSOMAL FRACTION PREPARED FROM THE PINK PORTION OF HARDERIAN GLANDS FROM RABBITS

Incubation system	Percent ^{3}H incorporated into hydroxyalkylglycerols*	
Complete†	23.3,	23.8
Complete minus dihydroxyacetone phosphate	2.5,	4.8
Complete minus CoA, ATP, and Mg^{++}	2.4,	3.0
Complete minus dihydroxyacetone phosphate, CoA, ATP, and Mg^{++}	1.9,	2.2
Complete except microsomes were placed in boiling water bath for 15 min before incubation	2.63,	2.64

* Liberated from total lipid extract after reduction with $NaAlH_2(OCH_2CH_2OCH_3)_2$.

† [1-^{3}H] Octadecane-1, 12-diol (0.4 μCi, 100 nmoles/vial), ATP (10 mM), CoA (100 μM), $MgCl_2$ (4 mM), dihydroxyacetone phosphate (1.66 mM), and microsomes of pink portion of the harderian gland (1 to 2 mg protein/vial).

dihydroxyacetone phosphate, CoA, ATP, Mg^{++}, and the microsomal fraction of the pink harderian gland were included in the incubations. (Table 42).

These experiments indicate that the biosynthesis of the ether linkage in hydroxy-substituted alkyl glycerolipids of the rabbit harderian gland can proceed in a manner analogous to that observed in cancer cells. We do not know how the hydroxy group is substituted on the fatty alcohol in this gland. Probably a hydroxy-substituted fatty acid is reduced to the alcohol by acyl reductases, enzymes now well documented in many biological systems. The natural occurrence of vaccenic acid and its double-bond location (C-11) indicates that suitable precursors of the hydroxy-substituted *O*-alkyl moiety in glycerolipids are available in mammalian cells.

THE METABOLISM OF ALKYLETHYLENEGLYCOLS IN MAMMALIAN CELLS

Alkylethyleneglycol lipids have been identified in nature by a Russian group. They occur as analogs of their glycerolipid counterparts. Essentially nothing is known about the metabolism of alkylethyleneglycols, but possibly they are formed from alkylglycerols by oxidation of carbon atom 3 on the glycerol moiety. Because the glycol lipids are closely related to the alkylglycerols, we (Fred Snyder, Boyd Malone, and Claude Piantadosi) examined a number of key reactions in which the alkylglycerols participate to determine if the alkylethyleneglycol lipids can be metabolized in the same manner. Our results demonstrate that the alkylethyleneglycols can serve as substrates for acyltransferases in rat liver and tumors (Table 43) and that the *O*-alkyl moiety can be cleaved by a tetrahydropteridine-requiring hydroxylase in rat liver microsomes (Table 44). However, even though the alkylethyleneglycols appear to be metabolized in a manner similar to alkylglycerols, we do not know whether the same or separate enzymes are involved.

TABLE 43

ACYLATION OF 1-*O*-[1-^{14}C]HEXADECYL-[2-^{3}H]ETHYLENEGLYCOL

System*	Homogenate source	^{14}C	^{3}H
		nMoles alkylacylethyleneglycol formed	
Complete	Rat liver	9.66 8.75	6.90 8.33
Minus ATP, CoA	Rat liver	10.07 11.00	11.17 7.06
Complete	Preputial-gland tumor	18.72 15.08	15.99 15.18
Minus ATP, CoA	Preputial-gland tumor	2.62 1.42	1.26 1.19

* Complete system contained Tris buffer (300 μmoles, pH 7.1), KCl (78 μmoles), ATP (30 μmoles), $MgCl_2$ (12 μmoles), NaF (125 μmoles), cysteine (25 μmoles), CoA (0.3 μmole), preputial-gland tumor homogenate (15 mg protein), or rat liver homogenate (21 mg protein) in a total volume of 3.0 ml, incubated for 20 min at 37°C.

TABLE 44

ENZYMIC CLEAVAGE OF 1-*O*-[1-^{14}C]HEXADECYL-[2-^{3}H]ETHYLENEGLYCOL

System*	Microsomal source	nMoles [1-^{14}C] palmityl aldehyde
Complete	Rat liver	22.54; 19.77
Minus $PteH_4$	Rat liver	0.02; 0.46
Complete	Preputial-gland tumor	0.03; 0.35
Minus $PteH_4$	Preputial-gland tumor	0.06; 0.07

* Complete system contained borate-KCl buffer (40 mM each, pH 9.0), $(NH_4)_2SO_4$ (24 mM), GSH (10 mM), $PteH_4$ (0.75 mM), rat liver microsomes washed three times (0.217 mg protein), preputial-gland tumor microsomes (0.496 mg protein) in a total volume of 1.0 ml, incubated for 10 min at 37°C.

URETHAN-INDUCED PULMONARY ADENOMA AS A MODEL FOR THE STUDY OF SURFACTANT BIOSYNTHESIS

The lung has only recently been thought of as an active organ with a wide range of metabolic functions. One is the production of pulmonary surfactant, the substance that lines the alveolar wall and maintains alveolar stability during compression of the lung. When surfactant production is insufficient, as in hyaline membrane disease or pulmonary artery occlusion, alveolar collapse ensues.

Researchers have collected surfactant by pulmonary artery perfusion, by lung lavage, and by sucrose gradient centrifugation of a whole minced lung. These various preparations are called "surfactant" by virtue of their ability to produce a typical hysteresis curve when area is plotted against surface tension as measured on a Wilhelmy balance. Chemically, they are mostly phospholipid, but some workers disagree on the amount of binding to proteins and carbohydrates and on the minor species of phospholipids present. Investigators do agree that lung surfactant, no matter how obtained, is unusual in that its major lipid (1,2-dipalmitoyl-*sn*-glycero-3-*O*-phosphocholine*) is saturated at both the 1- and 2-positions. This chemical structure is, however, well suited to surface activity, since disaturation lends a compactness to the glycerolipid molecule that permits a film containing it to undergo compression without "squeeze-out" of components and loss of integrity. Exactly how the lung produces so much disaturated phospholipid, when the specificity of the biosynthetic enzyme in most tissues tends to produce unsaturation at the 2-position of the glycerol moiety of phospholipids, is not known. No one knows where pulmonary surfactant is synthesized. Although the preponderance of data suggests that the alveolar type II epithelial cells are involved, other data indicate that synthesis occurs in the nonciliated Clara cells of the bronchioles, the type II cells serving only to control the surfactant level by phagocytosis.

Interpreting previous data has been difficult, since both lung lavage and whole lung preparations yield material derived from mixed cell populations. However, it is well known that the urethan-induced pulmonary adenoma in mice is an alveolar type II cell tumor, and we thought that a cell-free preparation of it might be ideal for studying the biosynthesis of pulmonary surfactant and related molecular species of lipid in a uniform cell population. A squamous-cell carcinoma of the lung was chosen for comparison with the adenoma to demonstrate which metabolic pathways for ester- and ether-linked lipids are characteristic of a neoplasm rather than of a specific cell population. Both the squamous-cell carcinoma and the adenoma are derived from the respiratory tract and are induced by a chemical carcinogen, but the adenoma is a primary lesion of well-differentiated, slowly proliferating epithelial cells while the squamous-cell carcinoma is transplanted and contains a variety of rapidly growing nondifferentiated cells.

Cathy Snyder,† Boyd Malone, Paul Nettesheim,† and Fred Snyder have obtained data demonstrating that the adenoma preparation does have a great capacity for synthesizing 1,2-dipalmitoyl-*sn*-glycero-3-*O*-phosphocholine, adding strong support to the theory that the type II alveolar cell is the source of pulmonary surfactant. Adenoma homogenates also produce alkyl glycerolipids from [1-^{3}H]hexadecanol, as do other tumors, including the squamous-cell lung carcinoma that we tested. The carcinoma, although actively utilizing palmitic acid, did not synthesize phosphatidylcholine nearly as much as the adenoma.

Adding Ca^{++} increased the synthesis of phosphatidylcholine from palmitic acid while decreasing total incorporation into lipids (Table 45). Since Ca^{++} inhibits CDP-base transferase activity, these data demonstrate that the major synthesis of disaturated phosphatidylcholine in the lung does not take place via the CDP-choline pathway nor by methyl transferase activity. Base addition probably occurs directly on phosphatidic acid or by an exchange mechanism, and our data indicate that disaturation on the glycerol moiety occurs before base addition when glycero-3-phosphate or dihydroxyacetone phosphate are used as precursors.

*Phosphatidylcholine.

†Oak Ridge National Laboratory.

1028168

TABLE 45

EFFECT OF THE ADDITION OF COFACTORS* ON THE INCORPORATION OF [1-^{14}C]PALMITIC ACID INTO VARIOUS LIPID CLASSES BY PREPARATIONS OF MOUSE LUNG

	FFA†	PC	PA	PE	TG	FFA	PC	PA	PE	TG
	Pulmonary adenoma homogenate					*Control mouse lung homogenate*				
Complete‡	4.0	39	25	3.2	9.6	54	7.6	12	3.0	9.5
+ CDP-choline	2.5	40	37	3.4	3.8	50	9.8	16	3.0	6.9
+ CDP-ethanolamine	3.6	32	34	9.9	5.7	51	7.5	16	3.8	9.6
+ $CaCl_2$	19.6	47	20	4.1	0.9	68	10.4	10	0.94	1.7
	Squamous-cell carcinoma homogenate					*Adenoma-bearing lung homogenate*				
Complete‡	5.6	9.7	34	4.5	20	61	12	10	4.8	1.4
+ CDP-choline	6.3	24	36	4.4	12	55	17	14	4.8	1.1
+ CDP-ethanolamine	6.0	11	33	11	19	54	15	11	6.0	2.0
+ $CaCl_2$	29	15	38	4.6	4	56	19	14	1.3	1.2

* When α-glycerophosphate, dihydroxyacetone phosphate, CoA, ATP, and Mg^{++} were omitted from the system, or when boiled homogenate was used, 99.1% of the activity remained in the fatty acid section.

† Abbreviations: FFA, free fatty acid; PC, phosphatidylcholine; PA, phosphatidic acid; PE, phosphatidylethanolamine; TG, triglyceride.

‡ The complete system contained homogenate (1 to 2 mg protein), 100 mM Tris buffer at pH 7.1, 100 μM CoA, 10 mM ATP, 4 mM $MgCl_2$, 1.67 mM dihydroxyacetone phosphate, 1.67 mM α-glycerophosphate, 33 μM [1-^{14}C] palmitic acid, and 33 μM [1-^{3}H] hexadecanol in a total volume of 3 ml; incubations were done at 37°C for 90 min. The vehicle used for hexadecanol and palmitic acid in our system was 20 μl of 95% ethanol. Other components were added to the complete system in some experiments: 20 mM $CaCl_2$, 3.3 mM CDP-choline, or 3.3 mM CDP-ethanolamine.

LIPID METABOLISM IN REPRODUCTIVE TISSUES

Although the metabolic pathways for the biosynthesis of alkyl and alk-1-enyl glycerolipids have been relatively well characterized in cancer cells, little is known about the turnover of the acyl moiety in these classes. Previous work has demonstrated that arachidonic acid is present in higher amounts in the ethanolamine plasmalogens than in the phosphatidylethanolamine of Ehrlich ascites cells. Rat testes are known to contain relatively large amounts of ethanolamine plasmalogens, and one of the histological changes in animals reared on diet deficient in essential fatty acid is the degeneration of spermatogenic tissue. Thus, to assess the possible role of plasmalogens in this deficiency, we examined the metabolism of tritium-labeled arachidonic acid in testicular tissue of rats maintained on an essential fatty acid-deficient and control diets (M. L. Blank, Robert L. Wykle, and Fred Snyder). We found that the essential fatty acid is retained to a greater extent in the testicular lipids of the animals fed the diet deficient in essential fatty acid. More specifically, it is conserved in the ethanolamine plasmalogens.This specific lipid subclass actually showed a net increase in the content of [^{3}H]arachidonic acid over the 72-hr period studied. The most plausible explanation is that the arachidonic acid is incorporated into the

1028169

plasmalogens by an acyl exchange reaction not involving the well known Kennedy pathway. We tested this theory by incubating various labeled substrates with appropriate cofactors and a postmitochondrial supernatant fraction of rat testes. We found [^{3}H]arachidonic acid was incorporated into the 2-position of preformed phosphatides under conditions where the CDP-ethanolamine pathway is completely blocked by calcium ions, results consistent with those expected if an exchange mechanism were responsible.

LIPID METABOLISM IN NERVOUS TISSUE

The conversion of *O*-alkyl moieties to *O*--alk-1-enyl groups in glycerolipids occurs in vivo and with cell-free systems. In tumor preparations, the cofactors involved in this reaction are ATP. Mg^{++}, and NADPH or NADH; the reaction, inhibited by cyanide but not by carbon monoxide, has a requirement for molecular oxygen. Thus the studies in cancer cells indicate that the biosynthesis of the *O*-alk-1-enyl bond is catalyzed by a mixed-function oxidase system similar to that involved in the desaturation of fatty acids.

Brain and other nervous tissue are also rich sources of alk-1-enyl glycerolipids. Since almost half of the ethanolamine phospholipids in brain exist as plasmalogens, we thought it important to determine if plasmalogens are formed in nervous tissue as they are in tumors. Furthermore, the correlation between myelination and plasmalogen formation emphasized that these lipids play an important role in developmental and degenerative processes affecting nerve cells.

In these studies, we (M. L. Blank, Robert L. Wykle, and Fred Snyder) found that ethanolamine plasmalogens were synthesized from [1-^{14}C]alkyl-*sn*-glycero-3-phosphorylethanolamine by mitochondrial supernatants of brains obtained from 12 to 14-day-old rats (3) (Fig. 51). The desaturation of the *O*-alkyl moiety to the *O*-alk-1-enyl moiety occurs on intact 1-alkyl-2-acyl-*sn*-glycero-3-phosphorylethanolamine agreeing with our earlier data obtained with tumor preparations.

CHEMICAL SYNTHESIS OF METABOLICALLY ACTIVE INTERMEDIATES INVOLVED IN LIPID BIOSYNTHESIS

We (Claude Piantadosi, Kun Chae,* Khalid S. Ishaq,* and Fred Snyder) (15) have recently described the synthesis of acyldihydroxyacetone phosphate which was originally characterized by Hajra and Agranoff and found to occur in guinea pig liver (J. Biol. Chem. 243, 1617, 1968). It is formed enzymatically by acylation of dihydroxyacetone phosphate in the presence of acyl-CoA, ATP, and Mg^{++} as cofactors. Agranoff and Hajra (Proc. Natl. Acad. Sci. 68, 411, 1971) also reported on the participation of the acyldihydroxyacetone phosphate pathway in mouse liver and Ehrlich ascites tumor cells, and suggested that this pathway plays an important role in these cells. Furthermore, Hajra (Biochem. Biophys. Res. Commun. 37, 486, 1969; and 39, 1037, 1970) and we (Robert L. Wykle, Claude Piantadosi, and Fred Snyder) (21) found that acyldihydroxyacetone serves as a precursor of alkyl glycerolipids.

* School of Pharmacy, University of North Carolina, Chapel Hill.

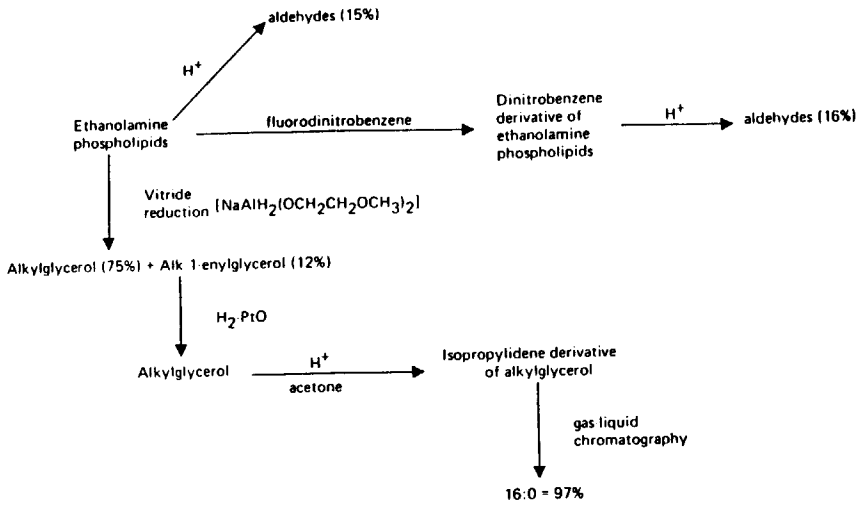
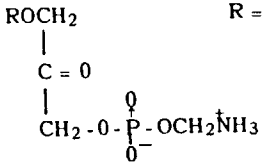


Fig. 51. Identification of [^{14}C]ethanolamine plasmalogens formed from [1-^{14}C]alkyl-sn-glycero-3-phosphorylethanolamine by a cell-free system prepared from rat brain. The percentages of ^{14}C in the indicated products from each procedure are shown.

The complete chemical synthesis of acyldihydroxyacetone phosphates is based on an earlier procedure for the synthesis of 1-*O*-alkyldihydroxyacetone derivatives. The cyclohexylammonium salts of 1-*O*-palmitoyl-2,2-dimethoxypropane-3-phosphate (Fig. 52), 1-*O*-acetyl-2,2-dimethoxypropane-3-phosphate, and 1-*O*-palmitoyl-2,2-dimethoxypropane-3-phosphorylethanolamine were prepared.

In related studies, we described the chemical synthesis and characterization of a new class of ether-linked keto phospholipids (Claude Piantadosi, Khalid S. Ishaq, M. L. Blank, and Fred Snyder) (16), 1-*O*-alkyl-3-*O*-phosphorylethanolamine-2-propanones:



The overall reaction sequence is illustrated in Fig. 53. We isolated ketal derivatives of the products and purified them by column chromatography on silica gel in an overall yield of 21 and 27%. The availability of these new keto phospholipids could be important in biosynthetic studies of acyl glycerolipids, plasmalogens, and related derivatives.

1028170

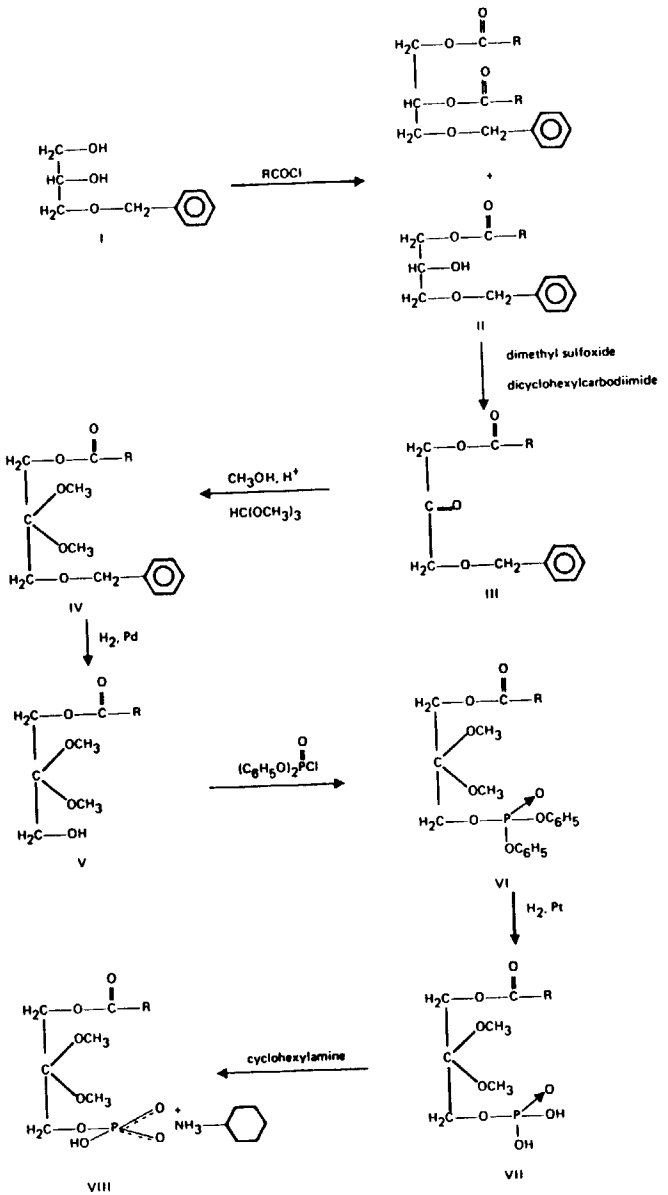


Fig. 52. Synthesis of 1-O-palmitoyl-2, 2-dimethoxypropane-3-phosphate cyclohexylammonium salt (R = $C_{15}H_{31}$).

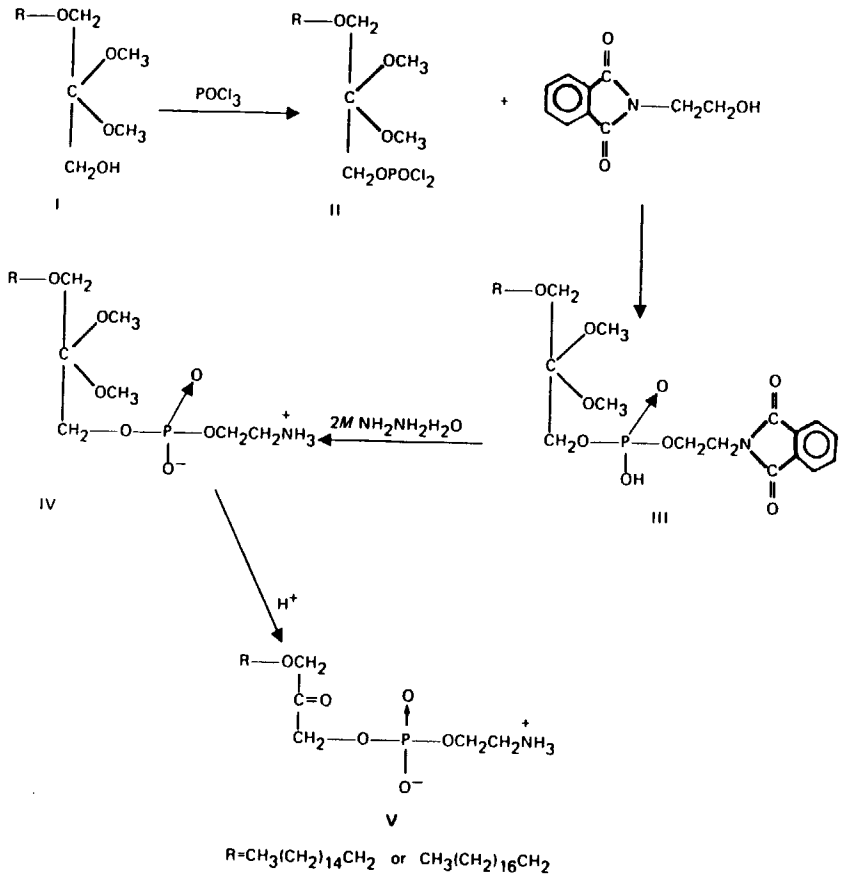


Fig. 53. Synthesis of 1-O-hexadecyl-2, 2-dimethoxypropane-3-O-phosphorylethanolamine and 1-O-octadecyl-2, 2-dimethoxypropane-3-O-phosphorylethanolamine.

ANALYTICAL PROCEDURES FOR DETECTING AND RESOLVING METABOLIC INTERMEDIATES AND THEIR DERIVATIVES

Detection of initial substrates and final products labeled with ^{14}C or ^{3}H present in metabolic systems is generally possible with most currently available radioassay instrumentation and techniques. In contrast, to detect the low

1028171

quantities of radioactivity found in many metabolic *intermediates* one often has a difficult and challenging problem, particularly if the functional groupings on the intermediary molecular species are very similar. Except for liquid scintillation assay and scraping techniques (1- and 2-mm zones), most radiometric procedures and instruments cannot detect activity levels only slightly above background nor resolve barely separable components with statistically good quantitation of their radioactivity.

An introductory lecture on radioisotopes by Fred Snyder at the Fifth International Symposium on Quantitative Flat-Bed Chromatography (Carlsbad, Czechoslovakia, September 1972) (35A) illustrated how very closely related labeled molecules produced in biochemical systems can be detected and assayed after chromatography on thin layers of silica gel. An application of this approach to the analysis of metabolites involved in the biosynthesis of alkyl glycerolipids is illustrated in Fig. 54. It emphasizes the problem of isotopic cross contamination caused by trailing of radioactivity from one compound into an adjacent zone containing a different compound. Techniques for detecting radioactivity are extremely sensitive compared to the detection of mass; therefore, the trailing of components is sometimes not obvious when the separated components on the chromatogram are visualized by spray reagents or iodine vapor. On the other hand, zonal profile analysis of the isotopic distribution along the lane will clearly show gross contamination of unlabeled components in the system. Unless the trailing of a radioactive peak is recognized, one would mistakenly assume that the radioactivity associated with a particular "spot" on a chromatogram is derived from that component visualized by the mass detecting reagent. Radioactive zonal profile scans reveal maximal information about cross contamination of components on the chromatogram.

SYNTHESIS OF FATTY ACID ESTERS OF SHORT-CHAIN ALCOHOLS BY AN ACYLTRANSFERASE IN MICROSOMES OF RAT LIVER

During the course of a series of experiments investigating the acylation of glycero-3-phosphate by microsomes of rat liver, it was noted that the label from fatty acids which had been dissolved in ethanol was extensively incorporated into an unidentified fraction. Subsequently, we identified this fraction as ethyl esters of fatty acids using silver-ion thin-layer chromatography and preparative gas-liquid chromatography (M. R. Grigor and Iverson C. Bell, Jr.*). The ethyl esters were formed when the microsomes were incubated in the presence of ethanol, radioactive fatty acids, CoA, ATP, and Mg^{++} ions (Table 46). The biosynthesis of ethyl esters was optimal at a pH near 8. Other primary short-chain alcohols were also esterified and the Km for the alcohol decreased with increasing chain length from methanol to hexanol. The requirements of CoA and ATP in these systems are consistent with those of an acyltransferase.

* Student trainee from Morehouse College, Atlanta, Georgia.

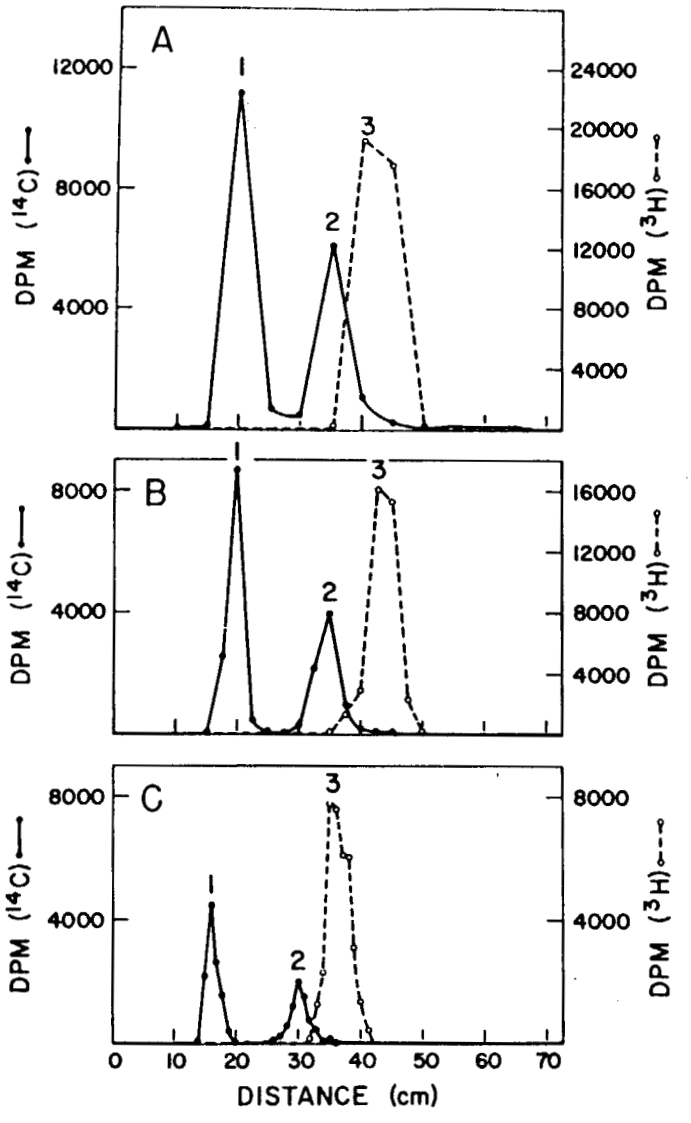


Fig. 54. A ^{14}C, ^{3}H-zonal profile scan of a model mixture of standards consisting of 1) [1-^{14}C] hexadecylglycerol, 2) [1-^{3}H] hexadecanol, and 3) [1-^{14}C] hexadecyldihydroxyacetone. Chromatography was carried out on thin layers of Silica Gel G developed in a system of hexane-diethyl ether-methanol-acetic acid (80:20:5:1). The letters A, B, and C designate the 1-cm, 5-mm, and 2-mm scans, respectively.

1028172

TABLE 46

SYNTHESIS OF ETHYL ESTERS BY MICROSOMES OF RAT LIVER

	Percent of recovered activity as ethyl ester*
Complete†	17.1, 18.5
Minus ethanol	0.7, 1.2
Minus CoA	0.4, 0.7
Minus ATP	0.8, 1.0
Minus Mg^{++}	2.9, 3.5
Plus NaF	17.6, 18.3
Enzyme heated 100°C for 15 min	0.2, 0.3

* Duplicate incubations.

† The incubation system contained 10 μM CoA, 10 mM ATP, 4 mM $MgCl_2$, 5 mg microsomal protein, 500 nmoles of (9,10-^{3}H)palmitic acid (0.5 μCi) as its potassium salt added dissolved in 10^{-2} M acetone solution, and sodium fluoride (20 mM) as indicated, in 3 ml of 0.1 M Tris-HCl buffer at pH 7.2. Incubations were carried out at 37°C for 5 min. The ethanol concentration was 0.28 M.

MARMOSET RESEARCH PROJECT

FURTHER STUDIES ON BLOOD FACTORS IN MARMOSETS

Previous studies demonstrated a blood factor on the red blood cells of marmosets. This appeared to be restricted to one subspecies of *Saguinus fuscicollis, S.f. illigeri*, and was accordingly noted as $_{s.f.}A^{i}$, where the subscript identified the genus and species and superscript indicated subspecies specificity. Subsequent blood factors in this and other races of *S. fuscicollis* can be noted in the same manner, using a capital letter symbol for the new factor and the appropriate superscript to identify the subspecies. This has been done for two factors now identified in *S.f. lagonotus* and are noted as $_{s.f.}B^{1}$ and $_{s.f.}C^{1}$ (N. Gengozian and Mary L. Patton). When data on *S. fuscicollis* are presented, the notation has been abbreviated by omitting the subscript, e.g., A^{i}. Animals lacking the specific factors have been identified with corresponding lower case symbols, i.e., ä, b, or c.*

In contrast to the intrasubspecies immunizations used to develop the immune reagents specific for factor A^{i}, blood factors B^{1}, C^{1} were detected with reagents made by immunization of *S.f. illigeri* marmosets with *S.f. lagonotus* red cells. All three reagents contained incomplete antibodies only, i.e., those detected with the use of Coombs antiserum directed against marmoset immunoglobulins. Tests of these reagents with red cells of different species of *Saguinus* marmosets and different races of *S. fuscicollis* revealed a strict limitation to their specificity

* Symbols for blood factors and their corresponding antibodies are printed in bold-face type; symbols for genes and genotypes, in italics; symbols for agglutinogens, phenotypes, and blood group systems, in regular type. The lower-case letter a with an umlaut, ä, is so designated to avoid confusion in the text.

(Table 47). Factor A^i, present only among *S.f. illigeri*, appeared with a frequency of approximately 26% among these wild-caught populations, while factor C^1 was present in only 16% (4 of 25) of the *S.f. lagonotus* marmosets tested.

TABLE 47

TESTS FOR BLOOD FACTORS A^i, B^1, AND C^1 IN AN IMPORTED POPULATION OF *S. FUSCICOLLIS* SSP., *S. MYSTAX*, *S. OEDIPUS*, AND *S. NIGRICOLLIS**

Species	Number of animals with phenotypes: A^i	ä	B^1	b	C^1	c
S. fuscicollis ssp.						
illigeri	26	85	0	35	0	31
lagonotus	0	35	7	18	4	21
nigrifrons	0	24	0	20	0	22
leucogenys	0	10	0	8	0	8
? *illigeri* / *leucogenys* ?†	0	10	0	5	0	5
S. mystax	0	18	0	10	0	7
S. oedipus	0	20	0	20	0	17
S. nigricollis	0	9	0	9	0	7

* Does not include animals chimeric for blood factors since genotypes could not be established for all.

† Taxonomic classification of these animals uncertain.

Hemopoietic chimerism in the marmoset has been demonstrated exclusively through sex differentiation: chromosome identification in metaphase plates or the drumstick of the mature neutrophil. The availability of monospecific reagents for blood antigens permitted tests for chimerism independent of sex, yet also revealed the relationship between red and white cell chimerism in heterosexual chimeras. The results with blood factor A^i in *S.f. illigeri* marmosets are shown in Table 48. We used two methods to quantitate red cell mixtures: 1) counting of free unagglutinated cells, i.e., type ä, in a Coulter counter, and 2) FITC conjugated antisera (immune reagents specific for factor A^i and the Coombs reagent, rabbit antimarmoset gamma globulin) and subsequently identifying fluorescein-tagged A^i red cells. Note that these two techniques agreed remarkably well. In four of six heterosexual blood chimeras the degree of white and red cell chimerism correlated directly. For example, animal No. 1 was an 18% white blood cell chimera and approximately 17% of its red cell population was phenotypically ä. Conversely, animal No. 3, which had 24% white cell chimerism, showed 79% of its cells to be phenotypically ä; therefore, the chimeric white cell population appears to be associated with the 21% red cell population typed as A^i. We may interpret the data for two other heterosexual chimeras (No. 2 and 4) to suggest that the percentage of white and red cell chimerism may be the same; on this premise, therefore, the probable phenotypes of the animal's own red cells can be estimated for these four. However, in animals No. 5 and 6 there is no apparent correlation between the white cell chimerism and the percentage of either A^i- or ä-

1028173

TABLE 48

QUANTITATION OF RED CELL CHIMERISM IN HETEROSEXUAL AND ISOSEXUAL BLOOD CHIMERAS

Animal No.	Sex	Percent white cell chimerism*	Percent red cells: Type ä†	Type A^i‡
1	Male	18	17	83 (84)
2	Female §	68	60	40 (41)
3	Male §	24	79	21 (20)
4	Male	4	86	14 (20)
5	Male	45	20	80 (82)
6	Female	12	64	36 (39)
7	Female §	–	57	52 (43)
8	Female §	–	55	41 (45)
9	Male §	–	n.d. ‖	– (95)
10	Male §	–	n.d.	– (97)
11	Male § ¶	–	18	82 (88)
12	Male §	–	n.d.	– (96)

* Percent cells of opposite sex found in blood lymphocytes by sex-chromosome analysis. Minimum of 100 metaphase plates examined for each animal.

† Percent cells unreactive with isoimmune reagent as determined by counting free, unagglutinated cells with the Coulter counter.

‡ Percent cells reactive with isoimmune reagent. Determined by subtracting percent ä-type cells from 100%. Values in parentheses obtained by counting A^i cells tagged with fluorescein-conjugated reagents.

§ Double brackets indicate co-twin pairs.

‖ Not done.

¶ Single bracket indicates co-twin died before testing.

type cells. In these, and also in the first four, we can positively identify animals bearing the genotype for blood factor A^i, or its absence ä, only through breeding studies. The last six animals, representing four sets of isosexual cotwins (two young died before testing) demonstrate red cell chimerism via the blood factor A^i.

Our breeding program has provided families for testing the inheritance pattern of the blood factors identified above. Since our colony consists predominantly of *S.f. illigeri*, data concerning inheritance of factor A^i are readily available; conversely, matings of *S.f. lagonotus* having blood factors B^1 and C^1 are limited. The following points may be noted in Table 49. Of 15 mating pairs of ä x ä animals, all 93 offspring were phenotypically ä. In 10 pairings of A^i and ä, a total of 15 offspring were ä, 16 were A^i, and 10 were (A^i),* i.e., chimeras. Only three pairs of A^i x A^i matings were available, and among these, two offspring were ä, eight were A^i, and two were (A^i). Note that in the single mating combination of an ä x (A^i) mating there were four ä offspring and four A^i, indicating that the chimeric father (A^i) was genotypically A^i. Although it is not

* To denote naturally occurring chimeric mixtures of A^i and a-type cells, we have used the symbol A^i in parentheses. This has also been done for factors B^1 and C^1 when these are in a population of cells lacking the respective antigens.

TABLE 49

PHENOTYPES OF OFFSPRING PRODUCED BY MATING *S. FUSCICOLLIS* SSP. WITH BLOOD FACTORS A^i, B^1, AND C^1

Mating combination	Number mating pairs	Offspring number and phenotype
	S.f. illigeri	
♂ x ♂	15	93 ♂
A^i x ♂ *	10	15 ♂, 16 A^i, 10 (A^i)
A^i x A^i	3	2 ♂, 8 A^i, 2 (A^i)
A^i x (A^i) †	6	3 ♂, 15 A^i, 17 (A^i)
(A^i) x (A^i)	1	1 A^i, 2 (A^i)
♂ x (A^i)	1	4 ♂, 4 A^i
	S.f. lagonotus	
bc x bc	4	8 bc
bc x B^1c ‡	3	3 bc, 7 B^1c
bc x bC^1	1	2 bc, 7 bC^1
B^1c x bC^1	1	2 B^1c, 2 B^1C^1, 2 bC^1
bc x B^1C^1	1	2 bc, 1 b (C^1)
	S.f. illigeri and S.f. lagonotus	
bc x A^i	1	2 A^ibc
A^i x B^1c	1	2 A^iB^1c
B^1C^1 x A^i	1	1 ♂B^1(C^1) §

* Includes reciprocal matings ♂ x A^i.
† Includes reciprocal matings (A^i) x A^i.
‡ Includes reciprocal matings B^1c x bc.
§ Single-born animal with chimerism for blood factor C^1.

apparent from the tabulations in Table 49, all (A^i) young, i.e., chimeras, were members of a co-twin pair or of triplets. Finally, matings of *S.f. illigeri* and *S.f. lagonotus* marmosets have produced several hybrid offspring in our colony; however, only two pairs have involved animals bearing their respective blood factors (last two mating pairs of Table 49). Mating of animals with factors B^1 and A^i resulted in a co-twin pair with each having both antigens on their red cells. Note the phenotype of the single-born animal produced by mating of an *S.f. lagonotus* having factors B^1, C^1 with A^i-type *S.f. illigeri*. The chimerism of this young for blood factor C^1 suggests that it had a co-twin which died in utero.

The blood factors identified appear to be inherited as simple Mendelian dominants; the family data compiled with factor A^i certainly support this interpretation and the limited information for factors B^1 and C^1 suggest a similar mode of inheritance. Animals typed as A^i, B^1, or C^1 may, therefore, be homozygous or heterozygous for the factor, e.g., A^i/A^i or A^i/a, while animals lacking the respective antigens may be designated as homozygous recessives, e.g., a/a, b/b, and c/c. Mating of such recessive homozygous animals has always yielded young lacking the respective blood factors; subsequent mating of these animals with those bearing the antigen has frequently resulted in young with the

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corresponding blood factor. As anticipated, the availability of more than one blood factor among a mating pair increases the probability of detecting chimerism. This was best demonstrated in the chimerism of the single-born animal resulting from the mating of an *S.f. illigeri* (type A^i) with an *S.f. lagonotus* (type B^1C^1); genotypically, the former animal must be heterozygous for factor A^i, i.e., A^i/a, and the latter heterozygous for factor C^1, i.e., C^1/c, to have produced the young with the phenotype ♂ B^1 (C^1). Chimerism in single-born animals has been detected before by sex-chromosome analysis and has been attributed to the death and resorption of a co-twin in utero. The present observation again emphasizes the universality of twinning and chimerism in the marmoset.

CHARACTERISTICS OF MARMOSET HEMOPOIETIC CELLS IN CULTURE

Previously we have shown that cells from marmoset hemopoietic tissue proliferate and differentiate in a liquid medium for an extended period. Although hundreds of cultures with differentiating granulocytic-, monocytic-, and megakaryocytic-series cells have been obtained from marmoset marrow, fetal liver, placenta, and blood, there have been broad quantitative and qualitative variations among the cultures. These seem to reflect an intrinsic biological status of the marmoset at the time the cells are harvested. An illustration of this was seen when we (Raymond P. Porter and N. Gengozian) attempted to culture blood cells from eight clinically normal marmosets weekly for several months. At one bleeding date, cells from three marmosets produced extensively proliferating cultures containing myelocytic and monocytic cells, and an extraordinary number of megakaryocytes (over 1,000) by days 8 to 10; the initial seed was 2×10^6 mononuclear cells. Cells taken on other dates from these three animals and the other clinically normal marmosets had produced cultures that were similar qualitatively but not quantitatively. During this same week, three newly acquired marmosets (still in quarantine) were discovered to have *Herpesvirus tamarinus* (HVT) lesions. Blood cells taken at sacrifice of these moribund marmosets likewise proliferated extensively in vitro with pronounced megakaryocytopoiesis. After the first 2 weeks of extensive proliferation, all six cultures proliferated at a diminished rate for a remaining 45 to 57 days. We do not know why the blood cells from three marmosets with lethal HVT disease and three non-HVT-infected marmosets from the colony coincidentally exhibited such extensive proliferation but modulation or stimulation of the circulating stem-cell population in vivo is suggested since variables in the culture medium were controlled. *Herpesvirus tamarinus* has been shown to exist in a carrier state (Murphy, B. L. et al. Lab. Anim. Sci. 22, 339, 1972) and perhaps this agent is responsible for the tendency to proliferate actively but we have not shown this to be true.

To substantiate the identity of the megakaryocytes, one of the cultures containing many such cells was incubated with ^{35}S since megakaryocytes synthesize sulfated glycoproteins. After cultures were incubated with ^{35}S and washed, the cells were pelletted, smeared onto microscope slides, and fixed, and autoradiograms (ARG) were prepared. After exposing the ARG for 7 days they were developed and stained. Multiple silver grains could be found only over the

cytoplasm of the large cells morphologically identified as megakaryocytes by Wright's stain (Fig. 55).

Another interesting feature of the marmoset megakaryocyte in vitro has been elucidated this year. Last year we reported that numerous nests or clusters of cells among the populations of mixed cells of marmoset marrow attach to the culture vessel. We observed that a large nucleus is always associated with these clusters and that the cell cluster is formed when a series of floating granulocytic cells attach to the broad upper surfaces of a "leptocytomembrane" of the cell with the large nucleus.

Using phase optics and suitable small culture vessels, with sequential observations we found that the usually free-floating, mature megakaryocyte will attach to the surface and begin to spread its cytoplasmic membrane over an ever-enlarging area in an irregular pattern. Attached megakaryocytes have attained a diameter of over 200 μm in several days; these attached forms usually disintegrate in 5 to 6 days. This surface-spreading feature of megakaryocyte has a counterpart in the normal mature platelet; the megakaryocyte attachment to the culture vessel has also been reported in human marrow cultures during the first 2 days of culture. The stickiness of the membrane of platelets may be manifested in the maturing marmoset megakaryocyte cytomembranes that, for reasons still unknown, grow well in vitro. Hypothetically this stickiness is the biological mechanism by which the clusters we see are formed in vitro, i.e., a cell membrane interaction phenomenon.

We have observed still another large, spreading cell that sometime grows among the adherent population of marrow cells (Fig. 56). When these multinucleate rather than multilobate, giant cells appear in a culture they are relatively



Fig. 55. Megakaryocyte from marmoset peripheral blood (8 days in vitro) cultured with ^{35}S. This ARG clearly shows the presence of label over the cytoplasm of this multilobate cell. Wright's stain X 900.

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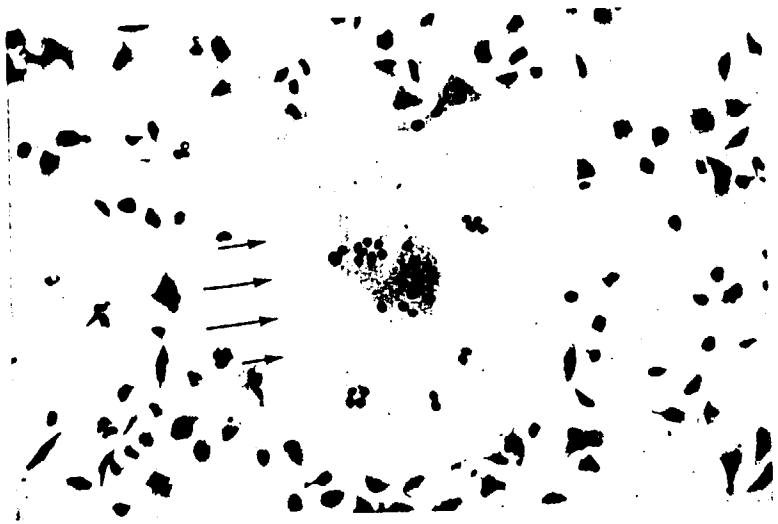


Fig. 56. Giant multinucleate cell growing among attached macrophagic cells in a culture of marmoset marrow. Clusters of nuclei are scattered throughout the cell; arrows point to left periphery of cytomembrane. Wright's stain X 100. See text.

numerous; however, they are found only in an occasional culture. They have been seen in cultures initiated from marrow and from blood. In contrast to the cluster-forming megakaryocyte, the nonadherent cell population does not attach to their cytomembrane. The precursor of this cell type and possible counterpart in vivo is obscure.

Because of the unpredictable, spontaneous culturability of marmoset marrow, the effect of growth-stimulating factors (GSF) on marmoset cells was investigated. Using marrow derived from 10 randomly selected normal marmosets, parallel cultures were initiated in both liquid and semisolid medium. As GSF we used dialyzed human urine or supernatant tissue-culture fluid from fibroblasts of fetal marmoset kidney (Metcalf D. In: Bone-Marrow Conservation, Culture and Transplantation, Proceedings of Panel, Moscow, 1968, International Atomic Energy Agency, Vienna, 1969, p. 3). Qualitative and quantitative differences between stimulated and nonstimulated cellular proliferation in the liquid and semisolid phase cultures were determined. The addition of GSF did not increase the number of colonies per 10^6 marrow cells but did increase the number of cells per colony, i.e., GSF only increased the rate of proliferation. The number of colony-forming units (CFU) ranged from 1/600 to 1/3000 of the nucleated bone marrow cells that had been plated. An interesting observation was made by comparing the growth of hemic cells by these two culture methods. In general, in soft agar, the individual colonies of marmoset marrow are made up of either granulocytic cell types or monocytic-macrophage cell types; mixtures of both cell types are also seen. The megakaryocytes do not produce colonies but

may be found floating free over the colony-containing agar surface if liquid medium is added. Marrow cells that proliferate extensively in liquid medium, when grown in soft agar produced an occasional colony type not observed in those that proliferated poorly in liquid medium. This third type of colony, developing from those marrows that proliferated extremely well, had a compact spherical form, whereas the macrophagic colonies grew in a broadly diffuse pattern and granulocytic colonies were made up of numerous clusters of semi-compact, shaggy-edged microcolonies. To further classify the cell type that made up the three kinds of colonies we removed cells of a macrophagic (?) colony from agar and placed it then in liquid medium with heat-killed yeast (*Candida albicans*). These cells quickly gorged themselves with the dead yeast cells showing their phagocytic function. Granulocytic (?) colony cells, microscopically have characteristic progranulocyte or doughnut-shaped prepolymorphonuclear to polymorphonuclear nuclear configuration. These cells are also capable of ingesting *Staphylococcus albus*. By 20 days some of the granulocytic colony cells grow up through the agar and form a monolayer colony on top. By contrast the cells from the spherical, tightly compacted, rapidly enlarging colonies had myeloblastic and promyelocytic morphology only (a stage in the development of this series of hemic cells that is apparently not motile). Occasionally a spherical, compact, marrow colony in agar with time begins releasing differentiated motile cells that extend from the central mass, presumably cells that develop a capacity for motility. The compact colonies, when present at all were always very infrequent (on the order of 1 per 500 to 1000 CFU). Thus, the distribution of this stem cell in marrow and circulating blood must be very sparse.

There appears to be a cyclic or modular phenomenon of marrow culturability in our marmoset colony, and it is interesting to speculate on the reason that hemic tissue, including blood cells, have cell types that at times may generate nondifferentiating precursor cells in vitro at the expense of their differentiation, but not in vivo. This may be due to intrinsic factors, such as a viral agent, or perhaps among the cell types in hemic tissue there is one that produces in vitro its own GSF.

CHARACTERIZING THE MIXED LEUKOCYTE CULTURE REACTION OF MARMOSET BLOOD LEUKOCYTES

Current studies of the mixed leukocyte response of marmoset blood were prompted by the need for a convenient means of measuring histocompatibility between the different species and subspecies. Previously we saw a marked variation in the ability of various animals to mount a first-set rejection of intrasubspecies foreign skin grafts. The general trend of the mixed leukocyte culture (MLC) of lymph node or spleen cells, while also indicating a variable response, demonstrated the feasibility of further developing the MLC for studies of hemopoietic chimerism and ultimately bone marrow transplantation (Donald D. Barnhart* and N. Gengozian).

* Oak Ridge Associated Universities Postdoctoral Fellow.

1028176

A modified, buoyant density gradient for separating blood lymphocytes provided a method permitting a reliable routine MLC in the marmoset. We determined the percentage yield of available lymphocytes from 5 ml of heparinized blood using gradients of varying density, and found that 1.117 ± 0.002 permitted recovery of 75 to 100% in an individual sample. Usually the cell preparations were essentially free of contaminating neutrophils.

Since the one-way MLC is conducted by interrupting division of that population of cells to be used as the antigen (stimulating) source, we compared the effects of mitomycin-C treatment and irradiation (2000 R, ^{137}Cs or X ray). Both methods appeared to be satisfactory although irradiation was more rapid and convenient. An initial question concerning the MLC reaction was the appropriate control system, i.e., could we culture autologous cells alone, measuring their background activity without any stimulating population, or must we include inactivated autologous cells, thus mimicking the cellular conditions of the MLC reaction? In several experiments we consistently observed that autologous cells cultured alone gave higher label (^{14}C-thymidine) incorporation (cpm) than when we examined the same cells with inactivated autologous cells. It occurred independent of the method used to inactivate the cells, i.e., mitomycin C or irradiation. The reason for this is not apparent but to properly control the MLC reaction we now routinely use a mixed autologous cell population as a measure of nonstimulation activity for our responding cell population.

Although we saw no differences between mitomycin C or irradiation in the control studies, it seemed important to investigate the effects of these two methods of cell inactivation in a semixenogeneic (intersubspecies) MLC reaction. We mixed blood cells of *S.f. illigeri* with blood cells of *S.f. lagonotus* previously exposed to 2600 R or mitomycin C. Little difference occurred between the methods (Table 50). Four cultures demonstrated higher stimulation indices after

TABLE 50

A COMPARISON OF THE EFFECTS OF IRRADIATION AND MITOMYCIN C ON THE MLC RESPONSE OF MARMOSET BLOOD LYMPHOCYTES AS MEASURED BY ^{14}C-THYMIDINE INCORPORATION:* A SEMIXENOGENEIC (INTERSUBSPECIES) COMBINATION

Treatment of stimulating population	Experiment number													
	1		2		3		4		5		6		7	
	cpm	Index†	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index
Irradiated §	4158‡	3.42	5304	2.63	2329	1.94	3264	2.48	6245	3.48	8450	1.47	2421	1.89
	1215‖		2011		1194		1316		1793		5710		1275	
Mitomycin C¶	5509	2.24	6943	2.19	4285	2.67	3508	2.60	5225	2.83	9136	1.24	2957	2.08
	2459		3146		1601		1346		1841		7321		1421	

* 4-Day cultures.

† The stimulation ratio determined from cpm of MLC divided by cpm of autologous cells.

‡ *S.f. illigeri* versus *S.f. lagonotus* cell combinations.

§ 2600 R.

‖ *S.f. illigeri* versus autologous cell combination.

¶ Mitomycin-C treatment for 30 min at 37°C. Washed 3X.

irradiation, one culture was higher with mitomycin C, and two showed little difference.

We determined the time of peak activity of the MLC response by ^{14}C-thymidine incorporation. Replicate semixenogeneic (intersubspecies) cell combinations were subjected to an 18-hr pulse of ^{14}C-thymidine on days 2, 4, and 6 as shown in Table 51. The maximum response of two cultures occurred on day 4, two showed little difference between days 4 and 6, while two were higher on day 6. As a group, however, for these six experiments, the average activity progressively increased from day 2 through day 6 in contrast to the allogeneic MLC responses among a more closely related group of animals, i.e., intrasubspecies combinations (Table 52). Thus MLC reactions between unrelated members of *S.f. illigeri* more frequently showed a peak reaction on day 2 of culture (5 of 11 experiments); indeed, in this series of experiments the average activities *decreased* from day 2 through day 6. Moreover there was a wide range of reactivity among the allogeneic combinations two (exps. 8 and 10) showing little interaction, and three (exps. 2, 9, 11) showing reactivity comparable to that in the genetically more disparate (intersubspecies) combinations of Table 51. These MLC data, presumably a measure of histoincompatibility, parallel our previous skin-graft-rejection data which showed a marked variation in rejection time of first-set skin grafts within members of one subspecies (*S.f. illigeri*, 13 to 64 days) and a lesser variation between intersubspecies combinations (*S.f. illigeri* versus *S.f. lagonotus*, 14 to 33 days).

The background activity of control cultures remained high, indicating possible nonspecific stimulation of the autologus cell mixture. The latter may act to decrease the sensitivity of the test thus limiting its predictive value of graft survival to interspecies or intersubspecies combinations. Earlier studies by Porter and Gengozian indicated several variables affected the level of labeled thymidine incorporation for cultured marmoset lymphocytes, i.e., serum source

TABLE 51

KINETICS OF A SEMIXENOGENEIC (INTERSUBSPECIES) MLC REACTION AS MEASURED BY ^{14}C-THYMIDINE INCORPORATION

Day of labeling	Experiment number												
	1		2		3		4		5		6		
	cpm	Index*	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	Average
2	14321		294		1208		636		200		3157		
		3.39		1.2		2.7		0.66		0.66		2.91	1.92
	422‡		243		443		958		299		1083		
4	2602		2333		5453		2456		1690		10030		
		3.65		3.79		5.4		2.84		2.11		6.88	4.11
	711		612		1005		862*		798		1457		
6	8562		4889		14603		3450		2630		10184		
		11.11		3.33		7.4		2.95		2.58		4.30	5.27
	770		1468		1972		1166		1007		2366		

* The stimulation ratio determined from cpm of MLC divided by cpm of autologous cells.
† *S.f. illigeri* versus *S.f. lagonotus* cell combinations.
‡ *S.f. illigeri* versus autologous cell combinations.

1028177

TABLE 52

KINETICS OF AN ALLOGENEIC (INTRASUBSPECIES) MLC REACTION AS MEASURED BY ^{14}C-THYMIDINE INCORPORATION

Day of labeling	Experiment number																							
	1		2		3		4		5		6		7		8		9		10		11			
	cpm	Index*	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	cpm	Index	Average §
2	2307†		14999		2158		3629		855		6938		3216		1074		2111		279		357		
		1.90		4.35		3.24		2.90		1.84		3.70		5.41		1.29		3.49		0.94		2.11	2.81
	1211‡		3445		660		1248		464		1873		594		828		641		295		166		
4	3410		16031		7674		12649		2466		10199		2069		2551		7874		1348		2555		
		1.72		3.87		1.69		1.01		4.75		2.20		2.44		0.56		4.74		0.93		5.16	2.64
	1972		4133		4539		12419		579		4622		845		4499		1659		1439		495		
6	4963		12534		12135		11583		4662		6578		5916		3894		8107		2723		4272		
		1.43		3.95		1.47		1.13		2.93		0.94		2.05		0.77		3.36		1.02		5.03	2.18
	3467		3176		8222		10213		1589		6949		2881		5035		2406		2655		848		

* The simulation ratio determined from cpm of MLC divided by cpm of autologous cells.
† *S.f. illigeri* versus *S.f. illigeri* cell combinations.
‡ *S.f. illigeri* versus autologous cell combinations.
§ Average for 11 experiments.

and concentration. Investigations now under way further substantiate these findings with blood cells in an allogeneic (intrasubspecies) MLC. Preliminary results indicate a high uptake of ^{14}C-thymidine by cells cultured with 2% fetal calf serum as compared to cultures containing autologous, allogeneic, or human AB serum. It should be noted that comparison of control cultures for thymidine uptake over a period of 6 days offers no evidence that the chimeric status of an individual leukocyte population plays a role in the observed high background activity.

COLONIC CANCER IN MARMOSETS

Last year we reported finding four instances of spontaneous mucinous adenocarcinoma in three imported cotton-top marmosets (*Saguinus oedipus oedipus*) and one colony-bred Goeldi's marmoset (*Callimico goeldii*). Although the primary sites were not recognized grossly at necropsy, we surmised from histologic sections showing the neoplasm arising in intestinal mucosa and the fact that peripancreatic lymph nodes contained carcinoma metastases, that these neoplasms had arisen in the upper small intestine of these animals. Since then we (C. C. Lushbaugh, Gretchen Humason, and N. Gengozian) have found three additional instances of this cancer and realize that our conclusion was wrong, and that these cancers actually originated in the large intestine. With these three new cases and review of the earlier ones we have demonstrated conclusively that all seven arose initially in the ascending colon and cecum. One animal, however, with multiple primary sites in the cecal mucosa also had multiple primary sites in the mucosa of the terminal ileum adjacent to the ileocecal valve (Fig. 57). Two of the three animals this year had metastases in pericecal nodes as well as in peripancreatic nodes. The third animal had a single lymph node metastasis in a node in the mesocolon adjacent to the primary site of this cancer in the ascending colon. In only one of these three cases was the primary site found grossly even though the wasting clinical course of the animals and their intermittent diarrhea made us suspect that all three had the disease. In one animal, as in the original four instances, we discovered the neoplasm only by finding, on histologic study, numerous metastases in cecal and peripancreatic lymph nodes. This primary site was then located by cutting and examining several hundred sections of large and small intestine carefully labeled as to anatomical location. Two separate, minute (1 mm in diameter and 2 mm in diameter), shallow, mucosal ulcers with mucinous carcinoma cells in their bases and borders were ultimately found in the cecum in mucosal folds of the ileocecal valve ring. The third instance (number seven in our series) was discovered fortuitously and as a result of our having used as a routine procedure in these necropsy studies, a technique for examining as much of the entire length and width of the gastrointestinal tract as time permits. In this technique, not original with us, after the opened intestines have been fixed flat in a pan overnight, we roll identified segments longitudinally (caudally) and embed them in paraffin. In this way, one miscroscope preparation can contain a section of 10 or more centimeters of intestinal tract. This animal was killed following development of sudden abdominal distention after a week of diarrhea and wasting. At necropsy it had a hugely dilated portion of ascending colon (Fig. 58) considered to be a paralytic ileus of unknown cause; no anatomical obstruction or vascular



Fig. 57. Montage of photomicrographs (∿30 X) of histologic sections of the cecum, terminal ileum, ileocecal valve, and adjacent lymph nodes (periodic acid-Schiff (PAS) stain). The unmarked arrows point to the multiple primary sites of adenocarcinoma in the cecal and ileal mucosa. The arrows marked with "m" indicate metastatic carcinoma in the nodes. The submucosal fibrosis in the cecal portion of the colon and the subperitoneal fibrosis around the ileum and nodes caused an obstructive mass that was grossly suspicious of cancer.

occlusion was present. The mucosal surfaces of the flattened ascending colon (Fig. 59) appeared grossly to be normal except for the greatly thinned, distended segment. After histologic preparation of a longitudinal strip of this portion of the intestine disclosed a 2 to 3 mm in diameter superficial ulcer (Fig. 60) with mucinous adenocarcinoma arising in its borders, we were able to identify in retrospect a minute defect in the mucosal pattern on the gross photograph (arrow, Fig. 59). We found after examining multiple sections that a regional node, identified in Fig. 58, contained a solitary lymph node metastasis. Lymph node metastasis

--1028178



Fig. 58. Gross photograph of the acute dilation of the segment of colon (co) that was found to be associated with a minute primary adenocarcinoma located (large area) just caudal to it. A single metastasis (met) was subsequently found in a lymph node (small arrow). The ileum (il) and cecum (ce) appeared to be normal.

remains the best proof of malignancy in experimental intestinal oncogenesis since local submucosal penetrations of epithelial cells that mimic local invasion can occur in benign inflammatory intestinal diseases (see below).

These experiences this year explain in part that our previous failure to recognize these neoplasms grossly was due to their minute size as well as to our unpreparedness. In reviewing the initial four instances to see if they were truly primary in the upper small intestine as we had reported, we found that none could be shown arising in a recognizable *small* intestinal mucosa. We had discounted this appearance earlier on the ground that mucosal simplification, known as

1028179

"intestinalization" in gastric carcinogenesis, commonly precedes neoplastic transformation in the gastrointestinal tract. This change destroys the various mucosal patterns on which histologic identification of the different regions of the intestinal tract depend. In our necropsy studies these mucosal landmarks were altered further by postmortem autolytic changes. Thus our erroneous deduction had been based chiefly on the numerous instances of metastatic cancer in peripancreatic nodes rather than cecal nodes; this finding seemed to us to imply an upper abdominal origin for the neoplasms.

In the one animal in which the primary site was found grossly and diagnosed directly this year (Fig. 57), submucosal lymphatic spread in the colon resulted in widespread local, submucosal, muscular and neural infiltrations, and

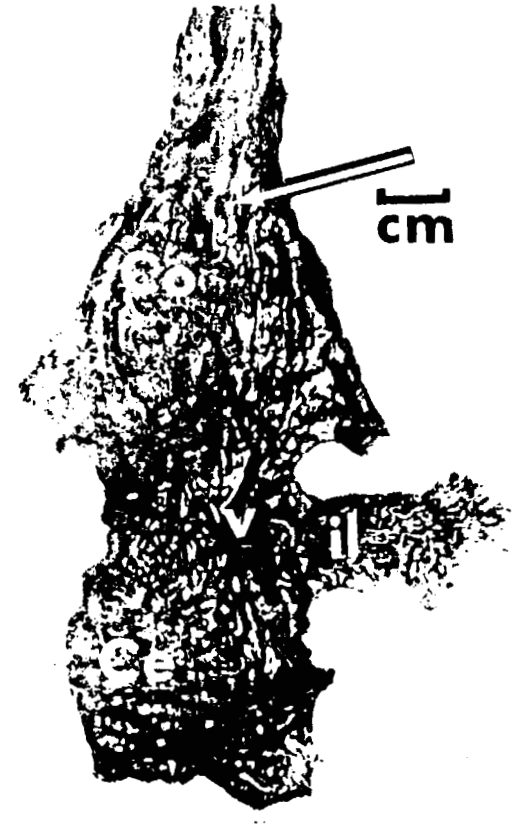


Fig. 59. Gross photograph of the opened and flattened cecum, ileum, and ascending colon of the cotton-top marmoset shown in Fig. 58. The arrow points retrospectively to the 2 mm in diameter, superficial carcinomatous ulcer that was overlooked at necropsy.

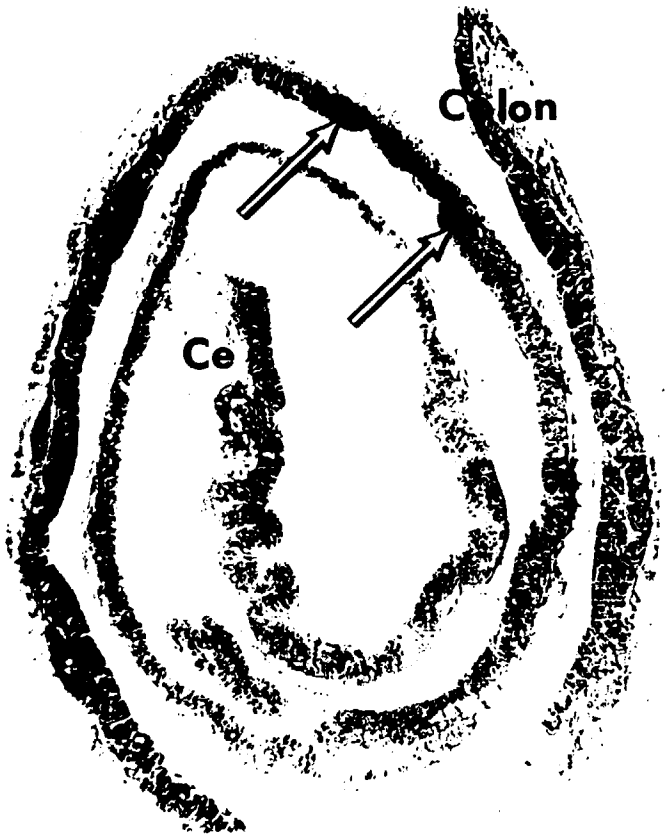


Fig. 60. Photomicrograph of a histologic section (periodic acid-Schiff and hematoxylin [PAS and H] stain) of a rolled longitudinal strip of the ascending colon shown in Fig. 58 and 59. The two arrows point to mucosal carcinoma cells that are heavily PAS-positive and located in the margins of the ulcer (shown by the single arrow in Fig. 59).

lymph node metastases. The latter were as advanced in distant (peripancreatic) as in local (pericecal) nodes. The explanation of this early and extensive metastatic involvement of mesenteric and peripancreatic lymph nodes from these small primary colonic cancers apparently lies in the anatomy of the mesenteries in the marmoset. Unlike the colon of man, neither the colon nor duodenum have "bare" areas with retroperitoneal attachments to the posterior abdominal wall. Consequently, the ascending colon of the marmoset would be unable to develop a significant collateral vascular drainage to bypass the mesenteric system. Most lymph drainage of the cecum and ascending colon

1028180

(supplied by the superior mesenteric arterial system) is therefore through the nodes of the coeliac axis which, because of another anatomical dissimilarity with man, are closely associated spatially with those that drain the pancreas and duodenum. The latter organs are also devoid of retroperitoneal bare areas and have mesenteries fused with that of the transverse colon. The extensive metastases in peripancreatic nodes are not therefore indicative that these carcinomas have originated in the upper small intestine (duodenojejunum) as we initially thought but merely point toward an origin somewhere in the intestine supplied by the superior mesenteric artery and its branches, Furthermore, in several instances, the desmoplastic subperitoneal fibrosis and peritonitis produced thick adhesions to structures of the posterior abdomen and linked together anatomically the right ureter and right colon in a way that made possible invasion of periureteral lymphatic channels.

Table 53 summarizes and brings up to date all our findings and interpretations of these histopathologic studies of spontaneous intestinal cancer in the marmoset. These cases are listed in chronological order of calendar time of death. Actual age was known only for the first animal to die with this disease because it was the only one of the series born in our colony. As shown, the "age" of the other animals, all imported, is stated as residence time in the colony. None were less than 1 year or longer than 6 years in residence; three lived less and three longer than 45 months in the colony, suggesting that at least 1 year and on the average about 3 to 4 years in the colony may usually be required for this disease to develop. Its occurrence in a colony-bred animal would seem to support the view that exposure to the causative agent does not have to occur in the wild. Two of the seven animals were female, forming a male-to-female ratio similar to that for the sex incidence of colon cancer in humans. All but one of the cancers occurred in the cotton-top species. None were found in *S.f. illigeri* and related species imported from the Amazon River basin even though about 10 *illigeri* were necropsied for every *oedipus*. The last four of the seven cases seem histologically to be a less advanced stage than the first three, which showed severe desmoplasia, ulceration, and extensive local invasion. Two of the last four animals (case 5 and case 7) were killed for necropsy and tissues for transplant when their clinical course suggested they might have the disease, and thus we obtained these cases in an early stage, but even then one animal (case 5) had numerous lymph node metastases. Our finding of multiple discrete primary sites in cases 4, 5, and 6 may be related to their earlier stage of development with death occurring before individual sites were either destroyed by ulceration or were joined together by extension through intervening normal mucosal islands. As indicated in the last three columns, all animals had various degrees of a chronic inflammatory disease of the colonic mucosa that causes local ulcerations, hyperplastic mucosal changes, and submucosal sclerosis. Our progress in histopathologic studies of the nature of this inflammatory disease and its incidence in the colony are described in the following paragraphs.

AN ULCERATIVE COLITIS ASSOCIATED WITH COLONIC CANCERS IN MARMOSETS

Our unique finding of malignant intestinal neoplasms occurring almost exclusively in one species of captive marmosets and not in any of the more

numerous of several closely related species in the ORAU marmoset research colony is difficult to explain. Our immediate approach to the question of the validity of the observation has been to restudy histologically the intestinal tracts of the 18 *Callimico goeldii* and the 48 adult *S. oedipus* that did not have intestinal cancer but died during the period when the cancers were found (Gretchen Illumason, N. Gengozian and C. C. Lushbaugh). Even if no other instances of neoplastic disease were to be disclosed, we might find lesser mucosal abnormalities in these colons, cecums, or terminal ileums conceivably related to the development of the carcinomas. In addition to restudying the two affected species, we also reviewed histologically 134 necropsies of *S.f. illigeri*, the principal species in our breeding program, but in which no instances of this type of cancer had been found. Eighty-four of these 134 necropsies were on adults of unknown age surviving in the colony for longer than 10 months after importation from the wild. These *S.f. illigeri* selected for more detailed study of their intestinal tracts seem to form a natural "control" group to study in contrast with our positive findings in the other two species.

Figure 61 is a histogram of the number of *S.f. illigeri* dying during succeeding 10-month intervals after arrival. The majority of imported marmosets of all species die within the first 20 months after arrival, usually from the effects of parasitic worms; the most debilitating is *Prosthenorchis elegans*, an acanthocephalid that lives as an adult in the terminal ileum of most marmosets (described in our colony previously by Drs. Nelson and Cosgrove). Apparently all heavily infested animals succumb within the first year. The available vermifuges do not affect this parasite, since surviving worms are found in animals dying after as long as 8 years in the colony. Reinfection of adults and new infection of colony-bred animals, however, does not occur because intermediate vectors such as cockroaches are completely controlled in the colony. This parasite erodes its way through the ileal wall where it attaches as an adult and renders the host unusually susceptible to enteric infections, peritonitis, pylethrombophlebitis, and hepatitis. Unlike many other intestinal worms (nematodes, for example), it does not provoke an epithelial response that results in tumor-like mucosal growths. For this reason, we believe that this kind of enteric infection probably does not play an etiologic role in the development of the intestinal carcinoma. Besides, as can be seen in Table 53 only 1 of the 7 animals with carcinoma had signs of this kind of worm infestation.

The histogram shows that after the first 10 to 20 months the death rate subsides sharply and then rises slowly, reaching its peak after about 7 to 8 years. This length of survival within the colony is in surprising agreement with the view of others that the "normal" life-span of these species is about 9 years if we can assume the animals average about 2 years old when trapped in the upper Amazon River basin. This survival curve suggests, however, that uncomplicated senescent death might not occur sooner than 11 or 12 years in a protected, optimal environment.

We are not able to state without reservations, even after this intensive histologic study, why each of these animals died. We are able to demonstrate, however, that more than half of those of all three species that survived the first 10 to 20 months have in common an intestinal disease not previously described definitively in them. This disease, found commonly in such laboratory animals as mice, rats, and hamsters, had been labeled benign adenomatous hyperplasia and

TABLE 53

CLINICAL AND PATHOLOGIC FINDINGS IN COLONIC CANCER OF MARMOSETS

Case No.	Date of death	Months: Age	Months: Residence time in colony	Sex, species	No. of primary sites	Invasion of: Intestinal region	Invasion of: Local intestinal structures	Invasion of: Mesenteric nerves	Invasion of: Other structures	Lymph node metastases: Total nodes examined: No.	Lymph node metastases: Chronic inflammation	Benign enteritis: Ulceration	Benign enteritis: Sclerosis and muscle hypertrophy
1*	2/6/68	45	45	Male *C. goeldii*	1	Colon	Massive	Many	Right ureter	5	9 Severe	Extensive	Massive
2	3/19/70	?	30	Male *S. oedipus*	1	Colon	Massive	Many	Right ureter, pancreas ?	1	14 Severe	Extensive	Massive
3	7/3/71	?	71	Male *S. Oedipus*	1	Cecum	Massive	Few	Both ureters, pancreas ?	7	14 Severe	Extensive	Some
4	8/17/71	?	25	Female *S. oedipus*	2	Colon (cecum)	Early	None	None	6	16 Moderate	Small	Little
5†	12/20/71	?	13	Male *S. oedipus*	2	Cecum	Early	None	None	11	14 Moderate	Small	Little
6	6/17/72	?	56	Female *S. oedipus*	7 Cecum 7 Ileum	Extensive	Few	None	None	5	16 Severe Focally mild	Numerous but small Superficial	Moderate None
7	9/1/72	?	49	Male *S. oedipus*	1 Ascending colon	Very early	None	None	None	1	10 Moderate	Minute	Early

* Only colony-bred animal.

† Only animal with *Prosthenorchis elegans* infestation of ileum.

1028181

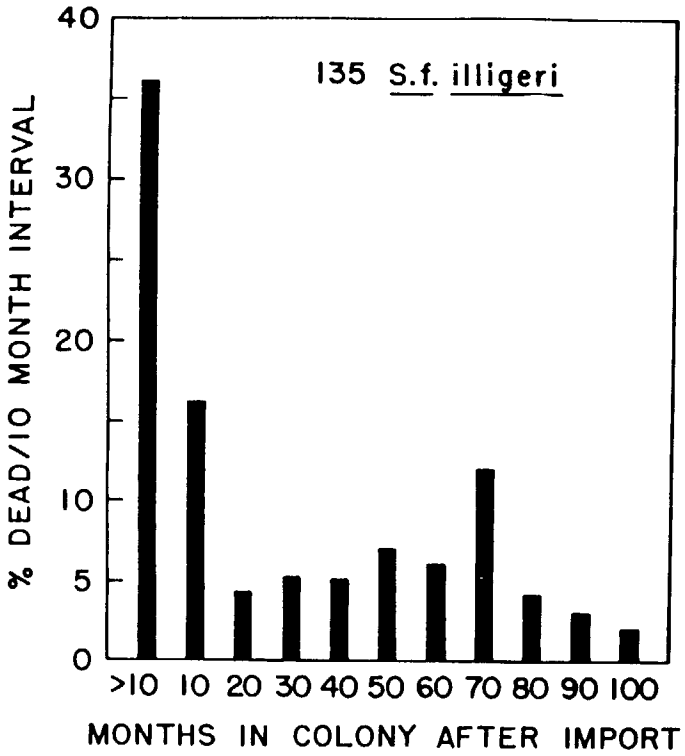


Fig. 61. Percent frequency distribution of death of 135 imported **S.f. illigeri** *marmosets in the ORAU research colony according to length of survival measured in 10-month intervals. The individual ages at time of arrival were unknown.*

enteritis, and in the past, during chemical carcinogenicity studies in rodents, has often been confused histologically with cancer. This disease becomes more extensive and locally destructive in the ileum, cecum, and colon as the marmosets age within the colony. In its more advanced stages. mucosal glands penetrate deeply into submucosal connective tissue in association with lymphocyte-rich submucosal cellular accumulations (Fig. 62 and 63). These atopic glandular structures lend this disease its falsely carcinomatous picture, more aptly described as "pseudocancer" because local "invasion" by mucosal glands is stopped by the external muscle coats; peritoneal spread and lymph node metastases do not occur.

The disease is definitely inflammatory and is most likely infectious. Our attempts to find a common bacterial cause by culture have revealed instead many different intestinal saprophytes and pathogens. These could be invaders perhaps secondary to the primary viral infection but we have no evidence for this

1028182

assumption. We have not established whether the acute large and small bowel enteritises reported in last year's Research Report as a common cause of death among neonatal colony-bred *S.f. illigeri*, are related to this disease of imported adult marmosets. However, in a still incomplete histopathologic study of such diseases of our colony-bred animals, we have found isolated instances of fatal, extensive, hyperplastic colitis. In one such instance in a 4½-month-old animal, the findings prove that the chronic colonic form of the disease can be acquired within 1 year of birth in our colony as would be expected if it is infectious.

The disease seems to begin in the cecum and to extend usually up the colon rather than retrograde into the ileum. Instances of chronic, hyperplastic ileitis, however, are found and are characterized by loss of the typical villous pattern of the mucosa and conversion of it to a colonic glandular pattern. Rarely this mucosal change or "intestinalization" is to be found in the stomach and duodenum. The mucosal cells become increasingly mucinous and through increased mitosis form hyperplastic glands. The lamina propria, particularly



Fig. 62. Photomicrograph of hyperplastic mucosal gland of the colon "invading" through an hiatus in the submucosal muscle into an area of chronic submucosal inflammation. The darkest areas are vacuoles of PAS and mucin in epithelial cells. (275 X)



Fig. 63. Photomicrograph of a more advanced submucosal "invasion" of hyperplastic colonic glands than in Fig. 62. These form a bottle-shaped diverticulum, a typical finding late in this chronic colitis of unknown cause. (275 X)

just beneath the crypts, becomes filled with lymphocytes and islands of plasma cells form. As the disease progresses and these cellular infiltrates become more extensive, all stages of plasmacytopoiesis can be found including the differentiation of large histiocytes (flame cells) heavily laden with brilliant periodic acid-Schiff (PAS)-positive glycoproteins (antibody protein?). In some instances, groups of hemocytoblasts, probably the precursors of plasma cells, infiltrate the connective tissues and appear like poorly differentiated malignant cells. Their resemblance to the undifferentiated forms of marmoset intestinal cancer, however, is only superficial since the latter usually have more abundant cytoplasm and contain vacuoles of mucin. Atopic hyperplastic glands in this inflammation always have a distinct basement membrane unless undergoing necrosis in the

1028183

midst of a granulocyte response. Mucosal ulcerations are common and elicit a reparative hyperplastic epithelial response. These hyperplastic glands, however, differ from their neoplastic counterpart in *S. oedipus* in that their cells always maintain lateral contact and form either normal glands or a simple, cuboidal, surface epithelium. These epithelial cells also differ from those of the neoplastic intestinal disease of *S. oedipus* by their inability to invade or infiltrate through smooth muscle. The downgrowths of epithelium always pass through the muscularis mucosae through the hiatuses for the arteries and veins of the mesenteric vasculature. Sometimes their apparent penetration is through pathologic faults created by locally destructive inflammation. We found no instances of mucosal cell penetration into the circular intestinal muscles. Grossly, as well as microscopically, the pericecal mesenteric lymph nodes have the appearance of the carcinomatous nodes of *S. oedipus*. However, on closer inspection in the inflammatory disease the masses of PAS-positive cells in the lymph node sinuses are histiocytes rather than carcinoma cells which typically have pleomorphic, enlarged, hyperchromatic nuclei displaced by a mucin-containing vacuole. The histiocytes in this inflammatory disease have small, benign nuclei usually centrally located in a granular PAS-positive cytoplasm. In some instances this PAS-positive material does seem to be ingested mucin obtained possibly from necrotic mucin-secreting epithelial cells. To date, however, we have been unable to prove this suspicion.

Histologic evidence is strong among the seven cases of marmoset intestinal cancer that some form of this intestinal inflammatory disease existed before carcinogenesis occurred. We have been unable to demonstrate, however, in some logical way that the carcinogenic change is related to it. While some instances of the cancer occurred in sites of a long-standing, desmoplastic, inflammatory process, in other cases submucosal fibrosis was minimal and mucosal hyperplastic changes were early and unaccompanied by downgrowths of glandular structures through the muscularis mucosae. In fact, the inflammatory, hyperplastic, adenomatous disease in many instances was most extensive and destructive in the *S.f. illigeri*, the noncancerous species. This observation suggests the possibilities that the disease, when extensive, may be either an immune-process protective against carcinogenesis or a parallel benign reaction elicited by a common etiologic agent but not a precancerous step in the carcinogenesis.

ULTRASTRUCTURAL ANATOMY

THE ROLE OF MACROPHAGES IN ^{67}GA LOCALIZATION

Part of the research effort in ultrastructural anatomy has been to elucidate the role of macrophages in ^{67}Ga localization in both normal and malignant tissues. In previous experiments using both light and electron microscopic autoradiography we showed that ^{67}Ga localizes in lysosomes or phagolysosomes and notably although not exclusively in macrophages. No evidence as yet other than autoradiographic data identifies the cell types that localize ^{67}Ga in either tumors or normal organs. It is noteworthy that histologic examination of a specific

organ, such as the thymus for AKR thymoma, when correlated with the localization of ^{67}Ga does not identify the cells that concentrate the radionuclide. In fact, such correlations can be entirely misleading. Neither does fractionation of cell homogenates by centrifugation reveal the cell type(s) that localize ^{67}Ga because no method yet devised can satisfactorily separate cells into pure populations before the preparation of cell homogenates. Therefore, the autoradiographic technique is the only available method to study cellular ^{67}Ga localization in organs or tumors.

Although the amount of ^{67}Ga taken up in the normal mouse by the approximately 2×10^6 peritoneal cells which are rich in macrophages is perhaps a rather small percentage of the total injected dose, these cells represent a potential model system to study the localization of ^{67}Ga in macrophages under different experimental conditions. We (D. C. Swartzendruber and Gary A. Wolfe) determined that ^{67}Ga localizes in large mononuclear histiocytes within 15 min after its intraperitoneal injection (Fig. 64). Mast cells, polymorphonuclear (PMN) cells (eosinophilic and neutrophilic in mice), and lymphocytes are by contrast only slightly labeled, even after 24 hr (Fig. 65), indicating that these latter cells concentrate small amounts of ^{67}Ga. The PMN cells contain many primary lysosomes, yet they are essentially unlabeled with ^{67}Ga. Since ^{67}Ga localizes in lysosomes or phagolysosomes of histiocytes and not in PMN cells, differences in

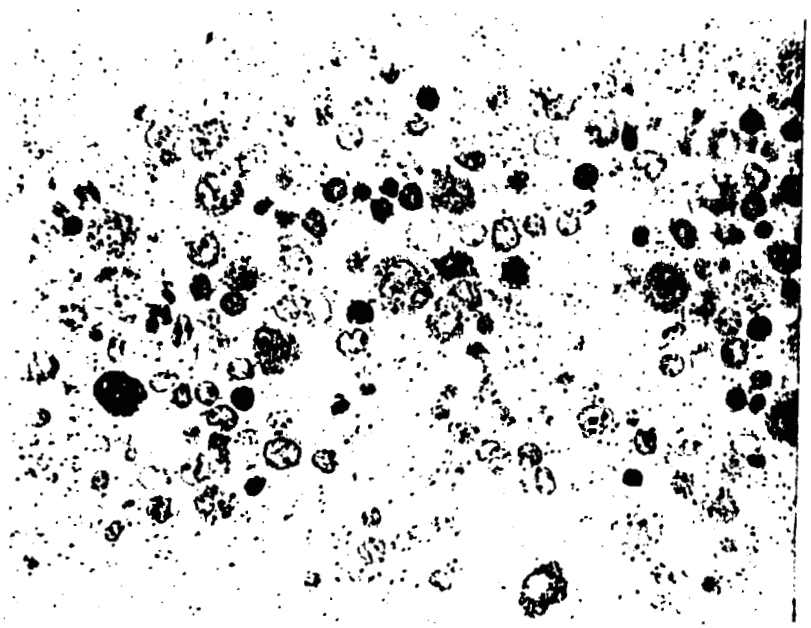


Fig. 64. Light microscopic autoradiograph of peritoneal cells from nonleukemic AKR mouse fixed 15 min after intraperitoneal injection of ^{67}Ga. Note labeling of large histiocytic cells. The two heavily granulated mast cells should not be confused with labeled histiocytes. 500 X.

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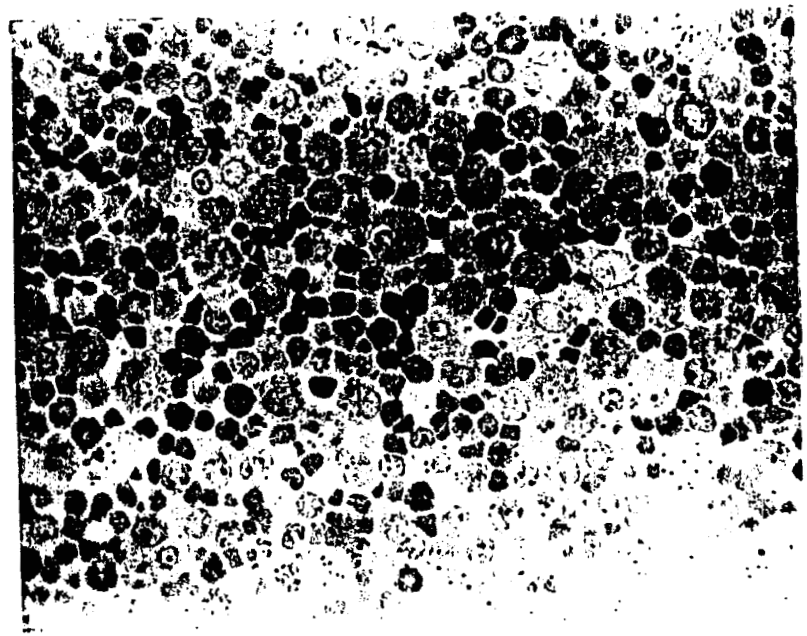


Fig. 65. Light microscopic autoradiograph of peritoneal cells from nonleukemic AKR mouse fixed 24 hr after intraperitoneal injection of ^{67}Ga. Note silver grains over histiocytes. 500 X.

lysosomes of different cell types or variations in specific lysosomal hydrolases of different populations of lysosomes may explain our data. Autoradiographs of peritoneal cells from leukemic (Fig. 66) or nonleukemic animals (Fig. 65) both show clearly large histiocytic cells labeled with ^{67}Ga, but with qualitative differences in the pattern of labeling. There are more silver grains per unit area in the cells from leukemic mice, and ^{67}Ga-labeled magrophages are more numerous in these preparations than from nonleukemic AKR mice. This different pattern of ^{67}Ga localization might be related to quantitative changes in macrophages as a result of activation by various antigens, e.g., tumor or viral antigens.

We have additional autoradiographic evidence for the participation of macrophages from other tissues in ^{67}Ga retention in AKR mice. Teased cells from thymuses of leukemic or nonleukemic mice were incubated with ^{67}Ga in vitro. Figure 67 shows an autoradiograph of teased cells from a leukemic AKR mouse after in vitro incubation at 37°C for 4 hr with ^{67}Ga in tissue culture medium 199 containing calf serum. Large phagocytic cells are clearly labeled with ^{67}Ga as shown in Fig. 67. Cells from a nonleukemic AKR mouse thymus treated under identical conditions are shown in Fig. 68. It should be emphasized that these studies are not intended to detract from the usefulness of ^{67}Ga in tumor detection by scintiscanning. Rather, they are intended to help elucidate the mechanisms of ^{67}Ga retention. Further, our autoradiographic data obtained from studies of ^{67}Ga localization in tissues and particularly macrophages of leukemic AKR mice

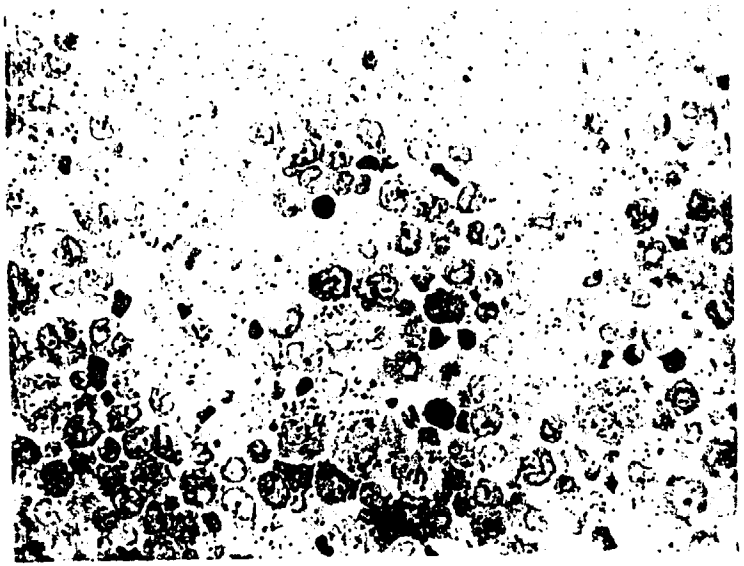


Fig. 66. Light microscopic autoradiograph of peritoneal cells from leukemic AKR mouse. Note large, labeled histiocytes. Cells fixed 24 hr after intraperitoneal ^{67}Ga injections. 500 X.

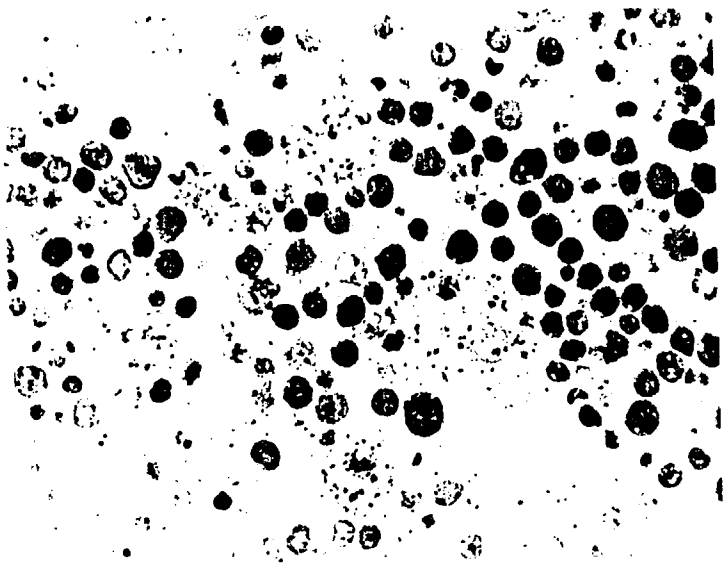


Fig. 67. Light microscopic autoradiograph of teased thymus cells from a leukemic AKR mouse incubated in vitro with ^{67}Ga for 4 hr at 37°C. Note the large, labeled macrophages. 500 X.

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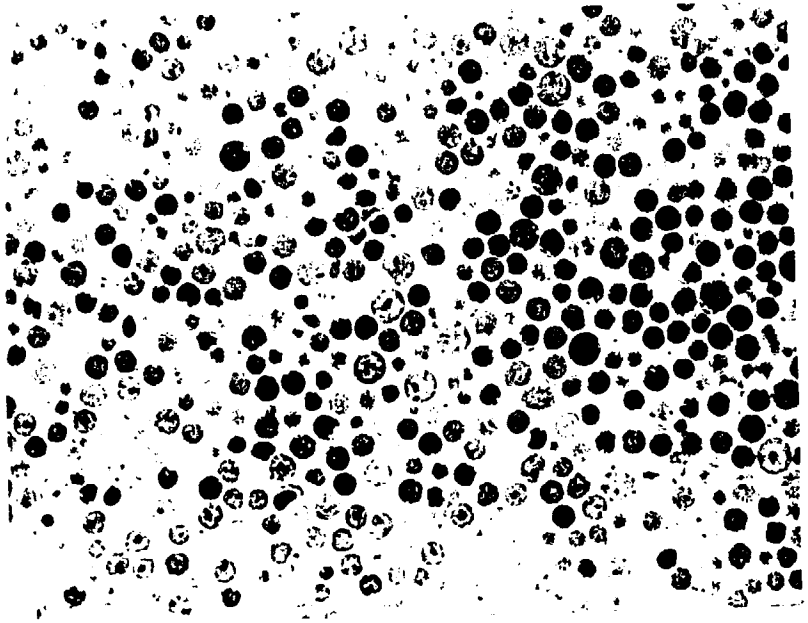


Fig. 68. Light microscopic autoradiograph of teased thymus cells from a nonleukemic AKR mouse and treated as in Fig. 67. Note absence of labeled cells. 500 X.

should not yet be generalized to other experimental tumor models or to human cancers. Our data suggest that caution is needed in interpreting ^{67}Ga uptake in malignant cells.

EFFECTS OF X IRRADIATION ON ^{67}GA LOCALIZATION

We are using another approach to study localization in cells by injecting ^{67}Ga after X irradiation. For example, macrophages are believed to be radioresistant, particularly at moderate X-ray exposures in the 450-R range and would survive while radiosensitive cells would disappear. We reasoned that acute X irradiation at low to moderate exposures might therefore enhance the uptake of ^{67}Ga by macrophages. Our experiments did not yield the anticipated results. Whole-body and tissue retention of ^{67}Ga was determined in adult $C3BF_1$ mice after acute whole-body X irradiation (D. C. Swartzendruber and K. F. Hübner). Whole-body retention decreased significantly after an exposure to 75 R (Fig. 69). Decreased retention in soft tissues was a transitory effect and recovery of the animal's ability to concentrate ^{67}Ga was dose dependent within the range of 100- and 950-R exposures (Fig. 70). Recovery occurred by day 3 after 100 R, and between days 6 to 8 after 450 and 950 R. The radiation effect of decreased retention was most pronounced when the radionuclide was administered 24 hr after irradiation. In

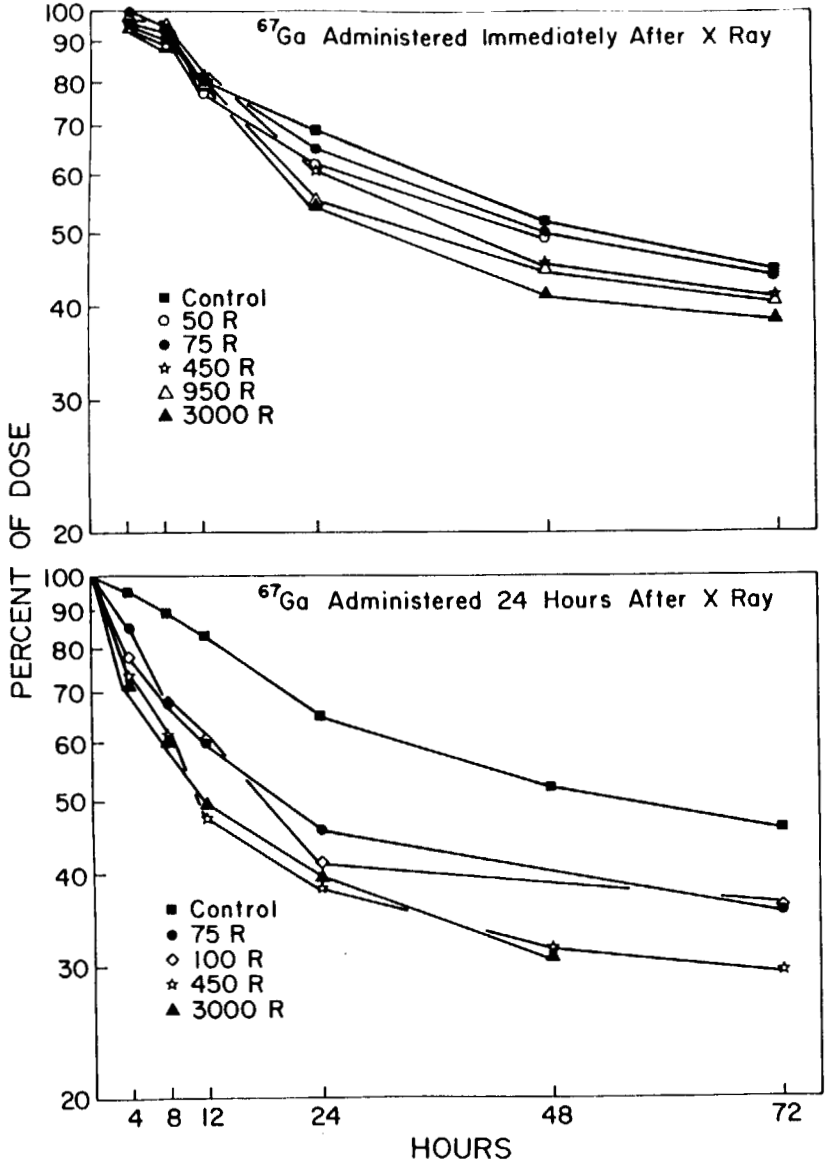


Fig. 69. Effect of total-body X irradiation of ^{67}Ga excretion as measured by total-body retention. Each point is an average of five mice. (Top) ^{67}Ga administered immediately after irradiation. (Bottom) ^{67}Ga administered 24 hr after irradiation.

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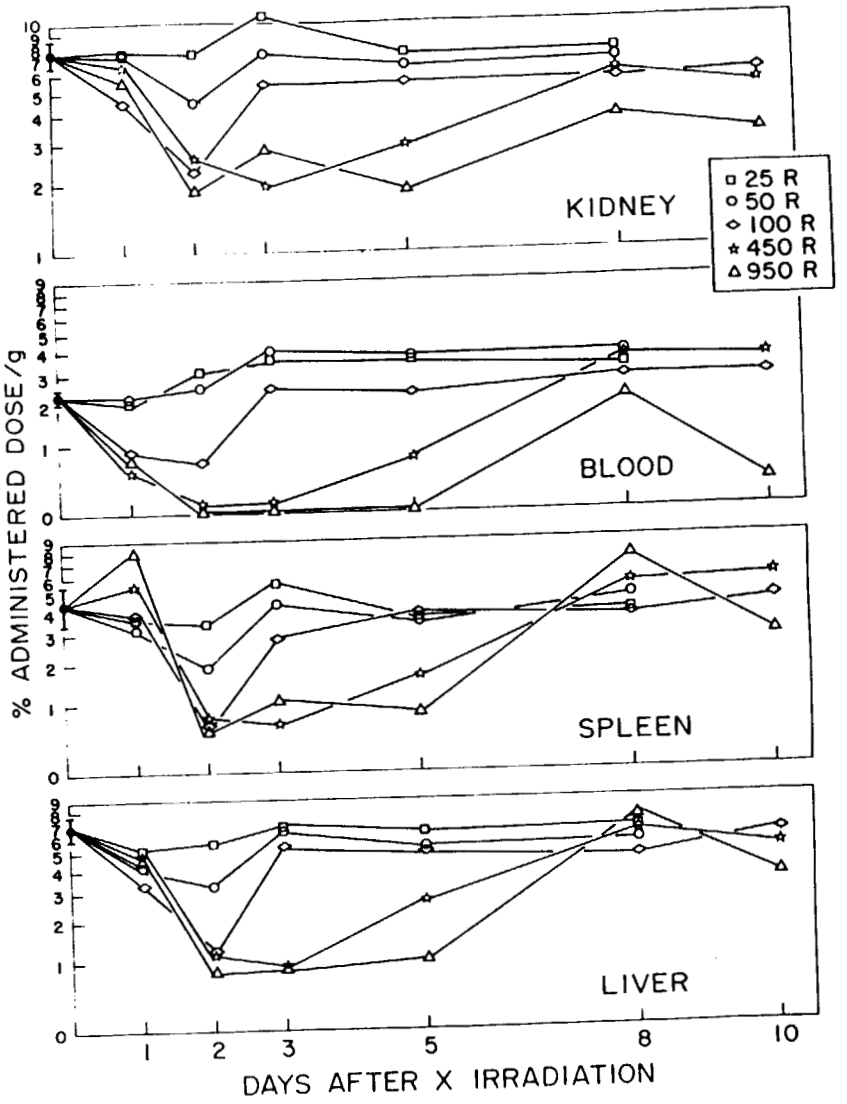


Fig. 70. ^{67}Ga concentrations in four representative tissues at intervals after total-body X irradiation. Tissue concentrations are averages of five mice per point, assayed 24 hr after intraperitoneal ^{67}Ga administration.

constrast to decreased uptake in soft tissues, ^{67}Ga concentration in whole femur increased after irradiation (Fig. 71). Blood levels decreased rapidly in irradiated mice (^{67}Ga injected 24 hr after 450 R) between 1 and 2 hr after giving the radionuclide. Two hours after injection, its concentration was approximately 15% of that in nonirradiated, control animals (Fig. 72). We conclude that decreased ^{67}Ga retention in mice after X irradiation is in part due to an effect on a relatively radiosensitive, as yet unidentified blood or serum component that normally binds the radioisotope. Other as yet unidentified factors may also be operative, however.

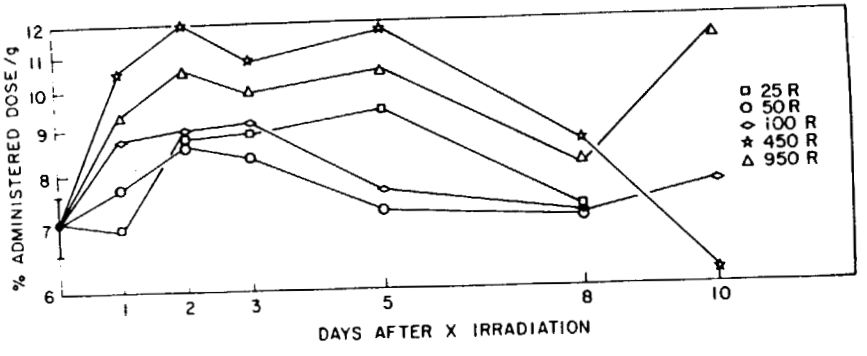


Fig. 71. ^{67}Ga concentrations in whole mouse femur at intervals after different X-irradiation exposures.

X-RAY ANALYSIS OF HEAVY METALS WITH A SCANNING ELECTRON MICROSCOPE

A continuing project of the ultrastructural anatomy program is a collaborative study with Helen Mateer Henson* involving ultrastructural identification of heavy elements, such as lead or cadmium, by X-ray analysis on a scanning electron microscope. The purposes are to precisely localize injected lead or cadmium within cells and/or cellular organelles, such as lysosomes, and ultimately to correlate the presence of these elements with evidence of cellular ultrastructural changes.

Adult $C3BF_1$ mice were injected intraperitoneally with lead acetate in doses of 80 mg/kg of body weight. At intervals after injections, peritoneal exudate (PE) cells were lavaged from the peritoneal cavity with saline and pelleted in centrifuge tubes. Small pieces of spleen, liver, and kidney were also removed and along with PE cells fixed in 3% glutaraldehyde buffered with 0.1 M sodium cacodylate. One group of tissues was postfixed in OsO_4 for correlative transmission electron microscopy. Both groups were dehydrated and embedded in Epon 812. Glutaraldehyde-fixed tissue sections were cut in the range of 2,000 to

* Oak Ridge National Laboratory.

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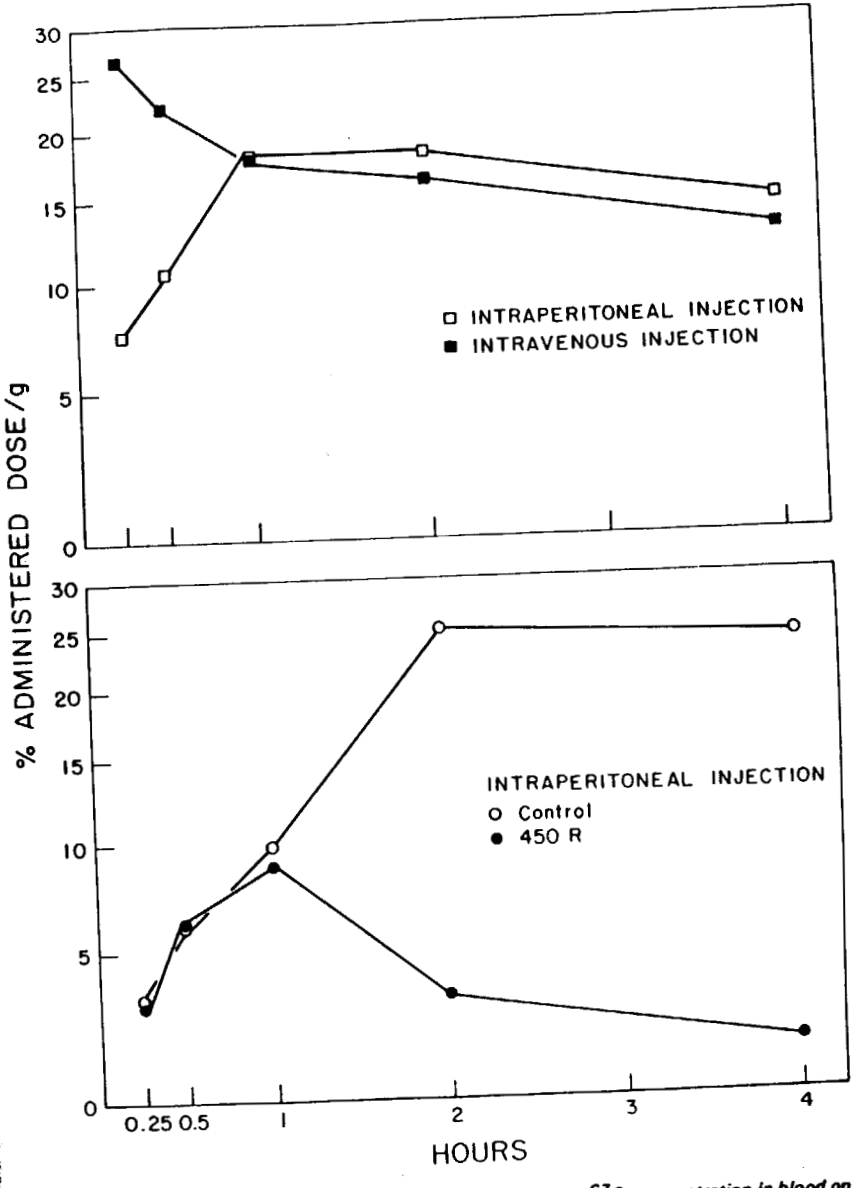


Fig. 72. (Top) Effect of intraperitoneal vs. intravenous injection on ^{67}Ga concentration in blood on nonirradiated mice. (Bottom) Effect of 450 R of X irradiation on ^{67}Ga concentration in blood; ^{67}Ga was injected intraperitoneally 24 hr after X irradiation.

5,000 A thick, mounted on 100-mesh molybdenum grids, coated with carbon, and examined in a scanning electron miscroscope equipped with an energy dispersive system for X-ray analysis. Macrophages from the PE contained aggregates of electron-dense particles surrounded by a single membrane. A direct, positive identification of these particles as lead was made on the basis of energy measurements of the characteristic X rays emitted when the sample was excited with the electron beam (Fig. 73). Operation of the scanning electron microscope in the transmission mode, coupled with the detection and analysis of X rays, enabled us to simultaneously localize and identify intracellular lead in PE macrophages. Correlative electron microscopic studies were done on thin sections of OsO_4-fixed

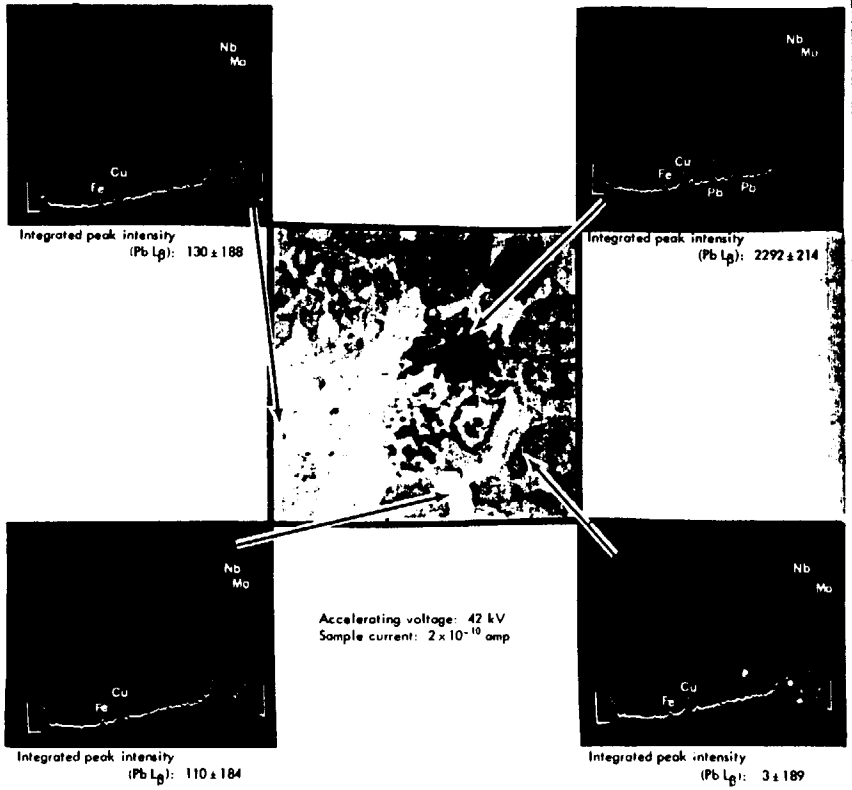


Fig. 73. Photograph in center is a scanning electron micrograph of a peritoneal macrophage taken in the transmission mode of operation. The arrows point to the areas of this section that were analyzed and the accompanying photographs indicate the visual display of elemental peaks for each area scanned. Note that the area containing the electron-dense material gave positive Lα and Lβ peaks whereas other areas scanned contained only background counts. The electron-dense material was therefore positively identified as lead. Glutaraldehyde-fixed material. 6,000 X.

1028188



Fig. 74. A conventional electron micrograph of glutaraldehyde- and OsO_4-fixed peritoneal cells from the same pellet as shown in Fig. 73. Note the electron-dense material in the macrophage corresponding to the material identified as lead in Fig. 73. 12,000 X.

tissues (Fig. 74). Eventually we hope to correlate changes such as mitochondrial swelling with a precise localization of the toxic metal.

INDUCED CYTOGENETIC ABNORMALITIES

FLUORESCENT BODIES IN INTERPHASE LEUKOCYTE NUCLEI FROM PATIENTS WITH LEUKEMIA

In 1970 a method was described by Caspersson et al. (Chromosoma 30, 215, 1970) for the differential staining of human metaphase chromosomes with the fluorochrome, quinacrine mustard (QM). Various segments of the long and short arms of the 22 pairs of autosomes and the pair of sex chromosomes bind QM more strongly than other regions, resulting in alternating brightly fluorescing versus weakly fluorescing bands. In metaphases from normal males, the most brightly fluorescing chromosome region is the long arm of the male sex chromosome, or the Y chromosome. Likewise, in approximately 50 to 80% of leukocyte interphase

nuclei from cytogenetically normal men a small, brightly fluorescing structure, the Y body, can be identified. The inactive, or heteropycnotic X chromosome of the normal female does not show differential fluorescence in leukocytes, although the female sex chromatin body does preferentially bind QM in nuclei of cultured fibroblasts and amniotic cells. Because of the presence of the Y body in male leukocyte nuclei and the absence of X chromosome fluorescence in female leukocyte nuclei, QM staining of blood smears has been advocated as a simple procedure for sexing of white blood cell preparations.

We have recently used this technique in an attempt to estimate the number of donor leukocytes in the circulating blood of a woman who received a marrow transplant from her brother (L. Gayle Littlefield, Lydia S. Corrill, Eugene E. Joiner, and Margo Steinman). The data from this study and subsequent follow-up cast some doubt on the reliability of using the percentage of cells with fluorescing bodies (F bodies) as a criterion for estimating the number of male nuclei in a mixed population of blood cells. This 53-year-old woman with acute leukemia was exposed to approximately 500 R total-body ^{60}Co radiation in preparation for a marrow transplant. On the following day she received 14.9×10^9 marrow cells intravenously from her brother who was matched in human leukocyte antigens. On several occasions after the infusion we obtained blood and marrow samples for evaluation of metaphases and interphase nuclei for the presence of the male Y chromosome.

Metaphase preparations: A marrow aspirate, obtained 5 days after the marrow infusion, was virtually aplastic and contained only pycnotic nuclei. By 20 days the marrow yielded dividing cells and of 50 metaphases evaluated, 49 could be definitely identified as Y bearing. Likewise, in an aspirate obtained on day 33, 54 of 56 metaphases had a Y chromosome. QM-stained slides of these preparations verified that the majority of the metaphases had the brightly fluorescing Y chromosome and the high percentage of male cells indicated that there had been a successful marrow graft. Aspirates were subsequently obtained 53 and 60 days after the transplant, but neither preparation was suitable for cytogenetic evaluation. In samples obtained on day 89 we were able to analyze seven metaphases, all of which were female. Blood and marrow smears at this time showed a recurrence of leukemia and probably the female metaphases in the direct marrow preparations were derived from the patient's malignant cell population.

We also initiated numerous phytohemagglutinin (PHA)-stimulated blood cultures to evaluate Y-bearing lymphocytes. Only two cultures were successful. In blood preparations on day 20 cultured for 72 hr, 50 metaphases were analyzed of which 41 were female, 35 had radiation-damaged chromosomes, whereas none of the Y-positive metaphases had radiation-induced aberrations. From blood samples obtained at 32 days we used 52-hr cultures to ensure harvesting a population of first division cells. Of the 35 metaphases scored in this preparation, five were male. As in the previous lymphocyte culture, the majority of the female cells were radiation damaged. The five normal, male lymphocytes were undoubtedly derived from donor cells. However, it was impossible to determine whether these lymphocytes were produced following a lymphocyte graft, or whether they were simply a circulating subpopulation of original lymphocytes transfused with the marrow infusion. Hoping to answer this question by determining the percentage

1028189

of male cells in subsequent preparations, we initiated 30 additional lymphocyte cultures during the next 6 weeks. Several mitogens, including PHA, concanavalin A, and pokeweed were tried but none of the cultures yielded analyzable metaphases.

Interphase preparations: As a second index of the number of male cells in circulating blood, smears were stained with QM and evaluated for fluorescent bodies. Control preparations from normal men and women were scored as well as slides from the patients. As shown in Table 54 the percentages of cells with F bodies in preparations from the patient are quite consistent in smears obtained from 40 to 75 days posttransplant (16.1% to 17.4%). Most of the F-positive cells in all of these preparations were granulocytes. On day 83 the number of cells with apparent F bodies increased sharply to 50.6%. Similar frequencies of positive cells (56.5% and 52.6%) were apparent in blood smears on days 84 and 85. This finding of an increase in the percentages of interphase cells with brightly fluorescing bodies is quite interesting in the light of the patient's hematological course. On the 75th day posttransplant, blood smears showed no blasts but did show a few young cells. By the 83rd day the patient had more than 90% blasts in her blood, coinciding with the increase in apparent F-positive cells in the QM-stained preparations. There are two possible explanations. Conceivably male (donor) cells were involved in the leukemic relapse. Leukemic transformation of engrafted donor cells has been reported in two other transplantation attempts. However, in our patient, metaphase preparations of marrow aspirates at the time of the relapse showed only female cells, indicating a recurrence of the original leukemic cell population. The incongruity between the sex of the interphase blast nuclei as indicated by the presence of fluorescing bodies and the karyotypic sex of the marrow metaphases suggested that these malignant interphase nuclei may have had different fluorescent characteristics than the nonmalignant cells, resulting in false positive observations of the Y body.

TABLE 54

PERCENTAGES OF NUCLEI WITH F BODIES IN BLOOD SMEARS FROM CONTROL MEN AND WOMEN AND A PATIENT RECEIVING A MARROW TRANSPLANT

	Day posttransplant	No. of cells	Percent F positive
CONTROL			
Males		479	65.2
		184	80.5
		209	72.0
Female		207	3.4*
PATIENT	40	298	16.1
	54	393	17.4
	61	552	16.3
	70	268	16.8
	75	82	17.0
	83	395	50.6
	84	145	56.5
	85	190	52.6

* False positive.

To determine whether similar results might be expected in QM-stained blood smears from other patients, we obtained blood smears from five men and six women with several types of leukemia to evaluate F bodies. From each coded slide we attempted to score 300 interphase nuclei, noting the presence or absence of fluorescing bodies. When F bodies were noted, we recorded the relative size of the nucleus, and the staining characteristics and number of F bodies. The results are shown in Table 55.

TABLE 55

PERCENTAGES OF NUCLEI WITH F BODIES IN BLOOD SMEARS FROM CONTROL MEN AND WOMEN AND PATIENTS WITH LEUKEMIA

Patient Sex, Number	Disease*	White blood cell count	Number cells scored	Percent F+ cells
♀- 1	CGL	121,600	600	11.2
♀- 2	CLL	75,300	301	9.7†
♀- 3	CLL	7,400	203	3.0
♀- 4	CGL	95,000	305	12.5
♀- 5	CGL	94,000	445	11.0†
♀- 6	CGL	27,300	303	6.0†
♀- 7	None		170	5.3†
♀- 8	None		160	0
♀- 9	None		250	0
♂-10	CLL	35,500	307	91.1‡
♂-11	CGL	66,900	300	35.0†
♂-12	AL	47,600	303	87.0‡
♂-13	CGL	265,600	302	90.2
♂-14	Leukemic lymphosarcoma	15,700	413	49.0†
♂-15	None		241	55.6
♂-16	None		200	60.0
♂-17	None		176	77.0

* CGL, chronic granulocytic leukemia; C11, chronic lymphocytic leukemia; AL, acute leukemia.

† Small or faintly stained.

‡ Multiple F bodies in approximately one third of the positive nuclei.

The percentage of nuclei with F bodies in preparations from women varied from 0 to 5.3% in smears from three controls, and from 3.0 to 12.5% in smears from six women with various types of leukemia. In the leukemic women, the lowest percentages of F-positive cells were obtained from two with the lowest white blood cell (♀-3, ♀-6). In three normal men the frequency of nuclei with F bodies ranged from 55.6 to 77.0% compared to a range of 35.0 to 91.1% in men with these sampled malignant blood diseases. In smears from two of the men, approximately one third of the positive nuclei had multiple brightly fluorescing bodies. F-positive nuclei are shown in Fig. 75.

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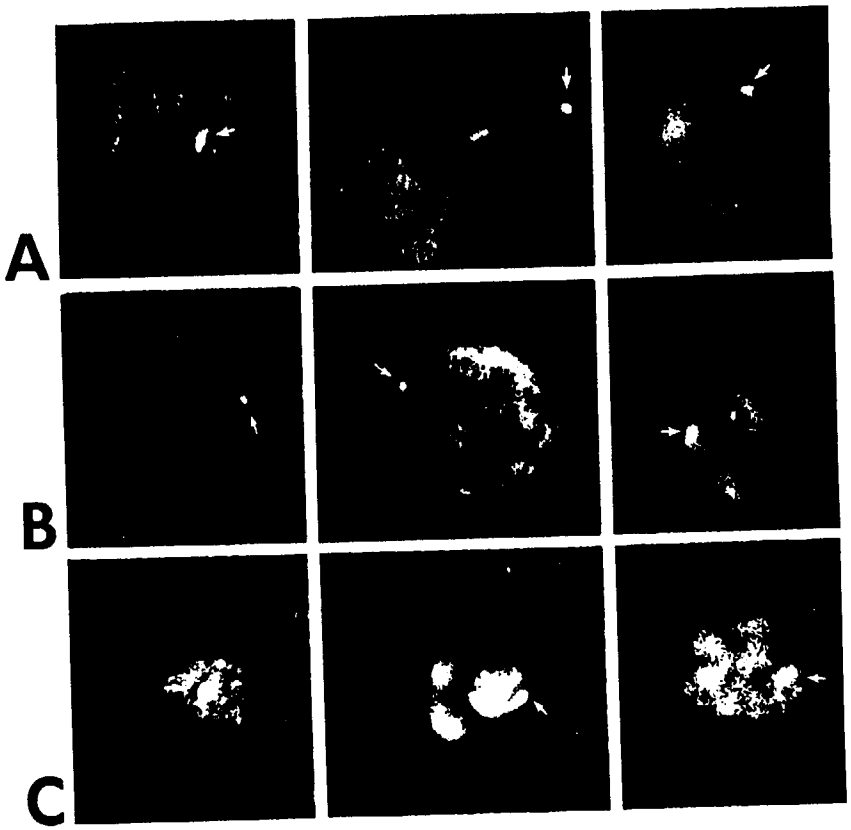


Fig. 75. Fluorescent bodies in interphase nuclei. (A) Nuclei from women with CGL, AL, and AL respectively. (B) Nuclei from men with CGL, malignant lymphoma, and CLL. Note multiple fluorescing bodies in the male patient with CLL. (C) Normal male nuclei. Note similar size and location of fluorescent bodies in all three groups.

Several observations concerning interphase fluorescence may be made from this study. First, some autosomal chromosome regions, e.g., the centromeric region of the chromosome 3 and the satellities of the large and small acrocentrics, frequently show bright metaphase fluorescence in some persons. Occasionally other investigators have noted very small fluorescing bodies in male and female interphase nuclei, and have suggested that these structures represent fluorescence of these autosomal regions. Generally, it is assumed that these regions of "secondary" fluorescence can be distinguished from the "primary" fluorescence of the Y body by virtue of the fact that it is more peripheral, larger, and more strongly fluorescent. We have not been able to use these criteria for identifying Y-bearing leukemic blasts. In the large vesicular

nuclei from leukemic men we have rarely observed the "unmistakable" brightly fluorescing Y body that may be seen in smaller, more condensed lymphocyte nuclei. Similarly, we have observed specific fluorescing bodies in large "immature" interphase nuclei from female leukemic nuclei that are indistinguishable from the structures observed in male leukemic cells. Although they appear smaller than the typical Y body when compared to the relative size of the nucleus, the actual size of the fluorescing bodies of the malignant cell is often very similar to the size of the Y in smaller nonmalignant lymphocytes. We have also noted that the frequency with which the F bodies are observed in leukemic cells varies in different preparations. Thus in slightly overstained preparations the bodies are obscured by generalized nuclear fluorescence, whereas in understained preparations, F bodies, including the Y of normal males, may not be bright enough to identify.

Probably the F bodies that we have noted in blood smears from the various women with leukemia, including the transplant patient, were derived from autosomal regions that were condensed during interphase. Since the frequency and intensity of autosomal fluorescence seems to vary in metaphases from different persons, it seems quite likely that the percentage of cells with F bodies in interphase is also quite variable.

Although nuclear sexing of some cell types by fluorescent staining may serve a useful purpose, i. e., evaluations of cultured amniotic cells in prenatal sex determination, or in screening blood smears from nondiseased persons for Y chromosomal abnormalities such as XYY males, our experience suggests that there may be serious drawbacks in its use for estimating the number of Y-bearing nuclei in mixed populations of leukocytes.

BANDING IN HUMAN LYMPHOCYTE CHROMOSOMES TREATED WITH TRYSIN AND UREA

Soon after the publication by Caspersson et al. (Chromosoma 30, 215, 1970) of the method for fluorescent staining of human metaphase chromosomes, several laboratories described techniques for the differential staining with Giemsa blood stain after treatment of fixed metaphases with hypertonic salt solutions or weak concentrations of proteolytic enzymes. Of the various techniques for Giemsa banding, two of the simplest procedures require immersion of slides in either trypsin or urea before staining. Although many of the published techniques for trypsin or urea banding superficially appear to be similar, there are numerous variations in specific steps of the procedures, e.g., concentration of enzyme, duration of treatment, temperature, pH of solutions, etc. Hence when a specific procedure does not produce banding in a particular group of slides, it is virtually impossible to know which step should be altered to obtain results.

During the year we have attempted to perfect a Giemsa-banding technique for use in our laboratory to identify abnormal malignant cell karyotypes (L. Gayle Littlefield and Margo Steinman). None of the published techniques that we have tried consistently produce banding, and frequently many modifications must be tried before we obtain good quality banding in a specific series of slides. In our initial attempts with lymphocyte chromosomes we varied the concentration, temperature, and pH of both urea and trypsin. After many trials we found that treatment at 37°C with either 0.025% trypsin (pH 7.5) or 4 M urea (pH 8.0) yielded good results more often than other modifications, but not consistently. During these staining attempts, it became apparent that the quality of banding varied in slides of different ages, and in slides from different persons. We also noted that the type of hypotonic solution to which the cells were exposed before fixation also seemed to affect the degree and clarity of banding. To further investigate the effect of the age of the slide and the type of hypotonic treatment on banding, we prepared replicate cultures and swelled the cells with either hypotonic media (3:1 distilled water to medium), 0.075 M potassium chloride (KCl), or 1% sodium citrate (NaCit). Slides of ages ranging from 1 to 30 days were then processed for Giemsa banding. Table 56 shows some of our results with the trypsin technique, and Table 57 shows comparable data from slides treated with urea before staining. In this series of experiments all slides were incubated at 37°C and stained in 5% Gurr's "R66" Giemsa stain made up in Sorensen's buffer at pH 6.8.

TABLE 56

BANDING AND CHROMOSOME MORPHOLOGY RESULTING FROM TREATING LYMPHOCYTE PREPARATIONS WITH TRYPSIN, pH 7.5, AT 37°C

Slide*		Treatment								
	Age (day)	Hypotonic media	Solution†	Duration (sec)	Banding	Spiralization	Fuzzy	Empty	Overly digested	Puff
G3	30	H_2O	Hanks'	30	++/+++	–	+	–	–	–
G2	16	H_2O	Hanks'	30	+/++	–	+/++	–	–	–
G6	16	NaCit	Hanks'	30	++/+++	–	+	–	–	–
G6	16	NaCit	Solution A	30	±	–	++	+++	+++	–
G6	15	NaCit	Dulbecco's	30	+++	–	±	–	–	–
G4	15	KCl	Dulbecco's	20	++	–	–	+	+	++
G4	15	KCl	Dulbecco's	30	+	–	–	++	++	++
G6	15	NaCit	Dulbecco's	25	++	–	±	–	–	–
G6	15	NaCit	Dulbecco's	35	++	–	+	–	–	–
G2	15	H_2O	Dulbecco's	30	+	–	++	–	–	–
G3	30	H_2O	Dulbecco's	30	+++	–	±	–	–	–

* Replicate slides of lymphocyte cultures from one person.

† 0.025% trypsin dissolved in calcium-magnesium free Hanks' balanced salt solution, in Dulbecco's phosphate buffered saline, or in 0.25% Gibco solution A.

Generally speaking the reaction that produces banding proceeds too rapidly in freshly made slides, frequently resulting in overly digested or fuzzy metaphases. Older slides are more resistent to both trypsin and urea, and usually yield better banding with less distortion of chromosome morphology. Also, slides prepared from cultures treated with water or NaCit as a hypotonic solution tend to yield better results than those treated with KCl. As shown in Tables 56 and 57, trypsin produces a more exaggerated effect on metaphase morphology than does urea, although the specific banding patterns are quite similar in good metaphases from the two types of treatments (Fig. 76 and 77).

1028191

TABLE 57

BANDING AND CHROMOSOME MORPHOLOGY RESULTING FROM TREATING LYMPHOCYTE PREPARATIONS WITH 4 M UREA, pH 8.0, AT 37°C

Slide*		Treatment								
No.	Age (week)	Hypotonic media	Solution†	Duration (min)	Banding	Spiralization	Fuzzy	Empty	Overly digested	Puffy
G2	3	H_2O	Dulbecco's	30	–	±	–	–	–	–
G2	3	H_2O	Hanks'	30	±	±	–	–	–	–
G2	3	H_2O	Sorensen's	30	–	±	–	–	–	–
G6	3	NaCit	Dulbecco's	30	–	+	–	–	–	–
G6	3	NaCit	Hanks'	30	±	+/++	–	–	–	–
G6	3	NaCit	Sorensen's	30	–	+	±	–	–	–
G4	3	KCl	Dulbecco's	30	–	+/++	+	–	–	–
G4	3	KCl	Hanks'	30	–	±	±	–	–	–
G4	3	KCl	Sorensen's		–	±	±	–	–	–
G6	3	NaCit	Hanks'	90	±/+	++/+++	–	–	–	–

* Replicate slides of lymphocyte cultures from one person.

† 4 M urea dissolved in Dulbecco's phosphate buffered saline, calcium-magnesium free Hanks' balanced salt solution, or Sorensen's phosphate buffered saline.

Although we have obtained some degree of banding after many types of treatments, we have not yet been able to consistently obtain usable metaphases from all preparations. Even in good preparations, frequently only a few metaphases will show optimum banding, and of these, often some chromosomes will be banded while others will not. Since many slides can be prepared from a single lymphocyte culture, it is usually possible after using several modifications of a banding technique to obtain a few metaphases that can be used for detailed karyotyping. In preparations in which the amount of material is limited and the mitotic index is low (i.e., direct marrow preparations), it may be impossible to obtain well banded metaphases. We are continuing our studies of those variables that affect the quality of banding in lymphocyte preparations, and hope to eventually devise a procedure that will consistently produce good results.

ORAL CONTRACEPTIVE STUDY

Statistical Analysis of Lymphocyte Data. During the last 3½ years our laboratory has studied the frequency of chromosomal aberrations in 840 lymphocyte cultures from control men and women, pregnant women, and women taking oral contraceptives (OC) (L. Gayle Littlefield, Eugene E. Joiner, Lydia S. Corrill, and Mary Beth Klepper). Although we have observed a significantly higher frequency of chromosome breakages in women taking OC, we have found that chromosome breakages are common in lymphocyte cultures from control men and women, and that the frequency of breakages is variable both in consecutive cultures from the same person and in cultures from different people. Because of the variability among cultures within all groups, it is extremely difficult to define

1028192

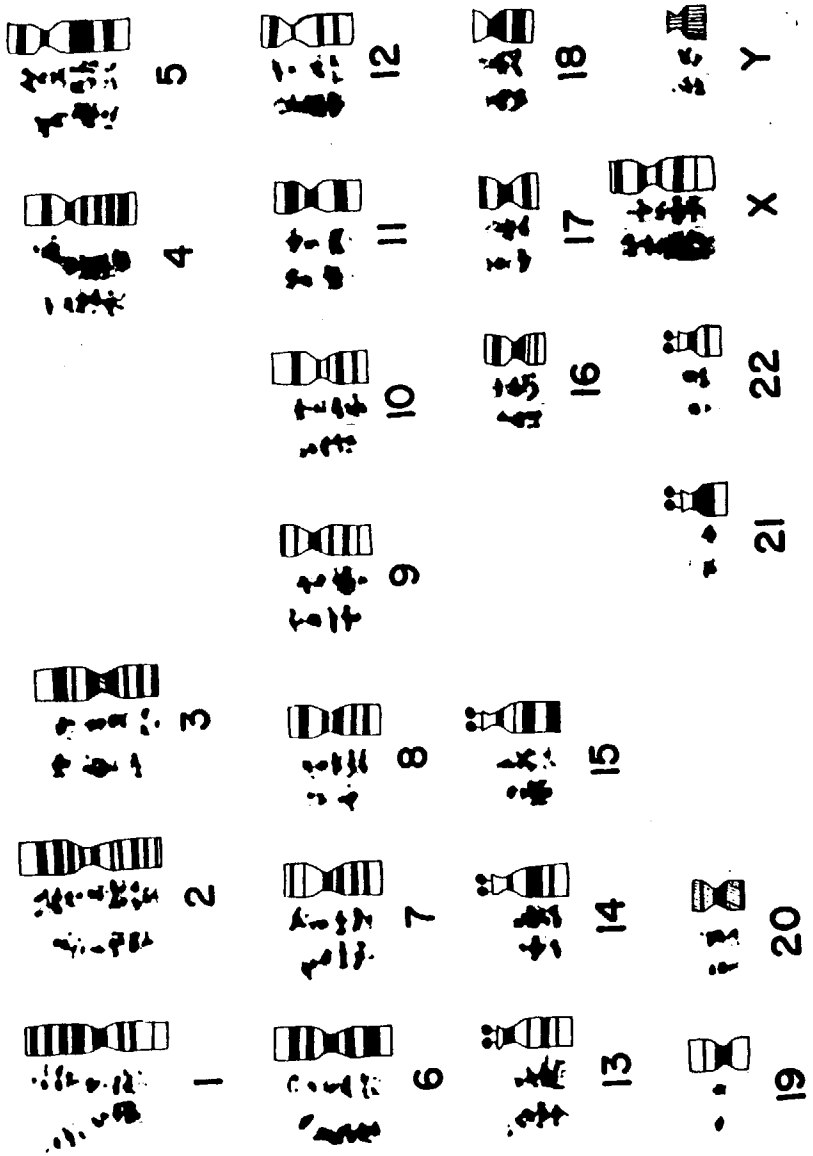


Fig. 76. Composite of two metaphases from the same person, showing trypsin-induced chromosome banding patterns. One homolog from each metaphase is on the left, and a diagram of the banding pattern on the right (0.025% trypsin in Dulbecco's phosphate buffered saline).



Fig. 77. Composite of two metaphases from two persons, showing urea-induced chromosome banding patterns. One homolog from each metaphase is on the left and a diagram of the banding pattern on the right (4 M urea in Dulbecco's phosphate buffered saline).

1028193

the exact effect of exogenous hormones on chromosome breakages. Obviously, in any in vivo study involving humans over a period of years, there are numerous extraneous variables that cannot be controlled, and which may affect experimental results. We have listed as many of them as possible in our study, and are currently attempting to determine by statistical analyses whether any of these contribute to the variability in the frequency of aberrations in this series (L. Gayle Littlefield, W. E. Lever,*, F. L. Miller,* and John B. Mailhes). From health sheets obtained from every subject at the time each blood culture was initiated, we have encoded information on the following independent variables: 1) date of culture (month and year), 2) age, 3) hormonal condition (never pregnant, never on OC; previously pregnant, never on OC, etc.), 4) number of pregnancies, 5) months since last pregnancy, 6) stage of current pregnancy (trimester), 7) type of pill, 8) months on pill, 9) months since last pill, 10) stage of menstrual or OC cycle, 11) infection during preceding culture period (viral, bacterial, cold, other), 12) medications during preceding culture period (aspirin, antibiotics, tranquilizers, narcotics, other), and 13) person analyzing slides. Likewise, for each culture we have encoded the following dependent variables: 1) number of abnormal cells, 2) number of abnormal chromosomes, 3) number of specific types of lesions (isolocus breaks, deletions, translocations, etc.), and 4) the distribution of lesions among various chromosomes or chromosome groups. By a variety of statistical tests and comparisons of cultures we hope to determine 1) whether there are differences in the types or frequencies of aberrations in cultures from women who have different hormonal histories (prior or current hormonal status), and 2) if these differences vary according to time on or time off treatment. We also hope to learn whether other incidental conditions, i.e., age of donor, season of year, etc., influence the frequency of abnormal metaphases in specific cultures, and, if so, to make appropriate data adjustments. At the conclusion of the analyses we hope to be able to determine more precisely the effect of OC use on lymphocyte chromosome breakages.

Fibroblast Studies. The great variability in the number of abnormal cells among different lymphocyte cultures from the same control subject, combined with sporadic instances of very high breakages, suggest that the lymphocyte may be very susceptible to various extraneous chromosome breaking agents (i.e., viruses, incidental medications, etc.). If this is so, then do OC simply cause increased breakages in a "reactive" cell, or are lymphocyte chromosomal breakages a useful monitor of chromosome damage in other somatic cells and gametes?

In an attempt to answer this question, we initiated a series of studies designed to determine the in vivo and in vitro effects of synthetic hormones on a second cell type, the skin fibroblast. During the study we have obtained skin biopsies for fibroblast culture from control women and women taking OC. In addition we have initiated several foreskin cultures from normal male infants for in vitro testing of estrogens and progestogens.

Chromosome aberration frequencies in fibroblast cultures from control women and women taking OC. There are several reasons for selecting fibroblasts as a second type of somatic cell for cytogenetic study. First,

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fibroblasts are the only somatic cells, other than lymphocytes, that can be readily obtained from humans and cultured for cytogenetic evaluation. Second, many replicate cultures may be obtained from a single primary cell line, allowing for the in vitro testing of many different substances in cultures derived from the same population of cells. One disadvantage in using primary fibroblast cultures for evaluating in vivo induced cytogenetic aberrations is obvious: Since fibroblasts must proceed through numerous cell divisions in vitro before a sufficient number of cells are available for cytogenetic evaluation, it is possible that some cells damaged in vivo might not survive in culture. During successive cell divisions, some aberrant cells may be eliminated while other abnormal stem lines may be propogated. Thus, the cell population that is finally analyzed may be different from the original population. In scoring cytogenetic aberrations in fibroblast cultures, one must not only look for typical types of chromosome breaks and rearrangements but also for repetitious lesions that could indicate an aberrant stem line.

With these considerations in mind, we obtained skin biopsies and blood from five women who were taking OC and from five women who had never taken synthetic hormones (John B. Mailhes, Barry S. Lazar, and L. Gayle Littlefield). Two lymphocyte cultures were initiated from each blood sample; one culture was supplemented with autologous serum and the other with fetal calf serum (FCS). The cells were harvested after 3 days according to the procedure routinely used in our laboratory. Primary fibroblast cultures were established from each skin biopsy (Mailhes, this report p. 177). All fibroblast cultures were initially maintained in a medium containing 20% FCS. When sufficient fibroblasts were accumulated for cytogenetic studies, we exposed replicate subcultures of rapidly dividing cells to the following types of serum: 1) 20% FCS, 2) 20% autologous serum, 3) 20% homologous serum (control), and 4) 20% homologous serum (OC user). Approximately 24 hr after adding the serum, we harvested the cultures for cytogenetic evaluation. We cultured four blood and skin samples from each woman during the course of 1 year to have representative data from each individual.

From this series of experiments we hope to learn 1) whether chromosomal breakages occur as frequently in fibroblasts as in lymphocytes, 2) whether the frequency of chromosomal aberrations or of derived chromosomal stem lines are higher in fibroblasts from women taking OC than in fibroblasts from control women, and 3) whether serum from OC users exerts chromosome breaking properties in vitro, either in previously exposed cells (fibroblasts from OC users) or in control cells.

Our preliminary cytogenetic findings on the frequency of chromosomal breakages are shown in Table 58. Since we have completed cytogenetic analysis of only a portion of the cultures at this time, we have insufficient data to validly compare the effects of OC (or serum from OC users) on breakages in cultures from the various women. However, we can make several observations based on our preliminary cytogenetic data and our experience in culturing the fibroblasts. First, with the data from the small numbers of cultures now available, it appears that "baseline" chromosome breakages occur with approximately equal frequency in lymphocytes and serially cultured fibroblasts. It should be noted, however, in the lymphocyte and fibroblast cultures examined thus far, the aberrations vary from 0 to 7% (excluding single chromatid lesions). We have not

1028194

TABLE 58

COMPARISON OF CHROMOSOME BREAKAGES IN LYMPHOCYTES AND FIBROBLASTS FROM CONTROL WOMEN AND OC USERS

		Control women			OC users		
Cell type	Serum treatment	Number donors	Number metaphases	Abnormal chromosomes /100 cells	Number donors	Number metaphases	Abnormal chromosomes /100 cells
Lymphocytes	Autologous	5	398	6.53*	3	428	4.21*
Lymphocytes	FCS	5	358	5.87	3	156	8.33
Fibroblasts	FCS	5	290	7.24	4	328	6.10
Fibroblasts	Autologous	5	441	5.90	4	547	5.12
Fibroblasts	Homologous (control)	5	409	5.13	3	421	5.46
Fibroblasts	Homologous (OC)	5	391	6.65	3	440	4.55

* Includes single chromatid lesions.

seen in either series of cultures the extremely high breakages (20 to 30%) occasionally noted in other lymphocyte cultures. Thus we do not know at this time if the fibroblasts would be as affected by extraneous agents which induce a very high frequency of breakages as lymphocytes. Second, it appears that stem lines of chromosomally aberrant cells may evolve in relatively short periods in fibroblast cultures from adult women. In the various fibroblast preparations of one biopsy from a control woman, 50 to 100% of the metaphases scored were hypodiploid, with a 45,XX,-D karyotype. These cells had been cultured for 75 days. Likewise, in fibroblasts from one OC user, 8 of 269 metaphases had an identical abnormal chromosome, in this instance a "B" chromosome with deleted short arms. These cells were analyzed after 40 days in culture. Data on the evolution of chromosomally aberrant stem lines may be important for two reasons. If we should find a higher percentage of chromosomally abnormal cell clones in fibroblast cultures from OC users, we would have reason to believe that the exogenous hormones did affect the fibroblast chromosomes in vivo, or that these substances predisposed fibroblasts to injury in vitro. Second, since fibroblast cultures derived from amniotic cells are widely used to determine the chromosomal complement of high-risk fetuses, it would be very useful to know how soon after initiation of the culture aberrant stem lines may be observed. In one reported case an abortion was performed on a normal male fetus based on an erroneous observation of a 45,XO karyotype in an abnormal clone of cultured amniotic cells.

We hope to complete the analyses of all cultures during the coming year. At the time we should have accumulated considerable amount of data both on the frequency of chromosomal breakages and the evolution of abnormal cell lines in fibroblast cultures from control women and OC users.

Cytogenetic studies in fibroblast cultures exposed in vitro to estrogens and progestogens. To determine whether the increased chromosome breakages that we had previously noted in lymphocyte cultures from OC users had been the result of a direct action of synthetic estrogens and progestogens on chromatin material, we studied chromosome aberrations in fibroblasts cultured in vitro with low, medium, and high levels of hormones (L. Gayle Littlefield, Eugene E. Joiner, John B. Mailhes, and Barry S. Lazar). Primary fibroblast cultures were established from a foreskin from a normal male infant. On day 55

we split four rapidly growing cultures into 18 replicates. Four hours after subculturing we treated duplicate flasks with various combinations of estrogens and progestogens; after approximately 23 hr exposure, all cultures were harvested for cytogenetic evaluation.

There were considerable differences in general cellular morphology and mitotic index (MI) between the various hormone-treatment groups and the control cultures. Because of the low MI in some preparations, we repeated several of the treatments in other subcultures derived from the same cell line. Subsequently we obtained scorable metaphases from all except the cultures treated with 100,000 and 200,000 ng/cc of the progestogen, dimethisterone. All slides were coded and scored for cytogenetic aberrations. In our previous study of chromosome breakages in lymphocytes, we considered a chromosome to be abnormal if it had a lesion involving both sister chromatids (i.e., isolocus breaks, deletions, and all classes of inter- or intrachanges). In the analyses of these preparations, we noted a high frequency of single chromatid lesions, and we have also included this type aberration in our data tabulations.

The specific estrogens and progestogens tested for chromosome breaking effects were mestranol and ethinyl estradiol, and norethindrone and dimethisterone, respectively. The combinations and concentrations of these steroids in several commercially available birth control pills are shown in Table 59. The cytogenetic findings in fibroblast cultures exposed to various concentrations of these hormones are shown in Table 60.

TABLE 59

CONCENTRATION OF ESTROGEN AND PROGESTOGEN IN SEVERAL OC

Brand Name	Estrogen	Protestogen
Combined *		
Enovid E (EN)	Mestranol 0.1 mg	Norethynodrel 2.5 mg
Ortho-Novum (ON)	Mestranol 0.1 mg	Norethindrone 2.0 mg
Ortho-Novum 1/80 (ON1/80)	Mestranol 0.08 mg	Norethindrone 1.0 mg
Ortho-Novum 1/50 (ON1/50)	Mestranol 0.05 mg	Norethindrone 1.0 mg
Ovulen (OV)	Mestranol 0.1 mg	Ethynodiol diacetate 1.0 mg
Norinyl (NOR)	Mestranol 0.05 mg	Norethindrone 1.0 mg
Norlestrin 1 (NO1)	Ethinyl estradiol 0.05 mg	Norethindrone acetate 1.0 mg
Norlestrin 2 (NO2)	Ethinyl estradiol 0.05 mg	Norethindrone acetate 2.0 mg
Sequentials†		
C-Quens	Mestranol 0.08 mg	Chlormadinone acetate 2.0 mg
Oracon	Ethinyl estradiol 0.08 mg	Dimethisterone 25.0 mg

* Estrogen and progestogen combined in one dose, average 20 consecutive pills per month.
† Estrogen alone taken for 15 to 16 days, followed by 5 days progestogen plus estrogen.

1028195

TABLE 60

ABERRATION FREQUENCIES IN FIBROBLAST CULTURES TREATED WITH ESTROGENS AND PROGESTOGENS

Treatment	Concentration	Cells scored	Cells with breaks			Chromosomes with breaks	
			TID+ISO*	ISO	TID	ISO	TID
	Steroid (ng/cc)†						
Mestranol Norethindrone	1X 8/160	100	16	7	11	8	13
Mestranol Norethindrone	10X 80/1600	100	14	4	11	5	14(+2)‡
Mestranol Norethindrone	100X 800/16,000	100	7	3	4	3	4
Ethinyl estradiol Norethindrone	1X 4/160	100	10	3	7	4	7(+1)‡
Ethinyl estradiol Norethindrone	10X 40/1600	100	11	2	9	2	13
Ethinyl estradiol Norethindrone	100X 400/16,000	100	12	6	6	6	6
Ethinyl estradiol Dimethisterone	1X 6/2000	100	10	1	9	1	9
Ethinyl estradiol Dimethisterone	10X 60/20,000	100	3	0	3	0	3
Ethinyl estradiol Dimethisterone	100X 600/200,000	---					
	Ethanol/cc§						
Normal control	---	100	12	7	6	7	6(+1)‡
Ethanol control	0.001	100	6	3	3	3	3
Ethanol control	0.01	100	12	4	8	4	9

* TID = chromatid, ISO = isochromatid.
† Steroid concentrations were figured on the premise that an average adult female weighs 55 kg with a mean extracellular volume of 230 ml/kg [0.1 mg of steroid ÷ (55 mg x 230 ml) = 7.905 ng/cc].
‡ Other abnormal chromosomes.
§ Two milligrams of steroids were dissolved in 0.2 ml absolute ethanol and diluted with appropriate amounts of culture media. Final concentration of alcohol in various cultures ranged from 1.4×10^{-5} to 5×10^{-3} cc Ethanol/cc culture medium.

Since the steroids were not soluble in water, we initially dissolved the hormones in 0.2 ml absolute ethanol and diluted this stock solution with various amounts of tissue culture medium to give the desired final concentration in the cultures. To determine whether the ethanol carrier affected the treated cultures,

we set up both normal control and ethanol control cultures. Of the three control preparations, the culture treated with 0.001 cc ETOH/cc ethanol/cc culture medium had the lowest number of breaks. The second ethanol control culture and the normal control preparation had very similar types and frequencies of breakages. Thus we can assume that the ethanol had no effect on breakages.

In the replicate fibroblast cultures exposed to the various estrogens and progestogens, the highest frequencies of breakages were seen in preparations treated with 1X and 10X concentrations of mestranol and norethindrone. In each of these cultures the increase in total breakages was the result of an increase in the frequency of chromatid-type lesions. Since the number of chromosomes with breaks did not increase with increasing concentrations of the hormones, the aberration frequency does not appear to vary directly as a function of dose. We observed the lowest frequency of breakages in the preparation treated with a 10X concentration of ethinyl estradiol and dimethisterone.

What explanations then might account for the inconsistencies in chromosome breakages (particularly of the chromatid type) among the cultures? First, all cultures were replicates of the same cell line of fibroblasts derived from a single person; thus it is not necessary to consider the possible variable of differences in aberration frequencies among cultures from different persons. On the other hand, since we were interested in determining whether high concentrations of several different hormones produced a large increase in chromosome breakages, we evaluated only one culture per treatment for this initial experiment. Thus, we cannot estimate the degree of random variation in aberration frequencies among different cultures exposed to the same concentration of hormones. It is certainly expected that one might score 100 metaphases from one group of slides from a culture and a second hundred metaphases from a second group of slides from the same culture and obtain different numbers of cells with aberrations. If the error due to random variation among metaphases from different slides should be large, then we would assume that there are no real differences between breakages in the control cultures and in cultures treated with the hormones.

On the other hand, the frequency of chromatid lesions in the 1X and 10X mestranol-norethindrone cultures (13 and 16%) appears to be too high to be the result of a random variation. If these concentrations of mestranol-norethindrone did in fact cause an increase in chromatid aberrations, there is a possible explanation for the apparent decrease in breakages in the cultures treated with higher concentrations of the hormones. Synthetic estrogens have been shown to inhibit cell division in HeLa cell and L cell fibroblasts. Typical effects are rounding up and release of monolayered cells from the floor of the culture flask and mitotic inhibition. These phenomena were observed repeatedly in the cultures exposed to concentrations of ethinyl estridiol and dimethisterone exceeding 60/20,000 ng/cc. Since mitotic inhibition is apparently linearly related to the concentration of the hormone to which cells are exposed, conceivably different subpopulations of cells were evaluated in the low, medium, and high exposure cultures. Thus, if an injured cell should not enter mitosis as rapidly as an intact cell, then, by harvesting all subcultures at the same time after exposure, we may have terminated the cultures exposed to the highest concentrations (the cultures with the greatest mitotic delay) before the more heavily damaged cells had reached division. Of course this hypothesis is purely speculative and additional studies in which cultures treated with high concentrations of hormones are serially harvested will be necessary to elucidate these preliminary findings.

1028196

A reliable procedure for the in vitro culture of human filbroblasts from small skin biopsies. In initiating fibroblast cultures from human skin biopsies several points must be considered. It is essential that 1) the procurement of the tissue sample be as painless as possible, 2) the excision leave no obvious permanent scar, and 3) the size of the biopsy be large enough for manipulation and preparation for in vitro culturing. During the course of this study we have obtained 91 skin biopsies from 15 women. We have used with varying degrees of success several published techniques for obtaining and culturing these biopsies, and have found the following modified procedure to be the simplest and most reliable (John B. Mailhes, Barry S. Lazar, and L. Gayle Littlefield).

We routinely obtain the skin biopsies from the midscapular region since this area is not highly innervated, and the resulting small scars are inconspicuous. As in any cell culture procedure, all instruments and solutions must be sterile and free of chemical contaminants, and aseptic techniques must be used throughout.

Procurement of biopsy: 1) Cleanse the local area with pHisoHex, swab twice with 70% alcohol, and anesthesize with Ethyl Chloride. (Ethyl Chloride does not interfere with cell growth, whereas biopsies obtained following intradermal injection of xylocaine frequently failed to grow in culture). 2) With a 2-mm diameter manual trephine incise a full thickness of skin (as indicated by minor superficial bleeding), lift with a fine-point forceps, and excise with sharp iris scissors. 3) Immediately transfer the biopsy to a 60-mm Petri dish containing 3 ml warm complete culture medium,* and place in a 37°C incubator until further handling.

In vitro cell culture: 1) Transfer the biopsy to a Petri dish containing complete culture medium and agitate to remove red blood cells. 2) Increase the surface area of the biopsy by cutting with a small sharp scissors but leave the sample intact. Repeated observations from different procedures have shown that this method gives a more rapid adherence to the culture flask. 3) With a Pasteur pipette or needle, transfer the biopsy from the Petri dish to a 25 cm^2 plastic Falcon flask, and with an accompanying drop or two of medium gently rub it on the floor of the flask. This enhances surface contact and facilitates adherence to the vessel. 4) Incubate at 37°C with a 5% CO_2 atmosphere for about 4 hr, then add 2 ml of complete culture medium to the flask. 5) Change the medium after 24 hr of incubation. Thereafter, we routinely change medium (decant and add 3 ml) every 48 hr or until cellular outgrowth is observed (at which time the medium is changed when the pH drops to 6.8 to 7.0). We usually notice cellular proliferation after 3 to 5 days in culture, and abundant outgrowth by 10 to 14 days after the biopsy is taken. At this time the biopsy may be moved to another area of the same flask to enhance cellular confluency.

Approximately 30 days are required to obtain a confluent monolayer of fibroblasts in a 25 cm^2 flask. Subsequently the cells may be readily subcultured and harvested for cytogenetic analyses by routine methods.

We feel that this procedure is superior to other techniques for the following reasons. Procurement of the biopsy is rapid, virtually painless, and produces a

* RPMI 1640 80%, FCS 20%, penicillin 50 units/cc, and streptomycin 50 μg/cc.

small wound requiring no suture. Cosmetically, it leaves a very small scar inconspicuous in this region of the back. For fibroblastic growth to occur from a biopsy tissue, it is essential that the primary explant become firmly adhered to the floor of the culture flask. Small pieces of tissue will generally float in the culture medium, and many methods (including implanting the biopsy in a plasma clot, covering the sample with sterile cellophane, or sandwiching the explant between cover slips) have been proposed to physically hold the tissue against the vessel floor, but these procedures are not necessary if the 2-mm biopsy is left intact and initially covered with a very small amount of medium. The explant will generally adhere within a few hours, and if other conditions are suitable, will produce good fibroblastic growth in about 2 weeks.

MISCELLANEOUS RESEARCH ACTIVITIES

RETENTION AND DISTRIBUTION OF GOLD THIOGLUCOSE IN MICE

Gold thioglucose (GTG) administered intraperitoneally to mice results in necrosis of a portion of the hypothalamus. It is generally believed that the affected area of the hypothalamus controls feeding because obesity, caused by hyperphagia, occurs after GTG administration. The affected area has been called the "satiety center." Although we and others have conducted many experiments dealing with the uptake of GTG by the hypothalamus, little is known about the distribution and retention of GTG in the rest of the body. A better understanding of the GTG distribution should help to explain why GTG is not always effective in producing hypothalamic lesions in normal animals and why insulin levels play such an important role in lesion development (Debons, A. F. et al., Amer. J. Physiol. 217, 1114, 1969).

In this study (Dawn D'Amico,* J. E. Carlton, Lewis R. Barber,† Roger J. Cloutier, and Evelyn E. Watson) GTG was intraperitoneally injected into young female C3BF$_1$ mice (0.8 mg GTG/g and approximately 1μ Ci ^{198}Au/g); groups of three animals were killed 1/2 hr, 2 hr, 6 hr, 18 hr, 1 day, 2 days, 3 days, 6 days, 10 days, and 17 days postinjection. The ^{198}Au content in whole blood, plasma, heart, pancreas, adrenals, kidneys, spleen, stomach, ovaries, liver, small intestine, large intestine, lymph nodes, intact brain, spinal cord, and gastrocnemius muscle was determined and recorded as the percent administered/g wet tissue. Figure 78 shows the ^{198}Au retention versus time in the blood plasma and in some of the organs studied; pancreas, kidney, and brain. Initially the GTG concentration in all tissues decreased rapidly. After a few hours the concentration in most tissues continued to decrease but at a slower rate. By the 17th day, the concentration in the pancreas, small intestine, large intestine, stomach, and spleen showed an apparent increase, probably resulting from the redistribution of gold in the body.

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1028197

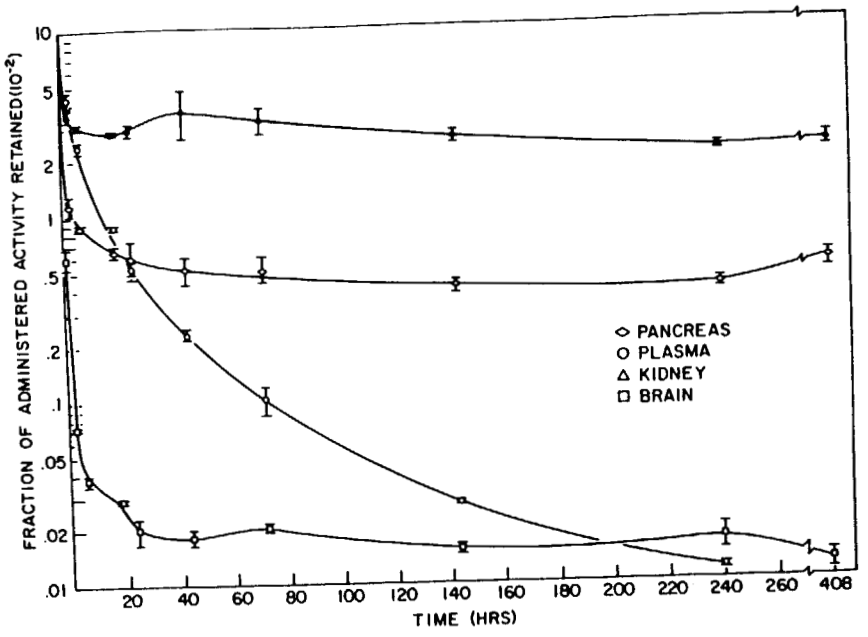


Fig. 78. Fraction of administered gold per gram wet tissue in pancreas, kidney, brain, and plasma when ^{198}Au thioglucose was given intraperitoneally to mice. Bars give ± 1 standard deviation.

Figure 79 shows the whole-body retention of ^{198}Au after ^{198}Au-thioglucose administration. The curve can be fitted with a two component exponential equation. Ninety-one percent of the gold has a half time of 0.7 hr while 9% has a half time of 373 hr. Future studies will compare the distribution and retention of GTG in control animals with its retention in insulin-treated animals.

WHOLE-BODY RETENTION OF ^{67}GA CITRATE

The radiation dose a patient receives from a radiopharmaceutical is dependent upon the retention of the radionuclide in the body. The retention of intravenously administered ^{67}Ga citrate in 112 patients with cancer was measured with the ORAU high-level whole-body counter (work supported in part by the U. S. Food and Drug Administration). This counter has an efficiency essentially independent of the patient's size and the radionuclide distribution. The biological half time (Tb) for stable gallium in the body was determined by fitting the retention curve with a series of exponential equations after correcting the data for ^{67}Ga radioactive decay (Evelyn E. Watson, Roger J. Cloutier, and W. D. Gibbs). We used our PDP-11 computer (Digital Equipment Corporation) to make the exponential least-squares fit to the whole-body counting results. The

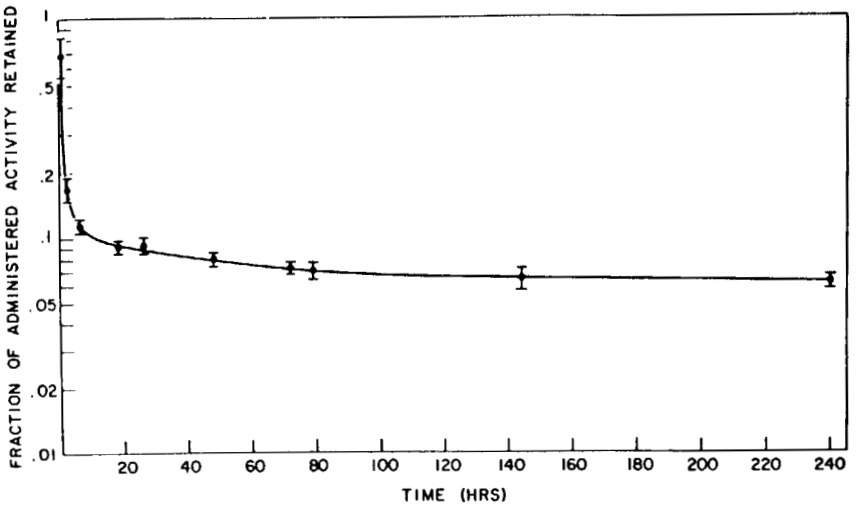


Fig. 79. Whole-body retention of gold when administered intraperitoneally to mice as ^{198}Au thioglucose. Bars give ±1 standard deviation.

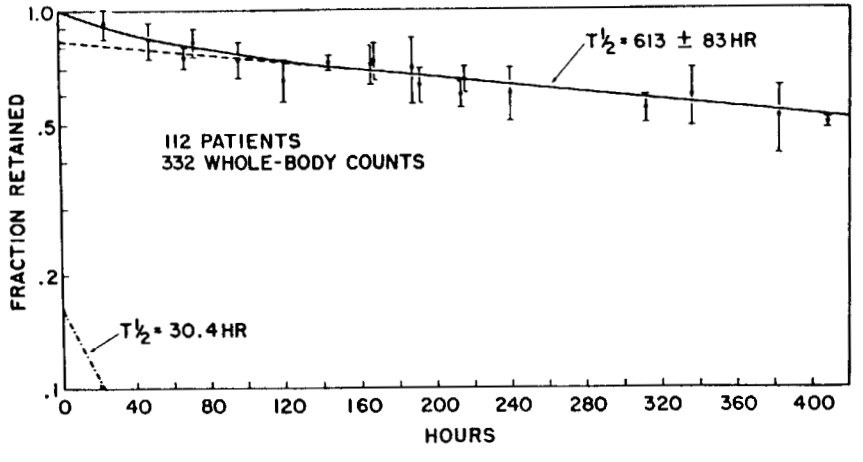


Fig. 80. Whole-body retention of gallium as determined from 332 whole-body counts of 112 patients who had been given ^{67}Ga citrate. Bars give ±1 standard deviation.

1028198

data are best fitted with two exponential terms. The short-lived biological component representing 17% of the administered gallium citrate has a half time of 30 hr. The remaining 83% has a half time of 613 ± 83 hr (Fig. 80).

A difference in retention was shown to be related to the patient's sex (Table 61). Because the study included few young patients and the data varied greatly, we could not show a significant difference related to the age of patients when the patients were grouped as <40 years and >40 years old (Table 62). As more data are accumulated at ORAU and elsewhere, we hope to determine whether differences in retention can be related to the patient's age or tumor mass.

TABLE 61

^{67}Ga RETENTION: COMPARISON BY SEX

	No. of patients	Short-lived component		Long-lived component	
		Percent	T_b (hr)	Percent	T_b (hr)
All patients	112	17	30	83 ±11	613 ± 83
Male patients	62	17	29	83 ±12	502 ± 88
Female patients	50	18	35	82 ± 9	776 ±156

TABLE 62

^{67}Ga RETENTION: COMPARISON BY AGE

	No. of patients	Short-lived component		Long-lived component	
		Percent	T_b (hr)	Percent	T_b (hr)
Patients <40 years	31	26	29	74 ± 6.8	607 ±127
Patients >40 years	81	13	28	87 ±11	583 ± 81

MISCELLANEOUS

PUBLICATIONS

From the Medical Division, Oak Ridge Associated Universities, 1972

Reprints, when available, and additional information on publications can be obtained from the Publications Office, Medical Division, Oak Ridge Associated Universities, P. O. Box 117, Oak Ridge, Tennessee 37830.

JOURNAL ARTICLES

Published

1. Berger, J. D. and Cloutier, R. J. *Health physics practices in laboratories using ^{3}H- and ^{14}C-labeled tracers.* Lipids 7, 604-610, 1972.
2. Blank M. L., Kasama, K., and Snyder, F. *Isolation and identification of an alkyldiacylglycerol containing isovaleric acid.* J. Lipid Res. 13, 390-395, 1972.
3. Blank M. L., Wykle, R. L., and Snyder, F. *The biosynthesis of ethanolamine plasmalogens by a postmitochondrial fraction from rat brain.* Biochem. Biophys. Res. Commun. 47, 1203-1208, 1972.
4. Brewen, J. G., Preston, R. J., and Littlefield, L. G. *Radiation-induced human chromosome abberation yields following an accidental whole-body exposure to ^{60}Co γ-rays.* Radiat. Res. 49, 647-656, 1972.
5. Gengozian, N. *A blood factor in the marmoset,* Saguinus fuscicollis *- its detection, mode of inheritance, and species specificity.* J. Med. Primatol. 1, 276-290, 1972.
6. Gengozian, N. *Inter- and intrasubspecies red cell immunizations in the marmoset, Saguinus fuscicollis ssp.* J. Med. Primatol. 1, 172-192, 1972.
7. Gottlieb, C. F. and Gengozian, N. *The humoral immune response in mice after neutron or X-irradiation at different dose rates.* J. Immunol. 109, 711-718, 1972.
8. Gottlieb, C. F. and Gengozian, N. *Radiation dose, dose rate, and quality in suppression of the humoral immune response.* J. Immunol. 109, 719-727, 1972.
9. Grigor, M. R., Moehl, A., and Snyder, F. *Occurrence of ethanolamine- and choline-containing plasmalogens in adipose tissue.* Lipids 7, 766-768, 1972.
10. Grigor, M. R., Pratt, R. D., and Snyder, F. *Acylation of cholesterol by microsomal enzymes from mouse preputial gland tumors.* Arch. Biochem. Biophys. 150, 371-375, 1972.
11. Hershman, J. M. and Edwards, C. L. *Serum thyrotropin (TSH) levels after thyroid ablation compared with TSH levels after exogenous bovine TSH: Implications for ^{131}I treatment of thyroid carcinoma.* J. Clin. Endocrinol. Metab. 34, 814-818, 1972.
12. Kniseley, R. M. *Marrow studies with radiocolloids.* Semin. Nucl. Med. 2, 71-85, 1972.
13. Littlefield, L. G. *An abnormal cell-line in a patient with acute granulocytic leukemia.* Cancer 29, 1281-1286, 1972.
14. Nelson, B., Hayes, R. L., Edwards, C. L., Kniseley, R. M., and Andrews, G. A. *Distribution of gallium in human tissues after intravenous administration.* J. Nucl. Med. 13, 92-100, 1972.
15. Piantadosi, C., Chae, K., Ishaq, K. S., and Snyder, F. *Synthesis of acyl dihydroxyacetone phosphates and related derivatives.* J. Pharm. Sci. 61, 971-974, 1972.
16. Piantadosi, C., Ishaq, K. S., Blank, M. L., and Snyder, F. *Synthesis of 1-O-alkyl-3-O-phosphorylethanolamine-2-propanone and related derivatives.* J. Pharm. Sci. 61, 607-610, 1972.

1028199

17. Porter, R. P. and Gengozian, N. In vitro *proliferation and differentiation of hemi precursor cells from marrow and blood of naturally chimeric marmosets.* J. Cel Physiol. 79, 27-41, 1972.
18. Soodsma, J. F., Piantadosi, C., and Snyder, F. *Partial characterization of the alkylglycerol cleavage enzyme system of rat liver.* J. Biol. Chem. 247, 3923-392 1972.
19. Vodopick, H., Rupp, E. M., Edwards, C. L., Goswitz, F. A., and Beauchamp, J. *Spontaneous cyclic leukocytosis and thrombocytosis in chronic granulocyti leukemia.* New Engl. J. Med. 286, 284-290, 1972.
20. Wykle, R. L., Blank, M. L., Malone, B., and Snyder, F. *Evidence for a mixed fun tion oxidase in the biosynthesis of ethanolamine plasmalogens from 1-alkyl-, acyl-sn-glycero-3-phosphorylethanolamine.* J. Biol. Chem. 247, 5442-5447, 1972.
21. Wykle, R. L., Piantadosi, C., and Snyder, F. *The role of acyldihydroxyaceton phosphate, reduced nicotinamide adenine dinucleotide, and reduced nicotina mide adenine dinucleotide phosphate in the biosynthesis of O-alkyl glycerolipid by microsomal enzymes of Ehrlich ascites tumor.* J. Biol. Chem. 247, 2944-2948 1972.
22. Yuhas, J. M., Stokes, T. R., and Lushbaugh, C. C. *Radiosensitivity and recover potential of human blood-forming tissues.* Radiology 105, 641-644, 1972.

In Press

23. Brown, D. H., Carlton, E., Byrd, B., Harrell, B., and Hayes, R. L. *A rate-zonal centrifugation procedure for screening particle populations by sequential produc recovery utilizing edge-unloading zonal rotors.* Arch. Biochem. Biophys.
24. Cloutier, R. J., Smith, S. A., Watson, E. E., Snyder, W. S., and Warner, G. G. *Dos to the fetus from radionuclides in the bladder.* Health Phys.
25. Cloutier, R. J., Watson, E. E., Rohrer, R. H., and Smith, E. M. *Calculating th radiation dose to an organ.* J. Nucl. Med.
26. Gengozian, N., Edwards, C. L., Vodopick, H., and Hübner, K. F. *Bone marrow transplantation in a leukemic patient following immunosuppression with anti thymocyte globulin and total-body irradiation.* Transplantation.
27. Kasama, K., Rainey, W. T., Jr., and Snyder, F. *Chemical identification and enzymi synthesis of a newly discovered lipid class--hydroxyalkylglycerols.* Arch. Bio chem. Biophys.
28. Hofer, K. G. and Swartzendruber, D. C. *^{67}Ga-citrate and ^{125}I-iododeoxyuridin as markers for* in vivo *evaluation of tumor cell metastasis and death.* J. Nat Cancer Inst.
29. Lee, T-c. and Snyder, F. *Phosphoglyceride metabolism of rat liver endoplasmic reticulum: Structural analyses, turnover studies, and enzymic activities.* Biochim Biophys. Acta.
30. Lee, T-c., Stephens, N., Moehl, A., and Snyder, F. *Turnover of phospholipids in plasma and microsomal membranes of rat liver.* Biochim. Biophys. Acta.
31. Littlefield, L. G. and Goh, K-o. *Cytogenetic studies in control men and women. I. Variations in aberration frequencies in 29, 709 metaphases from 305 cultures obtained over a three-year period.* Cytogenetics.
32. Littlefield, L. G. and Goh, K-o. *Cytogenetic studies in control men and women. II. Distribution of chromosome breaks.* Cytogenetics.
33. Otten, J. A., Tyndall, R. L., Estes, P. C., Gude, W. D., and Swartzendruber, D. C. *Localization of gallium-67 during embryogenesis.* Proc. Soc. Exp. Biol. Med.
34. Piantadosi, C., Chae, K., Ishaq, K. S., and Snyder, F. *The chemical synthesis of 1-^{14}C-octadecyldihydroxyacetone phosphate and 1-^{14}C-octadecyldihydroxy-acetone.* J. Pharm. Sci.

35. Porter, R. and Gengozian, N. *Immunologic responsiveness and tolerance of marmoset lymphoid tissue in vitro.* Transplantation.

35a. Snyder, F. *Thin-layer chromatographic behavior of glycerolipid analogs containing ether, ester, hydroxyl, and ketone groupings.* J. Chromatogr.

CHAPTERS IN BOOKS AND SYMPOSIUM PROCEEDINGS

Published

36. Barclay, T. R., Morris, A. C., Jr., and McDow, A. E., Jr. *Gamma-camera-to-computer interfacing—the easy way.* In: Proceedings of Second Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Oak Ridge, Tennessee, 1972, USAEC Report CONF-720430, 1972, pp. 369-375.
37. Beck, W. L., Stokes, T. R., and Lushbaugh, C. C. *Dosimetry for radiobiological studies of the human hematopoietic system.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971, E. A. Warman, ed. NASA Report NASA TM X-2440, 1972, pp. 974-980.
38. Cloutier, R. J. *Health physics education and training: past, present, and future.* In: Health Physics Operational Monitoring, Vol. 3, C. A. Willis and J. S. Handloser, editors. New York, Gordon and Breach, 1972, pp. 1305-1344.
39. Duggan, J. L., Beck, W. L., Albrecht, L., Munz, L., and Spaulding, J. D. *Studies of X rays induced by charged particles.* Advan. X-Ray Anal. 15, 407-423, 1972.
40. Edwards, C. L. and Hayes, R. L. *Localization of tumors with radioisotopes.* In: Clinical Uses of Radionuclides: Critical Comparison with Other Techniques, Proceedings of a Symposium, Oak Ridge Associated Universities, 1971, USAEC Symposium Series No. 27, CONF-711101, 1972, pp. 618-639.
41. Goswitz, F. A. *Bone marrow imaging.* In: Continuing Education Lectures, 1972; a Review of Nuclear Medicine, Atlanta, Southeastern Chapter, Society of Nuclear Medicine, 1972, pp. 10-1–10-15.
42. Goswitz, F. A. and Vodopick, H. A. *Dynamic radioisotopic studies in clinical hematology.* In: Clinical Uses of Radionuclides: Critical Comparison with Other Techniques, Proceedings of a Symposium, Oak Ridge Associated Universities, 1971, USAEC Symposium Series No. 27, CONF-711101, 1972, pp. 293-312.
43. Kniseley, R. M. and Goswitz, F. A. *Radiocolloid scanning.* In: Clinical Uses of Radionuclides: Critical Comparison with Other Techniques, Proceedings of a Symposium, Oak Ridge Associated Universities, 1971, USAEC Symposium Series No. 27, CONF-711101, 1972, pp. 275-292.
44. Lushbaugh, C. C. *Predicted levels of human radiation tolerance extrapolated from clinical studies of radiation effects.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971, E. A. Warman, ed. NASA Report NASA TM X-2440, 1972, pp. 398-415.
45. Lushbaugh, C. C. and Ricks, R. C. *Some cytokinetic and histopathologic considerations of irradiated male and female gonadal tissues.* In: Frontiers of Radiation Therapy and Oncology, Vol. 6, Human Radiation Tolerance. Proceedings of the 6th Annual San Francisco Cancer Symposium, 1970, J. M. Vaeth, ed., Basel, Switzerland, Karger, 1972, pp. 229-248.
46. McDow, A. E., Jr. *Code conversion by computer.* In: Proceedings of Second Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Oak Ridge, Tennessee, 1972, USAEC Report CONF-720430, 1972, pp. 335-341.

1028200

47. Ricks, R. C., Lushbaugh, C. C., McDow, E., and Frome, E. *Pulmonary-impedance power spectral analysis: A facile means of detecting radiation-induced gastrointestinal distress and performance decrement in man.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971, E. A. Warman, ed. NASA Report NASA TM X-2440, 1972, pp. 238-248.
48. Shively, J. M., Brown, D. H., Ball, F. L., Caton, J. E., Phares, E. F., Brantley, J. N., and Breillatt, J. P. *Partial characterization of the polyhedral inclusion bodies of* Thiobacillus neopolitanus. In: The Molecular Anatomy (MAN) Program, Second Semiannual Progress Report for Period September 1, 1971 to February 29, 1972. USAEC Report ORNL-4771, Oak Ridge National Laboratory, 1972, pp. 182-184.
49. Snyder, F. *Enzymatic systems that synthesize and degrade glycerolipids possessing ether bonds.* Advan. Lipid Res. 10, 233-259, 1972.
50. Snyder, F. *The enzymic pathways of ether-linked lipids and their precursors.* In: Ether Lipids: Chemistry and Biology, F. Snyder, ed., New York, Academic Press, 1972, pp. 121-156.
51. Snyder, F. *Ether-linked lipids and fatty alcohol precursors in neoplasms.* In: Ether Lipids: Chemistry and Biology, F. Snyder, ed., New York, Academic Press, 1972, pp. 273-295.
52. Webb, A. M. *Semiautomated determination of thyroxine (T_4) iodine.* In: Standard Methods of Clinical Chemistry, Vol. 7, G. R. Cooper, ed., New York, Academic Press, 1972, pp. 261-266.
53. Yuhas, J. M., Stokes, T. R., and Lushbaugh, C. C. *Multifactorial analysis of human blood cell responses to clinical total body irradiation.* In: Proceedings of the National Symposium on Natural and Manmade Radiation in Space, Las Vegas, 1971, E. A. Warman, ed. NASA Report NASA TM X-2440, 1972, pp. 233-237.
54. Zucker, S. and Webb, A. M. *Assay of muramidase activity in serum, plasma, or urine.* In: Standard Methods of Clinical Chemistry, Vol. 7, G. R. Cooper, ed., New York, Academic Press, 1972, pp. 9-17.

In Press

55. Akers, L. K., Beck, W. L., Jr., Cloutier, R. J., and Dickson, H. W. *Health physics training at Oak Ridge Associated Universities.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972.
56. Cloutier, R. J. and Watson, E. E. *Radiation doses from nuclear medicine procedures.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972.
57. Gengozian, N. and Patton, M. L. *Identification of three blood factors in the marmoset,* Saguinus fuscicollis *ssp.* In: Proceedings of the Third Conference on Experimental Medicine and Surgery in Primates, Lyons, France, June 1972, Basel, S. Karger.
58. Goswitz, F. A. *Radioactive vitamin B_{12} absorption tests and other studies.* In: Diagnostic Radiology, by R. Golden, Section 20, Diagnostic Nuclear Medicine, A. Gottschalk, J. L. Quinn, and E. J. Potchen, editors, Baltimore, Williams and Wilkins.
59. Hayes, R. L. and Edwards, C. L. *New applications of tumor-localizing radiopharmaceuticals.* In: Proceedings of the IAEA Symposium: Medical Radioisotope Scintigraphy, Monte Carlo, Monaco, October 1972, Vienna, International Atomic Energy Agency.
60. Lombardi, M. H., Beck, W. L., Jr., and Cloutier, R. J. *Survey of radiopharmaceutical use and safety in 69 hospitals.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972, Washington, US Bureau of Radiation Health.

61. Porter, R. P. and Gengozian, N. *Marmoset bone marrow.* In: Methods and Application of Tissue Culture, P. Krause and M. Patterson, editors, New York, Academic Press.

GOVERNMENT REPORTS

62. *Clinical Uses of Radionuclides: Critical Comparison with Other Techniques.* Proceedings of a Symposium held at Oak Ridge Associated Universities November 15-19, 1971. F. A. Goswitz, G. A. Andrews, and Manual Viamonte, Jr., editors, 1972, (USAEC Symposium Series No. 27, CONF-711101).
63. Morris, A. C., Jr., Barclay, T. R., Tanida, R., and Nemcek, J. V. *Design specifications for a miniaturized surgical probe.* USAEC Report ORAU-115, 1972.
64. Oak Ridge Associated Universities, Medical Division. Research Report, 1971, for the Year Ending December 31, 1971. USAEC Report ORAU-116, 1972.

MISCELLANEOUS PUBLICATIONS

65. Berger, J. D. Book Review: *Radiological Physics Examination Review Book,* Vol. 1, by C. G. Orton and C. H. Marshall, Medical Examination Publishing Co., Flushing, New York, 1971. Health Phys. 23, 259, 1972.
66. Goswitz, F. Book Review: *Manual of Nuclear Medicine Procedures,* W. H. Beierwaltes, J. W. Keys, Jr., and J. E. Carey, Cleveland, CRC Press, Division of Chemical Rubber Co., 1971. Ann. Intern. Med. 76, 1057-1058, 1972.
67. Humason, G. L. *Animal Tissue Techniques,* 3rd edition, San Francisco, W. H. Freeman, 1972.
68. Lushbaugh, C. C. Discussion. In: Frontiers of Radiation Therapy and Oncology, Vol. 6, Human Radiation Tolerance. Proceedings of the 6th Annual San Francisco Cancer Symposium, 1970, J. M. Vaeth, ed., Basel, Switzerland, Karger, 1972, pp. 143-144.
69. Snyder, F. (ed.) *Ether Lipids: Chemistry and Biology,* New York, Academic Press 1972.

ABSTRACTS

70. Beck, W. L., Stokes, T. R., and Wade, L., Jr. *Dosimetry for total-body irradiation accident.* Health Phys. 23, 419, 1972. Abstract P/76.
71. Brown, D. H., Swartzendruber, D. C., Carlton, J. E., Byrd, B. L., and Hayes, R. L. *Mechanism of gallium-binding in tumors: isolation and characterization of gallium binding-granules (GBG) in soft tissue tumors.* Proc. Amer. Ass. Cancer Res. 13, 37, 1972. Abstract 148.
72. Edwards, C. L., Hayes, R. L., and Nelson, B. *The "normal" ^{67}Ga scan.* J. Nucl. Med. 13, 428-429, 1972.
73. Goswitz, F., Morris, A., McDow, A., Kim, K., and Hodges, H. *A computer-interfaced four-probe counter and its application in a dynamic radiocolloid study.* J. Nucl. Med. 13, 433, 1972.
74. Lee, T-c., Piantadosi, C., and Snyder, F. *The subcellular distribution of ether lipids in tumors.* Proc. Amer. Ass. Cancer Res. 13, 58, 1972. Abstract 229.
75. Morris, A. C., Jr., Goswitz, F. A., Kim, K. A., McDow, A. E., Jr., Aaron, P. A., and Barclay, T. R. *Rapid analysis of dynamic radionuclide functions using the on-line computer.* J. Ass. Adv. Med. Instrum. 6, 180, 1972.
76. Porter, R. P. and Gengozian, N. *Megakaryocytes cultured from blood lymphocytes of naturally chimeric marmosets.* Blood 40, 588-589, 1972.

1028201

77. Ricks, R. C., Lushbaugh, C. C., and McDow, A. E., Jr. *Correlation of heart rate and CO_2 production with pulmonary impedance during exercise stress in man.* Fed. Proc. 31, 311 Abs, 1972. Abstract 544.
78. Ricks, R. C., Lushbaugh, C. C., and McDow, A. E., Jr. *Pulmonary impedance power spectral analysis: its correlation with heart rate and expired CO_2 during exercise stress in man.* J. Ass. Adv. Med. Instrum. 6, 186, 1972.
79. Snyder, F., Wykle, R. L., Blank, M. L., Lumb, R. H., Malone, B., and Piantadosi, C. *Enzymatic pathways for ether lipids.* Fed. Proc. 31, 454 Abs, 1972. Abstract 1342.
80. Stokes, T. R., Lushbaugh, D., Humason, G., and Lushbaugh, C. C. *^{59}Fe studies on the erythroblastic response of normal and splenectomized mice to low level continuous radiation.* Radiat. Res. 51, 472-473, 1972. Abstract Dc-5.
81. Swartzendruber, D. C. and Mateer, H. *Ultrastructural identification of intracellular lead by X-ray analysis on a scanning electron microscope.* J. Cell Biol. 55, 255a, 1972. Abstract 509.
82. Urso, P. and Gengozian, N. *Functional capacity of T, B, and A cells in allogeneic radiation chimeras.* In: Fourth International Congress of the Transplantation Society, San Francisco, 1972, Collected Abstracts, New York, Grune & Stratton, 1972, p. 300.
83. Watson, E. E., Cloutier, R. J., and Gibbs, W. *Whole body retention of gallium-67 citrate.* S. Med. J. 65, 1417, 1972.

MEDICAL DIVISION CONFERENCES*—1972

January 11: *Progress report on a recent human-marrow graft.* C. L. Edwards, N. Gengozian, K. F. Hübner, G. Charles Kingdon, L. Gayle Littlefield, and Helen Vodopick.
January 24: *Myeloid metaplasia.* Matthew Block, University of Colorado Medical Center, Denver.
January 25: *A metabolic basis for the selective localization of tritium from 1-^{3}H-fatty alcohols in tumors.* Roger H. Lumb, Department of Biology, Western Carolina University, Cullowhee, North Carolina.
February 8: *Progress report of work in the immunology group.* N. Gengozian.
February 22: *Decline of chromosome aberrations in pig leukocytes.* John B. Mailhes.
March 7: *The computer-interfaced, four-probe counter and its applications.* A. C. Morris, Jr. and F. A. Goswitz.
March 21: Progress report: *Clinical total-body irradiation.* C. L. Edwards.
April 4: Progress report: *Microbiology program at the Medical Division.* G. Charles Kingdon.
April 18: *Progress report of work in computer program.* A. E. McDow, Jr.
May 2: *Science, ethics, and materia medica: How scandium came to clinical trials for improvement of tumor scanning with ^{67}Ga.* B. Nelson, R. L. Hayes, and C. L. Edwards.
May 16: *Progress report of work in ultrastructural anatomy.* D. C. Swartzendruber.
June 6: *Using the PDP-8 computer.* Roger J. Cloutier.
June 13: *Progress report of work in marmoset research.* N. Gengozian.
June 27: *Ferrokinetic studies in chronically irradiated mice.* T. R. Stokes.

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* *Presented by Division staff unless otherwise noted.*

July 11: *The use of zonal centrifugation techniques for separating subcellular particles.* David H. Brown.
July 25: *Progress report of work in the biological chemistry program.* Fred Snyder.
August 8: *Antibody response to sheep red blood cells by spleen cells from allogeneic bone-marrow chimeras in vitro.* Paul Urso.
August 9: Summer Student Scientific Program:
The enzymic synthesis of diacylalkylglycerols by normal and tumor tissues. Seth Leo Alper.
The enzymatic synthesis of fatty acid esters of short-chain alcohols. Iverson Charles Bell.
The effect of intravenously administered ethiodol on the pulmonary distribution of carrier-free gallium-67 in rabbits. Marilyn L. Bell.
Programming for linear scanner clinical data. Ellen Brown.
A solution to the problem of automated protein analysis in the presence of sucrose. Tommy Caruthers.
Gold thioglucose: uptake and retention. Dawn D'Amico.
Ultrastructural study of mouse preputial gland tumor. Albert Gros.
Scanner to computer interfacing using optical electronic encoding. James Lucas.
Effects of in vitro sensitization of marmoset peripheral white blood cells utilizing chromium-51-labeled fibroblasts. Carolyn Rape.
The acceptance of heterogeneic hematopoietic graft in mice treated with Cytoxan and X irradiation. Kendell R. Sprott.
August 22: *Follow-up on recent marrow transplant.* C. L. Edwards.
September 12: *Progress report of work in the cytogenetics program.* L. Gayle Littlefield.
September 19: *Applications of the proportional counter photon camera in nuclear medicine.* C. J. Borkowski, Oak Ridge National Laboratory.
October 3: *Progress report of work being done by the Cooperative Group to Study Localization of Radiopharmaceuticals (^{67}Ga study).* C. L. Edwards and R. M. Kniseley.
October 17: *New radiation sources for possible use in radiotherapy.* Arthur H. Snell, Oak Ridge National Laboratory.
October 31: *A presentation (workshop) of two computer applications programs: Probit analysis and multipurpose plotting.* A. E. McDow, Jr.
November 14: *Progress report on physiologic effects of total-body irradiation.* Robert C. Ricks.
November 28: *Effect of irradiation on ^{67}Ga retention in mice.* D. C. Swartzendruber.
December 19: *Radiation doses in nuclear medicine.* Roger J. Cloutier.

LIPID SEMINARS–1972

January 20: *Practical capabilities of gas-liquid chromatography.* M. R. Guerin, Oak Ridge National Laboratory.
February 3: *Mass spectrometry and its application to the analysis of organic molecules.* W. T. Rainey, Jr., Analytical Chemistry Division, Oak Ridge National Laboratory.
February 29: *Glycoproteins and glycolipids of surface membranes.* Stephen Krauss, University of Tennessee Memorial Research Center, Knoxville.

1028202

March 16: *Kinetic studies of the enzymic reduction of alkyl-DHAP by microsomes of preputial gland tumor.* Kun Chae, Medical Division staff.
April 5: *The metabolism of plasmalogens.* Lloyd Horrocks, Department of Physiological Chemistry, Ohio State University, Columbus.
June 9: *Development of quantitative lung tumor system in mice.* Paul Nettesheim, Biology Division, Oak Ridge National Laboratory.
July 13: *Specific estrogen-receptors in human and experimental mammary carcinoma.* James L. Witliff, Department of Biochemistry, School of Medicine and Dentistry, The University of Rochester, New York.

REGIONAL LIPID MEETING*

Gatlinburg, Tennessee
November 2–3, 1972

GUEST LECTURERS

Dr. Salih J. Wakil Baylor College of Medicine Houston, Texas	*The role of lipids in the structure and function of membranes*
Dr. Raymond Dils Nottingham, England	*Control of fatty-acid chain termination by subcellular fractions of the lactating mammary gland*

INSTITUTION AND SPEAKER	TITLE OF TALK
Bowman Gray School of Medicine	
Patsy Wang	*Phospholipid metabolism in rabbit alveolar macrophages*
Moseley Waite	*Relationship of macrophage and liver lysosomal phospholipases*
Clemson University	
Oya Yazar	*Cyclopropane fatty acid biosynthesis in stringent and relaxed strains of Escherichia coli*

* *There were 10 sessions and 72 attendees.*

North Carolina State University

Carol Kepler	*Hydrogenation of oleic acid by rumen bacteria*
Ross Silcock	*Stimulation of glyceride synthesis in mouse adipose tissue*
Jody Keyser	*Biohydrogenation of long-chain unsaturated fatty acids. I. Search for a H_2 donor and co-factor in biohydrogenation of pumicic acid*
Fred Baker	*Biohydrogenation of long-chain unsaturated fatty acids. II. Preliminary purification and studies on the reductase enzyme*

Medical College of Georgia

School of Dentistry

Thomas R. Dirksen and George S. Schuster	*Lipid synthesis by bone and bone cell cultures*

Department of Medicine

Elaine B. Feldman and Daniel S. Feldman	*Cholesterol absorption: Solution-membrane interactions*
Terence Kuske	*Glycosphingolipids of lipoproteins*

Medical University of South Carolina

Haldor T. Jonsson, Jr.	*Quantitative evidence for the occurrence of essential fatty acid deficiency in the infant*

Oak Ridge Associated Universities

Cathy Snyder, Boyd Malone, Paul Netteschein, and Fred Snyder	*Urethan pulmonary adenoma as a model for the study of surfactant biosynthesis*
Merle L. Blank and Robert L. Wykle	*The turnover of acyl moieties in alk-1-enylacyl and diacyl ethanolamine-containing phospholipids in rat testes*
Boyd Malone, Claude Piantadosi, and Fred Snyder	*The metabolism of alkyl ethyleneglycols*

University of Florida

Melvin Fried	*Lipoprotein biosynthesis and metabolism in Type IV hyperlipoproteinemia*

1028203

University of North Carolina

Department of Biochemistry

Sharon Parker	*Effects of alcohol ingestion on beta-oxidation of fatty acids by rat heart*
Ronald C. Reitz	*Effects of alcohol on the TPNH oxidase catalyzed peroxidation of fatty acids*
Vidula Bangdiwala and Carl E. Anderson	*Plant glyceryl ethers*

Department of Medicinal Chemistry

Kun Chae, Claude Piantadosi, and Fred Snyder	*Ether-linked lipids in cancer cells: Alternate enzymic pathways for the biosynthesis of alkyldihydroxyacetone-P and alkylglycerol-3-P*

Department of Medicine

Robert G. Lamb and Harold J. Fallon	*An enzymatic explanation for increased hepatic triglyceride formation in rats fed high sugar diets*
Subash C. Jamdar and Harold J. Fallon	*Triglyceride biosynthesis in rat adipose tissue*
Dennis Traylor and Edward L. Hogan	*Brain gangliosides*

Vanderbilt University

Department of Biochemistry

Richard Whorton	*Subcellular fractionation of rat testicular tissue*
John G. Coniglio	*Studies of lipid metabolism in testicular tissue*

Department of Pharmacology

Murray Heimberg	*Regulation of hepatic lipoprotein metabolism*

Department of Physiology

H. C. Meng	*Brief review of lipid research program*
M. C. Wang and H. C. Meng	*Comparison of utilization of acetate, glucose, laurate, palmitate, and oleate by isolated perfused rat lung*
Albert Otten	*Incorporation of acetate and palmitate into complex lipids in newborn lambs and infant lungs*
M. D. Caldwell	*Human linoleic acid deficiency occurring during prolonged intravenous nutrition*

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Kendell R. Sprott
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Texas Woman's University
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1028206

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Investigator(s)	Title	Agency*	Current Period
	Active Interagency Agreements		
Drs. F. Snyder (ORAU) and C. Piantadosi (UNC)	Role of ether- and ketone-containing lipids during brain development	NIH-UNC	9/1/72- 8/31/73
Dr. R. Tyndall	A study of infections in irradiated patients	US Army	8/1/72- 7/31/73
Mr. R. Cloutier	Center for information on internal dosimetry of radiopharmaceuticals	FDA	5/1/72- 4/30/73
Dr. R. Kniseley	Joint AEC-NIH program for study of gallium-67	NCI	2/1/72- 1/31/73
Dr. C. Lushbaugh	ORAU study of therapeutic and accidental whole-body irradiation for NASA	NASA	1/1/72-12/31/72
Dr. L. G. Littlefield	Chromosome breakages in women taking oral contraceptives	NICHD	7/1/72- 6/30/73
	Active Grants		
Drs. C. L. Edwards and R. Kniseley	Gallium-67 as a tumor-localizing radioindicator	ACS	7/1/71- 6/30/73
Dr. F. Snyder	The occurrence, metabolism, and function of ether-linked glycerolipids in neoplasms	ACS	11/1/72-10/31/73
Drs. F. Snyder (ORAU) and C. Piantadosi (UNC)	Alkoxy and alkenyloxy glyceryl ethers and derivatives	NIH-UNC	1/1/72-12/31/73
Dr. F. Snyder	Biosynthetic mechanisms for ether bonds in lipids	NIH	5/1/72- 4/30/73
Dr. N. Gengozian	Immunology of hematopoietic chimerism	NIH	1/1/72-12/31/72
Dr. R. Hayes	Concentration of gallium by malignant neoplasms	NIH	5/1/72- 4/30/73
Dr. R. Wykle	Enzymic synthesis of plasmalogens in heart and brain	NIH	5/1/72- 4/30/73
Dr. D. Swartzendruber	Effect of irradiation on gallium-67 localization in animals	NIH	5/1/73-12/31/73
Dr. H. Goswitz	Regulation of granulopoiesis	NIH	1/1/73-12/31/73
Dr. G. Andrews	General Research Support	NIH	1/1/73-12/31/74
	Pending Grant and Contract Proposals		**Proposal Dates**
Dr. G. Andrews	Institutional research grant	ACS	7/1/73- 6/30/74
Dr. N. Gengozian	Studies of immunology of aging in the marmoset	NIH	1/1/73-12/31/73
Dr. L. G. Littlefield	Cytogenetic studies in rabbits exposed to synthetic hormones	NIH	7/1/73- 6/30/74
Dr. R. Hayes	ORAU-ORNL study of carbon-11 in nuclear medicine	NIH	5/1/73- 4/30/74
Dr. N. Gengozian	Colon cancer in the marmosets	NCI	Uncertain
Drs. C. Lushbaugh (ORAU) and A. Kretchmar (UTMRC)	Cell cycle parameters--bronchogenic carcinoma	NCI	4/15/72- 4/14/73

* *ACS* — *American Cancer Society*
FDA — *Food and Drug Administration*
NASA — *National Aeronautics and Space Administration*
NCI — *National Cancer Institute*
NICHD — *National Institute for Child Health and Human Development*
NIH — *National Institutes of Health*
ORNL — *Oak Ridge National Laboratory*
UNC — *University of North Carolina*
UTMRC — *University of Tennessee Memorial Research Center.*

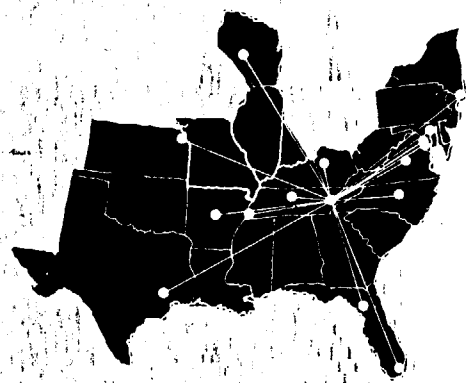
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1973 RESEARCH REPORT

ORAU-123



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Medical Division
Oak Ridge Associated Universities

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1973 RESEARCH REPORT

for year ending
December 31, 1973



Medical Division
Oak Ridge Associated Universities

Operating Under Contract With The United States Atomic Energy Commission

ON THE COVER—A map showing location of institutions active in *The Cooperative Group to Study Localization of Radiopharmaceuticals,** an organization of specialists in nuclear medicine collaborating under the sponsorship of Oak Ridge Associated Universities in a program to evaluate various radiopharmaceuticals. For details of this project see page 83.

Institution	Member
Duke University Medical Center	Robert H. Wilkinson
Marshfield Clinic	Robert H. Greenlaw
M.D. Anderson Hospital and Tumor Institute	Thomas P. Haynie
Memorial Sloan-Kettering Cancer Center	Richard S. Benua
NIH Clinical Center	Gerald S. Johnston
ORAU Medical Division	C. Lowell Edwards
Secretaries:	Gould A. Andrews
	Ralph M. Kniseley†
University of Arkansas School of Medicine	Charles M. Boyd
University of Florida College of Medicine	Frank H. DeLand
University of Kansas Medical Center	David F. Preston
University of Kentucky Medical Center	John B. Selby
University of Louisville School of Medicine	John K. McBain
University of Miami School of Medicine	Morton B. Weinstein
University of Tennessee College of Medicine	Ben I. Friedman
University of Virginia School of Medicine	Charles David Teates
USPHS Hospital, Baltimore	Linell Murphy
VA Hospital, Washington	Bertram J. L. Sauerbrunn
Vanderbilt University School of Medicine	A. Bertrand Brill

*Support for this work is received from Joint AEC-NIH Interagency Agreement No. 40-266-71 and American Cancer Society Grant No. CI-54.

†Present address: IAEA, Vienna, Austria.

NOTICE

This report contains preliminary data and was prepared primarily to record the year's work, and is not intended as a substitute for publication in the open literature. It is subject to revision and does not represent a final report. Please do not quote from or refer to this work in open literature without permission from individual authors to reference the material as a personal communication.

ORAU-123

Requests for additional information in reference to this report may be directed to:
Medical Division, Oak Ridge Associated Universities, Oak Ridge, Tennessee 37830

Printed in the United States of America. Price $7.60.
Available from: National Technical Information Service, Springfield, Virginia 22151.

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CONTENTS

ORGANIZATION OF RESEARCH iv

INTRODUCTION 1

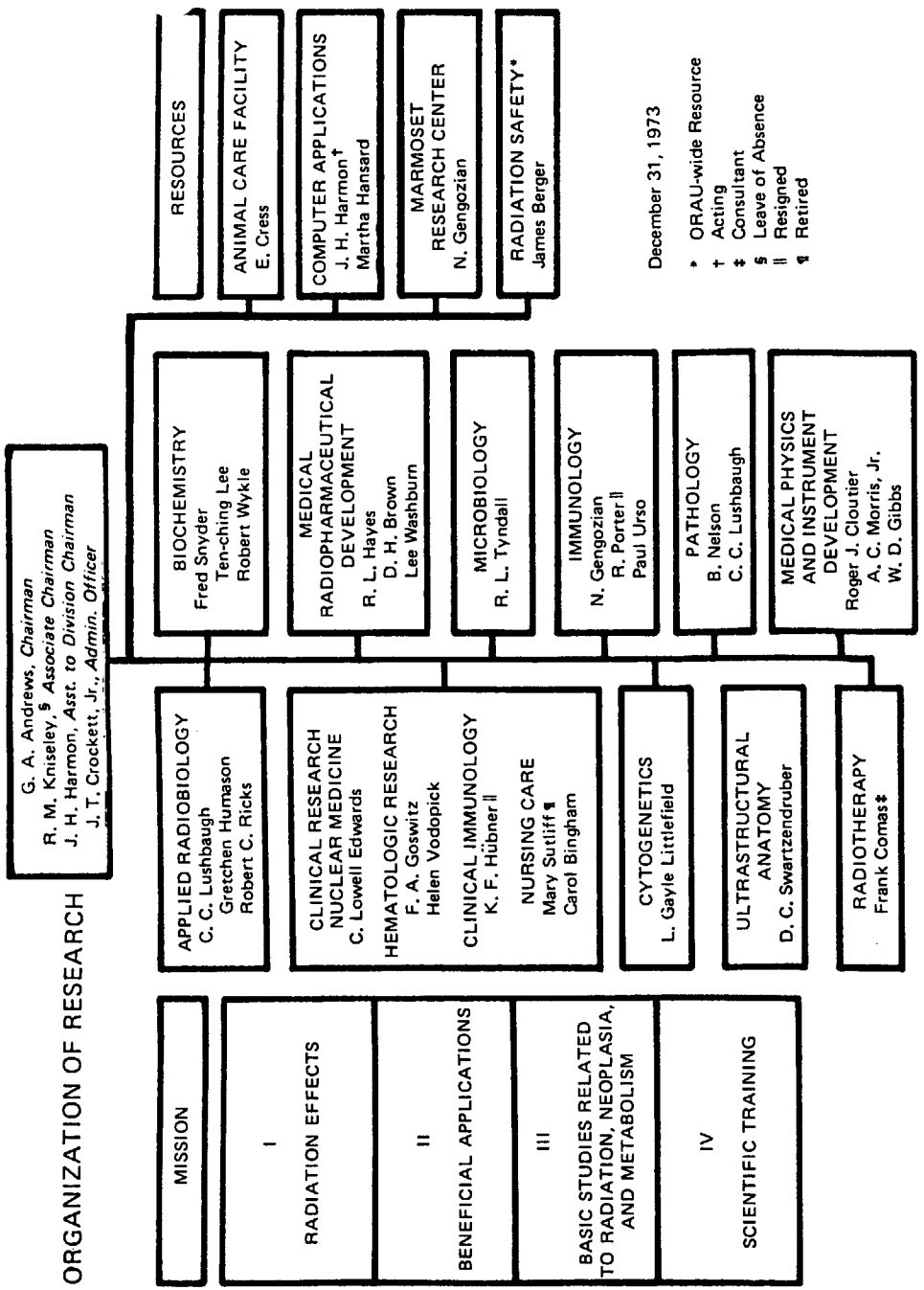
RADIATION EFFECTS AND RELATED TOPICS 4
- Summary of Accomplishments 4
- Clinical Observations 5
- Infections in Irradiated Patients 13
- Medical Physics and Instrumentation 23
- Applied Radiobiology 28
- Radiation Immunology and Marrow Transplantation 39
- Experimental Hematology 52

RESEARCH IN NUCLEAR MEDICINE 55
- Summary of Accomplishments 55
- Radiopharmaceutical Development 56
- Clinical Investigations in Nuclear Medicine 75
- Computer Methods 87

BASIC STUDIES IN RADIATION, NEOPLASIA, IMMUNOLOGIC RESPONSES, AND METABOLISM 91
- Summary of Accomplishments 91
- Biological Chemistry 93
- Marmoset Research Project 104
- Ultrastructural Anatomy 117
- Induced Cytogenetic Abnormalities 120
- Miscellaneous Research Activities 141

MISCELLANEOUS 145
- Publications 145
- Medical Division Conferences—1973 152
- Lipid Seminars—1973 153
- Regional Lipid Conference—1973 153
- Fellowships and Term Appointments 156
- Research Participants—1973 157
- Undergraduate Student Trainees—1973 157
- ORAU Medical Division Advisory Committee 157
- ORAU Medical Division Committee on Human Studies 158
- ORAU Medical Division Medical Radionuclide Committee 158
- Medical Committee, ORAU Council 158
- Medical Division Employees 159
- Consultants and Collaborators—1973 161
- List of Non-AEC Support 168

NOTE: Numbered references in the text may be located in the list of publications beginning on page 145.



1028214

INTRODUCTION

In this volume the Medical Division of Oak Ridge Associated Universities presents its annual report for calendar year 1973. The Division conducts biomedical research, under contract with the United States Atomic Energy Commission, with some additional support from other sources, both federal and private. The main research objectives lie in three closely related areas: (1) mechanisms of radiation injury, especially in man; (2) beneficial applications of radiation for diagnosis and therapy of disease, especially cancer; and (3) basic biological and physical problems arising from ongoing research and mainly related to cancer. In addition, the staff devotes a significant effort to training activities. In this report brief summaries beginning on pages 4, 55, and 91 highlight the accomplishments of the year.

This year was a difficult one because of financial limitations. Rising costs coupled with an AEC budget that did not increase proportionately made necessary a significant reduction in a number of personnel.

Senior Staff Changes. May 1973 saw the retirement of Mary Sutliff who had served 23 years as director of nursing for the Medical Division. She was a persistent worker for the good care of patients, devoted herself to the job at whatever hour of the day or night she was needed, shouldered difficult administrative problems with conviction and independence, and, by her persistent youthfulness, gave the old timers on our staff the illusion that very little time had gone by since the early days of the Division.

We were fortunate to obtain Carol Bingham to take on the duties of director of nursing.

In August 1973, Ralph Kniseley, Associate Chairman of the Division, started a two-year leave of absence during which he is serving as Director of the Life Sciences Division at the International Atomic Energy Agency in Vienna. During his absence, his duties at the Medical Division are being shared by a number of senior staff members.

Marion Garber, who had worked for ORAU as a librarian from 1950 until 1970, and as technical editor in the Medical Division the following three years, left the Division in September. Her dedicated and highly competent work has been a major asset to ORAU and the Medical Division.

Bill Nelson returned to the Division in July after having spent a year working at the University of Ulm, Germany.

Karl Hübner resigned in July having decided for personal reasons to move back to Germany from which he originally came. A rather extensive recruitment effort was undertaken to select a replacement; early in 1974 we learned that Dr. Hübner could return to the Division and in view of his previous fine performance we have arranged to have him do so.

Earlier in the year Raymond Porter, who had worked with our immunology group, left our staff to take a position at the Life Science Center at Nova University in Fort Lauderdale, Florida. Lee Washburn, who completed his Presidential Internship, stayed on as an extended temporary appointee with the plan that he will join the regular staff as soon as the carbon-11 project is funded.

The cytogenetics program underwent a significant reduction in size as a result of the conclusion of a very successful project carried out under contract with NIH. John Mailhes left to take a position with Dow Chemical Company in Freeport, Texas.

Earl McDow, who had worked effectively on developing the Medical Division's

computer resource, left early in the year to join a private company.

Organizational Change. The former Medical Physics group was reorganized and expanded to include medical electronics activities. It will be called the Medical Physics and Instrument Development Group, will be directed by Roger J. Cloutier, and will include staff members A. C. Morris, Jr. and W. D. Gibbs.

Guest Scientists and Trainees. A list of fellowship and term appointments can be found on page 156. These include one postdoctoral fellowship and three short-term medical student trainees, supported entirely by ORAU corporate funds, and a sabbatical visitor supported in part by ORAU. A list of AEC-supported summer student trainees is found on page 157.

Sources of Financial Support. Although the majority of the Division's funds continue to come from the U.S. Atomic Energy Commission, other sources of support have continued to expand. A list of contracts and grants, including several new ones, is found on page 168.

Staff Activities and Travel. During the year Fred Snyder was appointed as a member of the Editorial Board of *Biochimica et Biophysica Acta* and also of the *Reviews on Cancer* published by that organization. C. C. Lushbaugh was appointed to the Editorial Board for *Proceedings of the Society for Experimental Biology and Medicine*. In this year of the tight budget, only one overseas scientific trip was taken; this was by Fred Snyder who spoke at the 5th Harden Conference on Phospholipids at Wye College, Kent, England and who subsequently visited laboratories in Denmark and Germany.

Symposia. During the year the publication of the last ORAU symposium came out. This was entitled *Clinical uses of radionuclides: Critical comparison with other techniques* and was edited by Francis A. Goswitz, Gould A. Andrews, Manuel Viamonte, Jr., and Marion H. Garber. Copies are available as CONF-711010 from National Technical Information Service, U. S. Department of Commerce, Springfield, Virginia 22151.

The Division has agreed to serve as scientific host for the IAEA-USAEC symposium entitled *Dynamic Studies with Radioisotopes in Clinical Medicine and Research* to be held in Knoxville, July 15-19, 1974. This symposium will be a sequel to the symposium on the same subject held by the International Atomic Energy in Rotterdam in 1970. It will be concerned with all those applications of radionuclides in clinical medicine and research that involve investigation of the temporal patterns of uptake, metabolism, clearance or excretion of administered radioactive materials.

Planning Activities. A major amount of senior staff time was devoted to planning for the East Tennessee Cancer Research Center, a joint effort of four organizations: The University of Tennessee, which will administer the program; Oak Ridge National Laboratory; the University of Tennessee Memorial Research Center and Hospital; and Oak Ridge Associated Universities. During the year a good deal of progress was made and mutual interactions among the four groups increased considerably. There still remains a considerable uncertainty, particularly about how increased clinical research activities can be developed in both Knoxville and Oak Ridge; it does not appear feasible to center all clinical cancer research activities in either place. Toward the end of the year, efforts were undertaken to recruit a director for the Center.

A considerable amount of time was devoted also to other planning efforts, including how the Division might cooperate with Oak Ridge National Laboratory, especially the

1028215

Isotopes Division, on an enhanced radiopharmaceutical development program. Some planning was also devoted to the Division's possible participation in energy-related research if the Atomic Energy Commission or its successor takes on an expanded effort in this field.



Gould A. Andrews
Chairman

RADIATION EFFECTS AND RELATED TOPICS

A major objective of the Division's research is to elucidate radiation effects in the human being in order to better manage the rare accidental exposures that occur, and also to use total-body irradiation for therapy when it is useful. The therapeutic applications involve moderate doses used to suppress the proliferative activity in chronic hematologic disorders, and large doses for immunosuppression in preparation for marrow grafting. The work on total-body irradiation is approached from many angles, including dosimetry, physiological monitoring, prodromal effects, hematologic changes, cytogenetic effects, and the role of infection. Many aspects of this work are correlated with animal experiments going on at the Medical Division and at Oak Ridge National Laboratory.

Summary of Accomplishments

Further planning was devoted to the preparation for the care of radiation-accident victims. At the request of other AEC installations in Oak Ridge, plans were made for a decontamination facility that will eventually be available for accidental radioactive contamination of workers in the Oak Ridge area. The AEC has recognized the Medical Division as a center for the treatment of radiation accidents. Dr. Thomas Lincoln, Director, Health Division, Oak Ridge National Laboratory (ORNL) has agreed to be available to work with the Medical Division staff, particularly in problems of treating accidents involving internal intake of radioactive materials.

The use of moderate doses of total-body irradiation given at rather low dose rates was continued for the therapy of chronic leukemias, lymphoma, and polycythemia. In chronic granulocytic leukemia, several doses, dose rates, and fractionation patterns have been evaluated, particularly for their effect in the first six weeks after treatment. There appears to be no clear specificity of effect on any one cell type that is dependent on dose rate. As one would expect, with single exposures between 50 and 250 R (in air at midpoint of the radiation field) the higher exposures give greater hematologic effects and more prolonged remissions. The pattern of response of the leukocytes with time is different from that of normal leukocytes in accident cases. The overall survival for a group of patients with this disease treated in our studies compares quite favorably with that of the other series with chemotherapy.

Studies of bone marrow after total-body irradiation show a lesser degree of depression in cellularity than might be expected in relation to alterations in blood leukocyte and platelet values.

Studies related to infection in irradiated patients included reevaluation of the killing power of chronic granulocytic leukemia granulocytes for bacteria. It was found that in the test system when the number of granulocytes per unit volume of blood is constant, the radiation does not seriously alter the cell's killing power. In the small number of patients given antilymphocyte globulin and high doses of total-body irradiation for marrow transplantation, studies on serum and secretory proteins and on antibodies to viruses are reported. In animal work the radioprotective drug WR-2721 was shown to cause a reduction in infections after total-body irradiation.

The Information Center for Internal Radiation Dose from Radiopharmaceuticals has continued to expand its data base, to calculate internal doses using new techniques, and to respond to requests for this information from within and outside the Division. An X-ray beam monitor was built and a precision scan recorder was developed for printouts of scans after computer manipulation.

A summary of data on radioiodine uptake and whole-body retention of this nuclide showed a good correlation between the two.

Patients given 250 R of total-body irradiation (TBI) showed increases in carbon dioxide (CO_2) production during exercise following radiation exposure. Further studies on creatinine phosphokinase levels during irradiation showed a significant rise in five of seven patients given doses of from 100 to 250 R. A study of exercise tolerance in irradiated ponies, correlated with histopathology, suggested that changes in tolerance associated with irradiation are due to reversible metabolic effects rather than anatomical changes.

A patient with acute leukemia given a marrow transplant developed a severe graft-versus-host reaction, the first one seen at the Medical Division. The case showed that the particular regime used here does not prevent T-cell grafts, a possibility that was previously entertained.

In mice given marrow from allogeneic donors the resulting impaired immunologic mechanism was shown to be due to variable deficiencies in T-cell and B-cell function. Surprisingly, the immunologic reconstitution of chimera spleens could be achieved only with allogeneic cells, i.e., allogeneic to the chimera population. Several attempts to restore competence with syngeneic cells have failed, suggesting that the defect in these animals represents a major fundamental problem in immunology, the resolution of which should lead to a better understanding of the phenomenon of tolerance in animals or humans bearing allogeneic hemopoietic grafts. In another study it was shown that the administration of antithymocyte globulin before the infusion of lymphocytes into irradiated animals can adversely affect immunologic reconstitution even though all detectable cytotoxic factors of the serum had been eliminated before the cells were given.

Studies on granulocyte chalone revealed three separate fractions that can be derived from granulocytes and which cause reduced thymidine incorporation in cultured rat cells, but it is not clear which of these fractions have all of the criteria for chalones.

Clinical Observations

Therapeutic Total-Body Irradiation

During the past year we have treated (C. L. Edwards, F. A. Goswitz, Helen Vodopick, C. C. Lushbaugh, and G. A. Andrews) 20 patients with malignant hematologic disorders using total-body irradiation (TBI) as the only or principal therapy.

Chronic granulocytic leukemia (CGL)	9
Chronic lymphocytic leukemia (CLL)	4
Lymphosarcoma (LS)	3
Lymphosarcoma-cell leukemia (LSL)	1
Polycythemia vera (PRV)	2
Idiopathic thrombocythemia (IT)	1
	20

1028216

The treatments were given in two radiation facilities that have been described in previous reports and designated as (1) the low-exposure-rate total-body irradiator (LETBI) and (2) the medium-exposure-rate total-body irradiator (METBI). Through June 1973, a total of 60 treatments of 100 R or more had been given as continous exposures in LETBI (Table 1). Then the cobalt sources were adjusted to reduce the exposure rate from 1.5 R/hr to 0.8 R/hr providing daily exposures of approximately 16 R/day based on a 20-hr day. Treatments in LETBI at 1.5 R/hr have been designated as LETBI-I and those at 0.8 R/hr, LETBI-II. Five patients have been treated with exposures of 100 R or more in LETBI-II.

1. 66-year-old woman	CGL	150 R
2. 72-year-old man	LSL	100 R
3. 72-year-old man	LSL	100 R
4. 59-year-old woman	PRV	100 R
5. 48-year-old man	CLL	100 R

For several years the Medical Division has been evaluating the effects of TBI, given at different dose rates, in patients with chronic proliferative disorders of the bone marrow. Major emphasis has been placed on collection of complete data on the short-term effects of individual exposures of from 50 to 250 R. Very careful and systematic hematologic follow-up has been done, especially during the first 6 weeks after each individual exposure, and assessments are being made of this extensive collection of data to derive information on relative radiosensitivity of the various diseases at different stages of their evolution, on the relation of radiation dose to effect, on the significance, if any, of dose rate, and on kinetic implications of time patterns of response. With this kind of information the therapeutic application of TBI in these diseases can best be developed.

The number and types of TBI treatments of 10 to 250 R given at ORAU in METBI and LETBI through 1973 are shown in Table 1. Of the 188 treatments given to 139 patients, 82 were single exposures of 50 or 100 R in METBI. These were all given prior to 1968 and provided preliminary data that helped in selecting exposures for later treatment protocols. These treatments were seldom the only treatment of these patients and are distinct from the maintenance-type exposures of 10 to 60 R in METBI that were repeated at intervals to maintain a desired blood count.

The rest of the TBI treatments were given in either of two forms: (1) Induction-type treatments were given as daily exposures of 10 to 30 R in METBI or protracted exposures in LETBI until the prescribed total exposure of 100 to 250 R had been given. Usually, treatments were prescribed in this manner for previously untreated patients in whom a fairly prompt reduction in the blood counts was desired. A waiting period of 6 weeks or more was then necessary before determining the need for further therapy. (2) Maintenance type therapy consisted of exposures of 10 to 60 R given at intervals of a few days to several months depending on the response to previous exposures and based on an estimate of amount of radiation necessary to achieve or maintain the desired blood count or other effect.

From 1967 until 1973, 60 protracted exposures of 100 to 250 R were given in LETBI-I to 39 patients. These treatments were separated by a period of observation during which time periodic examinations were made to assess the response to the therapy. Because we tried to avoid overtreatment, the prescribed exposures were usually too small to restore the blood counts completely to normal. During the earlier phases of the TBI project, further therapy was withheld until after the disease had definitely relapsed and the blood counts had returned toward the pretreatment levels. Recently the prescribed induction

1028217

exposure has been followed by small maintenance doses at irregular intervals soon after maximum effect has been seen. Thus, we are now using TBI not only to induce remissions, but to maintain them after a satisfactory response has been achieved. This accounts for the increased number of small exposures given during the past year.

In addition to the five LETBI-II treatments in 1973, two patients were given induction treatments in LETBI:

1. 44-year-old-man	CGL	250 R
2. 69-year-old-man	CGL	150 R

TABLE 1

TBI TREATMENTS OF 50 R AND GREATER THROUGH 1973

Exposure per treatment (R_x)	METBI (1.5 R/min)					LETBI I (1.5 R/hr)		LETBI II (0.8/hr)	Totals
	Single acute exposures	10 R/day (3 days/week)	10 R day	30 R day	60 R day	10 R day	30 R day	16 R day	
Chronic Lymphocytic Leukemia									
50	15					1	4	1	15
100	20								26
120		1							1
150		1							1
Lymphosarcoma and Lymphosarcoma Cell Leukemia									
50	5	1*	1			1	3‖	2 ¶	7
100	22	1†	1			2 ‡			30
150		1		1			4 ¶		4
250				1					5
Chronic Granulocytic Leukemia									
50	8				3		6		8
60				1 §			1		3
100	4						9	1	11
120				13		1			1
150						2	8°		24
200				1					2
250									9
Polycythemia Rubra Vera							5	1	13
100	7			3			8		11
150							4 ✓		4
250									
Idiopathic Thrombocythemia							1		2
100	1			2			7		9
150									
Total	82	5	2	22	3	7	60	5	186

- • Patient received 60 R
- † Patient received only 90 R
- ‡ One patient received only 140 R
- § 25 R/day
- ‖ One patient had lymphosarcoma cell leukemia
- ¶ Two patients had lymphosarcoma cell leukemia
- ° One patient received only 222 R
- ✓ One patient received 230 R.

and three patients were given induction therapy in METBI:

1. 72-year-old woman	CGL	150 R
2. 42-year-old woman	CGL	150 R
3. 55-year-old woman	PRV	150 R

Patient 1 of the METBI group had received TBI on three previous occasions but the interval between treatments was so great that her white blood cell count had increased to over 70,000 mm^3. When the disease has relapsed to this extent the prompt suppression achieved with the induction program is more desirable than the gradual reduction seen with maintenance exposures.

The largest number of TBI treatments administered during 1973 were of the maintenance type. Eleven patients received from 1 to 40 treatments of 30 R in METBI, two received single treatments of 60 R, and seven received from 1 to 10 treatments of 10 R in an effort to achieve or maintain the desired blood values.

		No. of Rx
60 R METBI		
1. 72-year-old man	CGL	1
2. 40-year-old woman	CGL	1
30 R METBI		
1. 74-year-old man	CGL	40
2. 55-year-old man	CGL	21
3. 40-year-old woman	CGL	18
4. 44-year-old man	CGL	13
5. 67-year-old man	IT	10
6. 69-year-old man	CGL	9
7. 47-year-old woman	CGL	7
8. 73-year-old man	LS	6
9. 71-year-old man	CGL	5
10. 66-year-old woman	CGL	4
11. 42-year-old woman	CGL	1
10 R METBI		
1. 68-year-old woman	CLL	10
2. 73-year-old man	LS	9
3. 69-year-old man	CLL	5
4. 81-year-old woman	CLL	5
5. 86-year-old man	LS	1
6. 47-year-old woman	CGL	1
7. 66-year-old woman	CGL	1

The treatments in both facilities were well tolerated with only occasional symptoms, chiefly anorexia and lethargy, and rarely nausea. Most of the METBI treatments were given on an outpatient basis.

The selection of patients, the type, and the dose of radiation have not been random. For one thing, our objectives are different for various diseases. For instance, we have treated no patients with IT with 250 R because our objective in this disease is merely to keep the platelet count down to 500,000/ mm^3 or less. Empirically, we have found that the

1028218

platelet counts in these patients can be effectively lowered with exposures of less than 250 R. On the other hand, many patients with CLL or LSL had thrombocytopenia prior to therapy and were excluded from this treatment. Even in the absence of initial thrombocytopenia we used only lower doses distributed over a longer period of time for these patients because radiation is more apt to significantly suppress the platelets in these diseases. With very slowly accumulated TBI exposures, it has been possible to suppress the white blood cell count in CLL without producing severe thrombocytopenia. However, as yet, we have not achieved a complete remission in CLL patients with TBI alone.

In PRV, as in CGL, a number of factors entered into the decision as to when to radiate and at what dose. The objective of the treatment was often to lower the red blood cell counts to normal, but we had to be attentive to the platelet count and the cellularity of the marrow also. Usually, these patients were treated initially with an induction-type course of TBI of 150 to 250 R, depending somewhat on the height of the blood counts, the marrow cellularity, and the patient's symptoms. Once the remission had been achieved in PRV or CGL, it could usually be maintained by small doses at intervals as needed. The frequency and size of the radiation doses required to maintain a steady state in these diseases appears to vary a great deal from patient to patient, presumably reflecting some kinetic aspect of the disease.

Since 1957 we have treated 30 CGL patients with 50 induction-type treatments of TBI. Many of the patients had received other forms of therapy, i.e., chemotherapy or radiation to spleen or liver and, for the most part, until recently there has been no effort to achieve a complete remission of the disease using TBI alone. Table 2 shows the clinical

TABLE 2

REMISSION RESPONSE TO 50 TBI TREATMENTS OF 30 PATIENTS WITH CGL

	Total	Complete remission	Partial remission	Improved	No change
250 R	9	2	3	4	–
150 R Fract. METBI	13	1	4	7	1
150 R LETBI	9	4*	1	4	–
100 R METBI	5	–	3	2	–
100 R LETBI	6	–	1	5	–
50 R METBI	8	–	1	7	–

*One patient developed a pancytopenia before the remission.

response to each treatment. For purposes of this study we have defined a complete remission as requiring (1) normal hemoglobin (>13 gm), (2) normal total WBC (<15,000), (3) normal platelet (<400,000), (4) normal to only slight increase in the marrow cellularity with a G:E ratio of less than 10:1, (5) an absence of blasts or progranulocytes on the blood smear, and (6) a spleen no larger than 10 cm below the costal margin. A partial remission requires normal blood cell counts with 2% blasts or progranulocytes on the blood smear, a G:E ratio of less than 20:1 with normal to increased marrow cellularity. Any improvement in cell counts without achieving the specific criteria for remission was classified as "improved status." The remission rate appears to be dose-related inasmuch as no complete remission was achieved by single treatments of less than 150 R. On the other hand, the frequency of remissions at 150 R compared with 250 R seems to justify the caution taken to avoid excessive marrow suppression.

In only two instances did the radiation result in pancytopenia. One patient, a 69-year-old woman, received two exposures of 150 R with an interval of only 2 months. The second exposure was followed by a period of pancytopenia requiring transfusion of blood components before she recovered and remained in complete remission for nearly 2 years. The other patient was an 88-year-old man with a WBC of only 31,000 before treatment of 100 R in METBI. His pancytopenia lasted 2 months.

Other significant side effects of the radiation have not been seen. We have seen no cataract formation and we have not seen the Addison type of hyperpigmentation and hanition described in patients treated with busulfan.

Survival data indicate that our patients are living about as long as, but not significantly longer than, patients treated by other good, modern methods. Hidden within survival data, however, is the duration of the blast cell crisis. In every instance the blast cell crisis introduces a new life style much less desirable than that enjoyed through the less aggressive phase of their illness. As with most groups of CGL patients, most (73%) of ours have died in the blast cell crisis. The important question, whether the blastic transformation occurs earlier after TBI than after other treatments, is yet to be answered.

Human-Marrow Changes After Treatment With TBI

In the 1971 Research Report we gave an account of the marrow changes in 30 patients who were given 44 treatments with continuous irradiation at the low-dose rate of 1.5 R/hr in the LETBI-I facility. Last year we reported on the marrow changes of 12 patients who received 14 treatments (12 in LETBI, 2 in METBI) at total exposures of 30 R/day.

During 1973, we (F. A. Goswitz, C. C. Lushbaugh, and Helen Vodopick) compared the marrow before treatment and approximately 18 days after the start of treatment in nine patients who got 10 treatments (Table 3). Two of these 10 treatments were administered in the LETBI-I facility at the 30 R/day dose rate. Thus, we have an accumulative total over several years of 60 treatments (to 39 patients) in LETBI with continuous exposures of 100, 150, 222, or 250 R. Related data on these patients are in this Research Report in the Therapeutic Total-Body Irradiation section. Since one marrow aspirate was omitted, the marrow study is limited to 59 treatments. Three of our 10 treatments in 1973 were with 150 R over a 5-day period at the medium-dose rate of 1.5 R/min. Together with the two studies listed in the 1972 Research Report we have a total of five in this group. Five of 10 treatments in 1973 were received by four patients in our LETBI-II unit, at the dose rate of 0.8 R/hr or approximately 16 R/day. All marrows were interpreted in the same manner as described in the 1971 Research Report.

In general, the degree of marrow cellularity and the decrease or absence of fat are the features most related to radiation. Table 3 shows changes in marrow in 10 patients treated in 1973. To avoid platelet suppression in the lymphoproliferative disease, we treat most patients with fractionated, rather than a single protracted course of irradiation. Treatments with 10 R in patients with chronic lymphocytic leukemia may reduce the absolute blood lymphocyte count 5,000 to 10,000 per mm^3, while the same dose for treating chronic granulocytic leukemia will have no observed effect in the blood or the marrow. A minimum of 30 R would be necessary to produce detectable reduction in the absolute blood granulocyte count and the marrow G:E ratio in most instances.

Since a statistically adequate number of patients with CGL has been studied in the LETBI-I facility, we wish to summarize our experiences of the CGL patients treated with

1028219

TABLE 3

MARROW DATA IN PATIENTS TREATED WITH TBI*

Previous R	Disease	Treatment number, facility	Cells		Fat		Erythroid G:E ratio		maturation		Granulocytic maturation		Lymphocytes		Megakaryocytes/ platelets		Iron	
			b	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*
								Patient Treated With 250 R										
0	CGL	1†	↑↑↑	↑↑↑	A	A	24.0	10.0	N	N	L↑↑	L↑	A	A	↑↑/↑↑	↑↑/↑	A	↓↓↓
								Patients Treated With 150 R										
†‡†	CGL	1†	↑↑↑	↑↑↑	↓↓↓	↓↓↓	165.6	7.0	N	N	N	L↑	↓↓	↓↓↓	N/N	N/N	↑	↑↑↑
0	CGL	2†	↑↑	↑	↓↓	↓	50.1	21.8	N	N	L↑↑	N	↓↓↓	↓↓↓	N/N	N/N	↓↓↓	↓↓↓
C†‡	CGL	3‡	↑↑↑	↑↑	↓↓↓	↓↓↓	96.5	1.3	N	R↑	L↑↑	L↑↑	↓↓↓	↓↓↓	↑↑/↑↑↑	↑/N	A	A
0	CGL	4§	↑	↓	↓↓↓	↓↓	18	4.5	N	R↑	L↑	L↑	↓↓↓	↓↓	↑/↑	N/N	↓↓↓	A
0	PRV	1†	↑↑	↑	↓↓	↓	1.0	1.2	L↑	N	L↑↑	L↑	↓	↓	↑↑↑/↑↑↑	↑/↑	A	A
								Patients Treated With 100 R										
0	CLL	1§	↑↑	N	↓↓	↑	0.67	0.6	N	N	N	N	↑↑↑	↑↑↑	N/N	N/N	↓↓↓	A
0	LSL	1§	↑↑	↑↑	↓	↓↓	0.26	1.4	N	N	R↑↑	N	↑↑↑	↑↑↑	↓/↓	↓↓/↓↓	↑↑	↑
§	LSL	2§	↑↑↑	↑↑	↓↓↓	↓↓	0.38	3.1	N	N	N	N	↑↑↑	↑↑↑	↓↓/↓↓	↓↓↓/↓↓↓	↑↑	↑↑
†‡§§	PRV	1§	↑↑↑	↑↑	↓↓↓	↓↓	0.98	2.7	L↑↑	L↑	L↑	N	↓↓↓	↓↓	↑↑↑/↑↑	↑/N	A	A

*Symbol abbreviations

↑ = increase, ↓ = decrease; *a* = after irradiation, *b* = before irradiation

A = absent, C = chemotherapy, N = normal, L = left shift, O = none, R = right shift

CGL = chronic granulocytic leukemia, CLL = chronic lymphocytic leukemia

LSL = lymphosarcoma cell leukemia, PRV = polycythemia rubra vera

†Treated in METBI
‡Treated in LETBI I
§Treated in LETBI II

TBI. Table 4 illustrates the marrow changes between the pre- and postirradiated specimens after 100 R in METBI and 150 R in LETBI. In general, there was less reduction in cellularity than we anticipated even in patients who showed a pronounced reduction in blood leukocyte count at the time the posttreatment specimen was obtained. There was a definite change in the G:E ratio categorized as pronounced if the reduction between marrows was greater than 75%, moderate if greater than 50%, and slight if greater than 25%. All five patients who were treated with single exposures of 100 R in METBI had a fall in the G:E ratio. After 150 R protracted treatment in LETBI, the G:E ratio fell in seven of nine instances. Histiocyte iron, for the most part, remained absent. A moderate-to-pronounced reduction in megakaryocytes occurred in five of the nine treatments with 150 R and in two instances after treatment with 100 R in METBI. Table 5 reveals the changes after 150 R in METBI and 250 R in LETBI. Because higher doses were administered, these changes are greater. Ten of the 13 treatments in METBI resulted in slight to moderate reduction in marrow cellularity of patients given 150 R. Five of seven treatments after 250 R in LETBI produced a moderate decrease in marrow cellularity. The fall in the G:E ratio occurred after 10 of 13 treatments with 150 R in METBI and after all seven treatments with 250 R in LETBI. Megakaryocytes decreased after 11 of 13 treatments with 150 R in METBI and after all seven treatments with 250 R in LETBI. Fat content increased in nine of 13 patients treated with 150 R in METBI and in all patients treated with 250 R in LETBI.

TABLE 4

MARROW CHANGES IN CHRONIC GRANULOCYTIC LEUKEMIA

	AFTER 100 R METBI (5 treatments)				AFTER 150 R LETBI (9 treatments)			
	None	Slight	Moderate	Pronounced	None	Slight	Moderate	Pronounced
Cellularity	2	3	0	0	2	7	0	0
Fat	1	4	0	0	3	5	1	0
Megakaryocytes	1	2	2	0	1	3	3	2
Platelets	1	2	2	0	3	2	3	1
Histiocyte Iron	3	2	0	0	8	1	0	0
G:E Ratio	0	2	2	1	2	1	2	4

Figure 1 tries to simplify the changes in the G:E ratios in CGL before and after treatment with these various doses of TBI in the METBI and in the LETBI-1 facility. No significant change was observed in the average value of the G:E ratio before and after treatment for those patients given 50 R in METBI and 100 R in LETBI. A definite decrease in the G:E ratio resulted after patients are treated with 100 R in METBI or 150 R in either facility. The marrow will still remain hypercellular, however, even after single treatments with 250 R in LETBI at the dose rate of 30 R/day or after fractionated doses totaling 250 R at the 30 R/day dose rate in METBI. These findings demonstrate that more than a single exposure of total-body irradiation or a single course of treatment at the doses previously used is almost always necessary to bring the G:E ratio near normal in a patient with CGL.

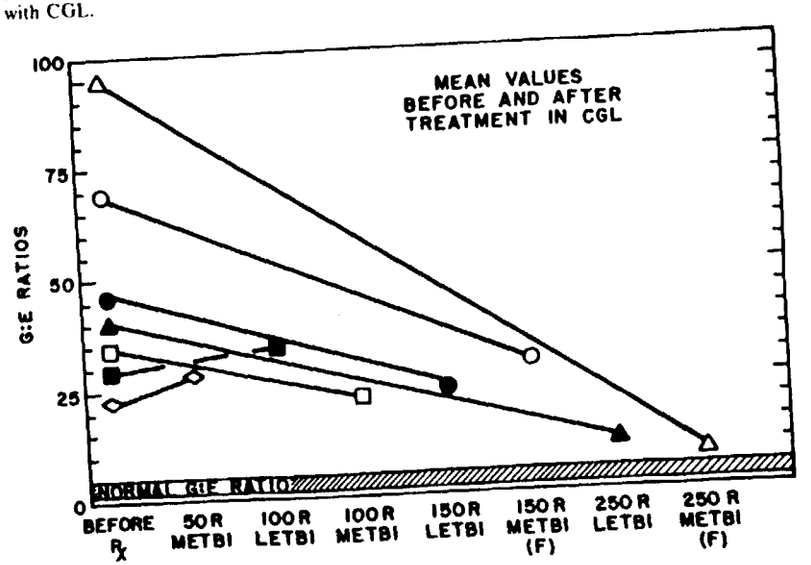


Fig. 1 Average marrow G:E ratios obtained in patients with chronic granulocytic leukemia before treatment and approximately 18 days after the start of various treatments with TBI. (F) means fractionated doses given at 30 R/day.

1028220

TABLE 5

MARROW CHANGES IN CHRONIC GRANULOCYTIC LEUKEMIA

	AFTER 150 R METBI (13 treatments)				AFTER 250 R LETBI (7 treatments)			
	None	Slight	Moderate	Pronounced	None	Slight	Moderate	Pronounced
Cellularity	3	6	4	0	2	2	3	0
Fat	4	6	3	0	0	4	2	1
Megakaryocytes	2	4	5	2	0	3	4	0
Platelets	4	5	4	0	3	3	1	0
Histiocyte Iron	5	4	4	0	3	2	1	1
G:E Ratio	3	1	4	5	0	2	3	2

Infections in Irradiated Patients

Effects of TBI on Antimicrobial Defenses

Introduction

We are primarily concerned with both the pragmatic problem of microbial infection in leukemia patients and with the more theoretical problem of leukemia ontogeny. Concerning the problem of microbial infections, we have concentrated our efforts on elucidating the effects of TBI on antimicrobial defenses. Concerning leukemia per se, we have concentrated our efforts on studying the relationship of embryogenesis and leukemogenesis.

If we are to better protect patients from the deleterious side effects of leukemia and/or treatment of leukemia, we must elucidate the pathogenesis of the infections that occur in patients with bone-marrow failure and impaired immune defenses.

The major objectives of this aspect of the work are to study postirradiation infection in patients undergoing radiation therapy and in other patients with similar cellular deficiencies by defining factors involved in the increased susceptibility of the host, by characterizing the pathogens responsible for the infection, and by seeking suitable methods for the prevention and treatment of infections in those patients.

Effects of TBI on Phagocytosis and Killing of Bacteria by Blood Granulocytes

These studies (W. G. Tankersley, Shirley Colyer, and R. L. Tyndall) were carried out on six patients with chronic granulocytic leukemia given therapeutic TBI. Unlike previous interpretations of similar data, the present results and reinterpretation of previous data indicate that the reduced killing capacity of blood granulocytes following TBI is most likely a reflection of the therapeutic reduction in excessive number of cells and is not a deleterious effect on granulocytes on a per cell basis.

Figures 2 and 3 show a typical example of previous data obtained from leukemic patients, but plotted in relation to bactericidal capability of blood cells from normal donors and also in relation to the patient's leukocyte count at various intervals following TBI. These results are typical of most patients previously studied.

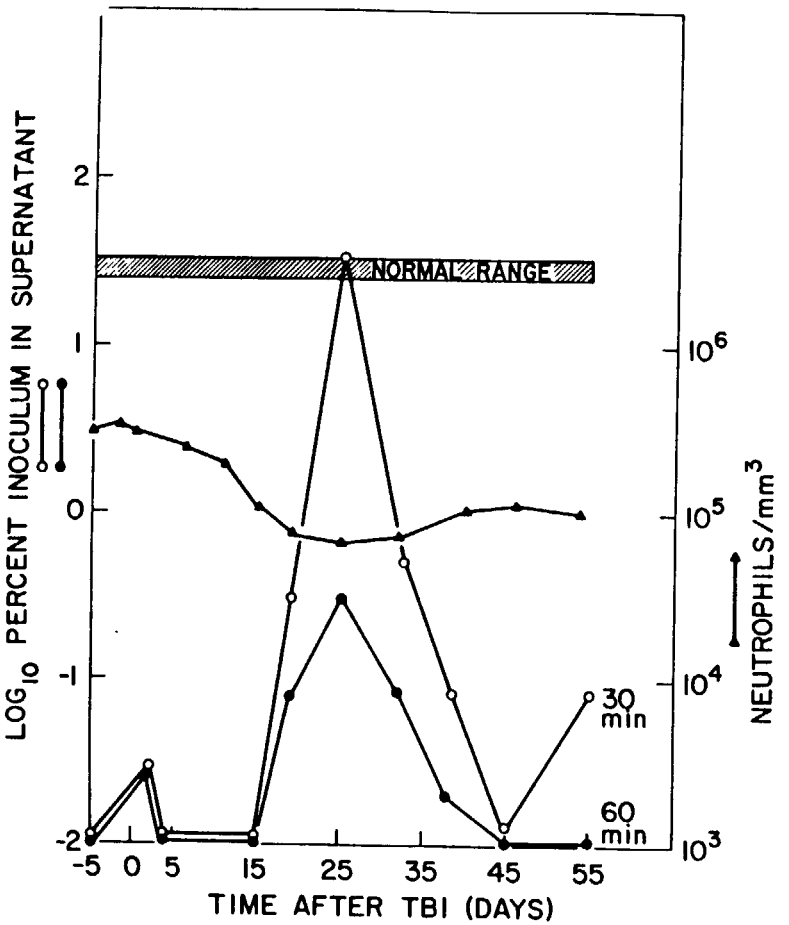


Fig. 2 Clearance of *Pseudomonas aeruginosa* after 30- or 60-min incubation with neutrophils from a CGL patient receiving 150 R TBI. Normal range is the average clearance in 30- to 60-min by neutrophils from normal donors.

Of interest is the observation that prior to TBI therapy the ability of blood from such patients to clear both *Pseudomonas* and *Staphylococcus* in an in vitro test system is markedly greater than blood from normal donors. This enhanced clearance is often 2-3 logs greater than in blood from normal donors. Following TBI therapy, the efficiency of clearance diminished relative to pre-TBI levels. The decrease generally paralleled the drop in total white blood cells (WBC) associated with the therapy.

As the therapeutic effect of TBI became apparent around days 10 to 15 after irradiation and the number of leukemic blood neutrophils began to diminish (Figs. 2 and 3), a

1028221

concomitant decrease in bactericidal capability occurred so that by day 25 after TBI, when the white cells reached their lowest levels, the clearance of bacteria was least efficient and most nearly approximated that in normal blood. By day 45 after TBI when the peripheral white cell count began its "abortive rise," there was again a concomitant enhancement of bacterial clearance. In view of this reinterpretation of previous data and in view of Solberg's recent study (Solberg, C.O. Acta Path. Microbiol. Scand. 80, 10-18, 1972) showing that the critical variable in the in vitro destruction of bacteria by human leukocytes was the number of leukocytes/mm^3 of blood, we altered our protocol for analyzing the phago-

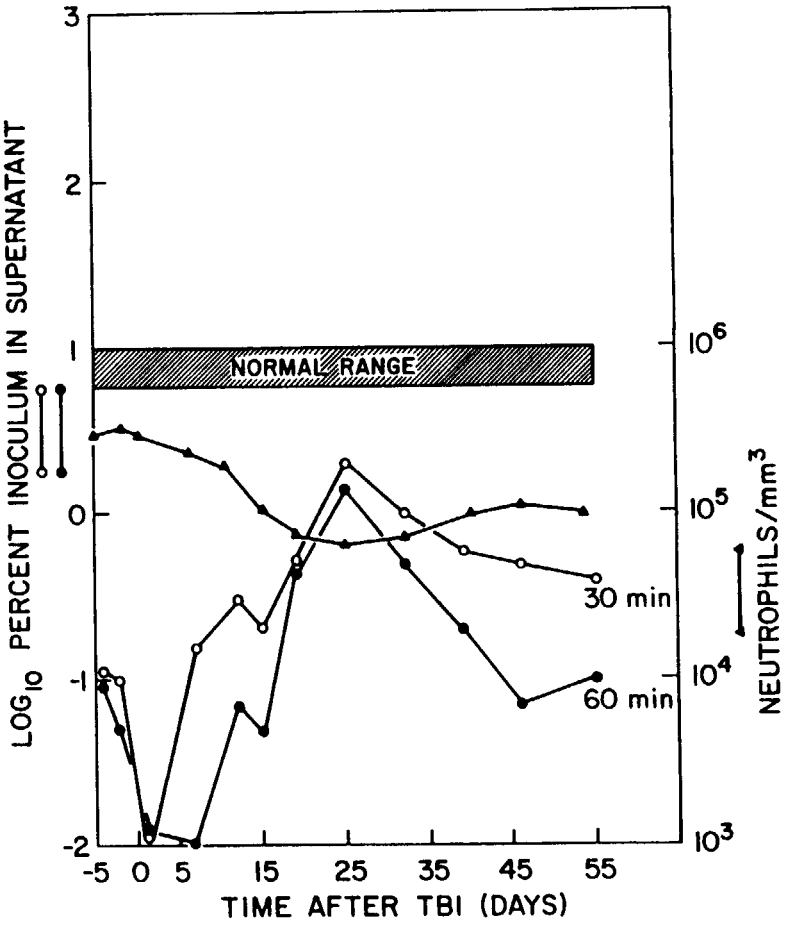


Fig. 3 Clearance of *Staphylococcus aureus* after 30- to 60-min incubation with neutrophils from a CGL patient receiving 150 R TBI. Normal range is the average clearance in 30- to 60-min by neutrophils from normal donors. [Patient: S.S.]

cytic and bactericidal potential of leukocytes after TBI. Blood from patients was analyzed by adding bacteria at a ratio of two bacteria for each granulocyte without diluting the blood and also by diluting the blood with autologous plasma to white cell counts approximating normal levels, i.e., 5000/mm^3, then adding bacteria at the same 2:1 ratio. When blood from patients exposed to TBI was so studied, it was even more apparent that the effects of TBI on impairment of phagocytosis was mostly due to the drop in levels of WBC associated with TBI therapy. Thus when white cell levels were diluted to approximately normal values the bactericidal activity of the blood also approximated normal values (Fig.

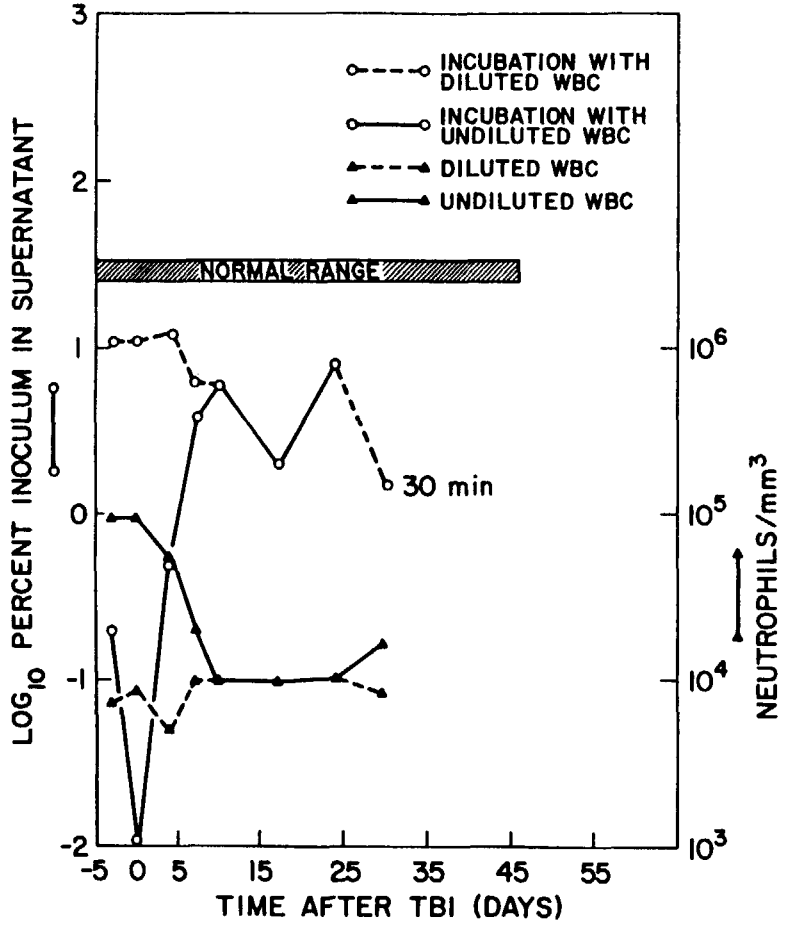


Fig. 4 Clearance of *Pseudomonas aeruginosa* after 30-min incubation with undiluted neutrophils diluted to levels comparable to those in normal human donors. Chronic granulocytic leukemia patient received 150 R TBI. Normal range is the average clearance in 30- to 60-min by neutrophils from normal donors.

4). Subsequent tests of phagocytic ability of TBI patients' blood cells, adjusted so that the number of neutrophils/mm^3 was normal, confirmed the data in Fig. 4 that TBI does not interfere with the individual cell's ability to destroy bacteria but merely reduces the total number of cells. It should also be noted that granulocytes from the CGL patients are as effective as normal granulocytes in destroying bacteria in vitro, supporting the therapeutic use of such cells in septicemia patients with severe neutropenia.

Effect of TBI on Serum and Secretory Proteins

A previous study of patients receiving 100 to 300 R TBI showed various degrees of serum protein diminution following TBI. Proteins analyzed included immunoglobulins, transferrin, albumin, C-reactive protein, and secretory IgA. More recently these determinations have been restricted to patients receiving TBI in excess of 400 R. The present study (Stuart Chaskes) involves two such patients.

Detailed data of serum protein profiles from one such patient are presented. Patient R.O. was an acute granulocytic leukemia patient in blast cell crisis. This patient was given antilymphocyte serum (ALG), 500 R TBI (40 R/min), and bone-marrow transplantation. Data in Table 6 show the alterations in various serum proteins following TBI. As was

TABLE 6

EFFECT OF 500 R TBI (40 R/min) AND BONE-MARROW TRANSPLANTATION ON A PATIENT'S SERUM PROTEIN LEVELS

Time (Days)	Serum Proteins (gm/100 ml)					
	T.P.*	Albumin	$Alpha_1$	$Alpha_2$	Beta	Gamma
-10	6.18	3.30	0.43	0.86	0.56	1.03
- 5	5.85	3.32	0.38	0.86	0.59	0.70
+ 2	5.71	3.25	0.42	0.73	0.47	0.84
+14	5.85	3.16	0.36	0.67	0.67	0.94
+23	5.00	2.87	0.25	0.45	0.59	0.84
+28	6.40	3.07	0.39	0.66	0.83	1.45
+39	6.85	3.77	0.24	0.53	0.83	1.47
+46	7.05	3.83	0.27	0.66	0.82	1.48
+52	6.06	3.22	0.27	0.64	0.75	1.18
+60	6.18	3.16	0.41	0.50	0.63	1.49
Normal range	6.5-8.5	2.7-4.8	0.13-0.43	0.52-1.02	0.65-1.45	0.60-1.78

*Total protein.
Patient: R.O., M

seen in previous patients, the levels of total protein, beta and gamma globulins decreased during treatment with ALG and immediately after TBI. By days 28 to 39 after TBI, however, the level of total serum proteins had returned to pretreatment levels or in the case of beta and gamma globulins to above pretreatment values. Even during the terminal illness the blood levels of albumin, $alpha_1$, beta, and gamma globulins remained at levels comparable to or above the pretreatment values. Only the $alpha_2$ globulin levels fell significantly at this time. This pattern differs from that previously reported (patient M.M.) where albumin, beta, and gamma globulin levels dropped significantly in the terminal stages. More akin to the last patient M.M. and unlike his own serum patterns are the profiles of secretory protein observed in patient R.O. As seen in Fig. 5, the levels of IgA

1028222

in saliva of R.O. continually dropped during ALG and following TBI treatment. The total protein, albumin, and IgG levels similarly fell during this period. This loss of secretory protein is particularly marked because most patients show a concomitant salivary dehydration which would ordinarily increase the concentration of salivary protein. By day 30 after treatment, levels of these proteins rose dramatically, but then fell sharply by day 60 after treatment. Evidence that these patterns indicate alterations in the product of secretory cells per se and are not a reflection of altered serum levels is the maintenance of serum levels of gamma globulin in the presence of precipitous loss of salivary IgG and IgA and absence of IgM.

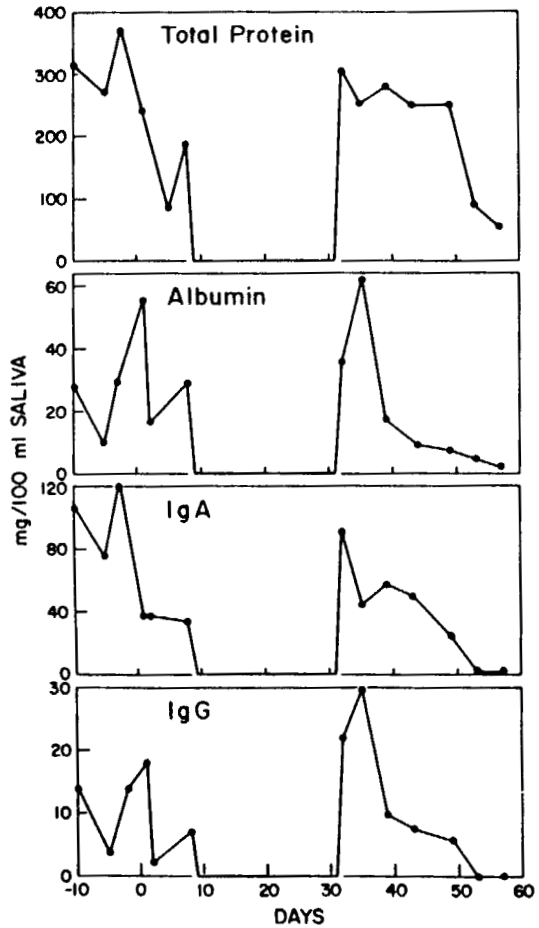


Fig. 5 Changes in salivary protein concentrations in a patient receiving 500 R TBI (40 R/min) and bone-marrow transplantation. (Patient: R.O.)

1028223

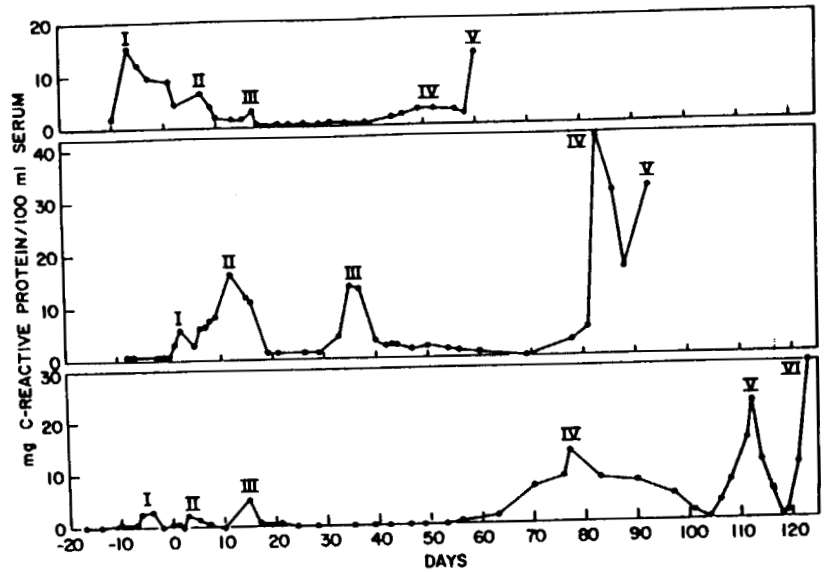


Fig. 6 Correlation of C-reactive protein concentrations with infections or inflammatory responses.

A (top). It is thought that the first peak (day -7 to 0) corresponds to a severe inflammatory episode elicited after ALG treatment. The latter part of the second peak and the entire third peak coincided with a *Candida albicans* infection of the mouth. Herpesvirus infection also occurred at this time. The small fourth peak is not known to correspond to any inflammatory-type response. Finally, the fifth peak corresponds to a fatal septicemia caused by *Proteus* sp and *Pseudomonas* sp.

B (middle). Five distinct peaks occurred during the study of this patient. Peak one (day 2) can be correlated with a urinary tract infection caused by the *Klebsiella-Enterobacter* group. The second peak corresponds to an episode of otitis and pharyngitis. The third peak corresponds to hepatitis (unknown etiology) along with a second urinary tract infection (*Klebsiella-Enterobacter* and *Proteus* sp). A third urinary tract infection (*Proteus* sp) on day 49 did not elicit a CRP peak. The fourth peak (83 days) corresponds to blast cell crisis and the fifth peak to a pneumonia and septicemia caused by *C. albicans*. *Candida albicans* was isolated from the sputum 8 days before death.

C (bottom). The first small peak may have been caused by a mild reaction to ALG. Small peaks II and III cannot be traced to an infectious agent or inflammatory-type response. CRP levels were grossly elevated from 60 to 122 days. During this time interval, three large peaks (IV, V, and VI) occurred. The patient is thought to have had a *Pneumocystis carinii* infection during this time interval. The autopsy revealed a pneumonia and the last peak (VI) may correspond to it. During the 60-122-day time interval the patient had both cardiac and kidney irregularities that may have contributed to CRP levels. The patient (day 68) had white patches on the uvula which resemble those seen in Vincent's angina. Additionally, the infectious agents of Vincent's angina (fusiform bacillus and spirochetes) were seen in a saline mouth wash. Peak IV (74-76 days) coincides with a *Pseudomonas aeruginosa* urinary tract infection, peak V (day 112) also coincides with a urinary tract infection (*Proteus* sp, *Pseudomonas aeruginosa*, and *Klebsiella-Enterobacter*).

Following high therapeutic doses of TBI, the levels of C-reactive protein in patients' sera usually show more similarities among various patients than do the profiles of other serum proteins. Most importantly, increased C-reactive protein levels are good indicators of infection and/or inflammatory response. As seen in Fig. 6, the concentration of the protein in three patients showed marked and distinct peaks, many of which were associated with infection. Production of C-reactive protein was nonspecific in that it related to bacterial, mycotic, viral, and parasitic infections. Terminal illness also was always correlated with a pronounced rise in serum concentration of C-reactive protein.

Effect of TBI on Specific Immune Responses

Analysis of herpesvirus and cytomegalovirus (CMV) antibodies in sera of two patients receiving TBI doses equal to or in excess of 500 R have been made (R. L. Tyndall) in cooperation with Dr. John Stewart of the Center for Disease Control in Atlanta, Georgia. As seen in Table 7, patient R.O. showed a persisting level of anti-CMV antibody from day 16 through 60. Antiherpesvirus antibody levels, however, increased between days 16-39 and subsequently declined by day 60. In patient P.T., antibodies against CMV decreased progressively from their highest level prior to TBI to their lowest level at day 21 following TBI. Samples after day 21 are still to be analyzed. Conversely, while levels of anti-CMV antibody were dropping in patient P.T., antiherpesvirus antibody was synthesized after TBI. The sera of the marrow donor to patient P.T. showed a low level of antibody to CMV and no significant amount of antiherpesvirus antibody.

TABLE 7

EFFECT OF TBI (40 R/min) ON ANTIVIRAL ANTIBODIES

Patient	Dose (R)	Days prior to (−) or after TBI (+)	Antibody Titer* Cytomegalovirus CF	Cytomegalovirus IHA	Herpesvirus CF	Herpesvirus IHA
R. O.	500	+16	16	256	32	2048
R. O.	500	+39	16	256	>126	>4098
R. O.	500	+60	16	256	64	>4098
P. T.	694	−30	>256	1024	16	< 8
P. T.	694	+18	>256	512	8	512
P. T.	694	+21	256	512	8	512
Marrow donor for P. T.	NA	NA	128	128	<16	< 8

*Titer expressed as reciprocal of the highest dilution giving a positive result.
NA = Not applicable; serum for antibody analysis obtained at the time when marrow was transplanted into patient P. T.

On comparing the migration inhibitory factor (MIF) reactions (Stuart Chaskes and K. F. Hübner) of lymphocytes from patient P.T. and his donor, there is evidence of loss of some specific reactivity in the patient's lymphocyte following TBI and marrow transplant together with concomitant suggested acquisition of reactivity, indicative of donor lymphocyte competency (Table 8). For instance, prior to TBI and marrow transplantation the donor lymphocytes reacted strongly to all antigens, i.e., PPD, mumps, histoplasmin, dermatophytin, and dermatophytin "O". Because of limited numbers of lymphocytes, patient P.T. was tested against only three antigens. Of these, he reacted to PPD and histoplasmin but not to mumps prior to TBI. Following TBI and marrow transplantation, however, significant reactivity was seen against mumps and histoplasmin in spite of the fact that the patient had less than 1150 lymphocytes/mm^3 of blood. Due to these extremely low numbers of lymphocytes only the two antigens could be tested. In these MIF tests an inhibition of macrophage migration by more than 20% was considered a positive reaction.

TABLE 8

IN VITRO ASSAY OF DELAYED HYPERSENSITIVITY (MIF) OF A PATIENT RECEIVING 694 R TBI (40 R/min) AND A BONE-MARROW TRANSPLANT

Source of lymphocytes	Date	Percent Migration Inhibition* Mumps	Histoplasmin	PPD
Donor	4/12/73	53.1	60.2	65.1
Patient (P.T.)	5/30/72	11.7	62.0	47.2
Patient (P.T.)	2/13/73	NT	47.0	NT
Patient (P.T.)	3/26/73†	0.0	49.9	33.1
Patient (P.T.)	6/11/73†, ‡	23.4	20.7	NT

Patient: P.T., M—TBI of 694 R given on 4/9/73.
*A greater than 20% inhibition is considered a positive result.
†Patient receiving prednisone.
‡White blood cell count $<2500/mm^3$ with approximately 45% lymphocytes.
NT = not tested.

Microbiological Studies on a Patient in an Ultraclean Environment After TBI

The Medical Division maintains a laminar-air-flow (LAF) facility that provides an ultraclean environment for patients with increased susceptibility to infection. The LAF facility is a restricted entry, self-contained unit composed of two patient rooms and a clean preparation and anteroom. The patient rooms are equipped with high-efficiency filters that cover the entire wall of one end of the room and which are capable of excluding all particles over 0.3 micron in size. All unsterile material and all personnel, except medical personnel, are rigidly excluded from the patient's room. All foods and materials are sterilized or effectively excluded from the patient's environment by appropriate sterile coverings. The room is sterilized by fogging with an antiseptic solution (Osyl) and allowed to air for 24 hr before the patient is placed in it.

Medical personnel are allowed into the LAF based on need to enter. Sterile gown, cap, mask, boots, and surgical gloves are required. Personnel are instructed to remain downwind of the patient at all times.

Microbiological studies (W. G. Tankersley and Shirley Colyer) have continued on the use of ultraclean environments during the patient's occupancy. The microbial contamination level of surfaces, furnishings, fixtures, and medical personnel in the LAF rooms and preparation rooms were determined by contact plate at least once a week. The microflora of the patient's nose, throat, mouth, skin, and feces were cultured two times each week; blood and urine samples were also examined.

1028224

While the individual flora of the two patients varied, as would be expected, the number and variety of organisms isolated from these patients was markedly reduced. These overall data indicate that we cannot, nor should we expect to, completely eliminate bacteria from the patient's environment. However, by appropriate prophylaxis, antibiotic therapy, and the cleanroom environment we can greatly reduce the level of microbial contamination and thereby greatly protect the patients from the chance of a serious infection.

Chemical Protection of Natural Defense Mechanisms During TBI

Because infections occurring after irradiation at times have a rapid course and high mortality rate, it is important to protect or enhance the host's natural defense system during exposure. A number of chemicals experimentally can modify the effects of irradiation.

Our preliminary studies with MEA and AET suggested that these chemicals could afford significant protection against postirradiation infection in CD-1 mice. Either of the drugs (400 mg/kg) provided excellent protection against spontaneous infection in mice exposed to 800 R cobalt-60 (^{60}Co) TBI. Under these conditions approximately 20% of the protected mice showed evidence of spontaneous infection. In contrast, up to 80% of the unprotected animals were infected sometime during the 21-day study.

We have recently (W. G. Tankersley and R. L. Tyndall) turned our attention to the radioprotective drug WR-2721 because of its relatively lower toxicity and its possible use in human clinical trials. As seen in previous studies with MEA and AET, mice treated with this drug (400 mg/kg) immediately prior to TBI similarly showed a marked reduction in spontaneous infections as determined by bacteriological tests of liver and spleen tissue. Mortality was likewise reduced. Since the peak of infection in mice following TBI coincides with the peak mortality period, it is assumed that bacterial septicemia is the cause of death. Since this is an assumption and cannot be proved, we altered the experimental protocol in order to prove that WR-2721 could inhibit fatal bacterial infections following TBI. To do this we irradiated CD_1 mice with 600 R using a ^{60}Co source. Some irradiated mice were inoculated with WR-2721 (400 mg/kg) 30 min prior to TBI. Six days after irradiation, the mice were challenged with approximately an LD_5 of *Pseudomonas aeruginosa*. Mice receiving only irradiation or bacteria survived, whereas, in the absence of WR-2721, mice receiving both 600 R and *Pseudomonas* showed 95% mortality which was reduced to 10% with WR-2721 treatment (Table 9). These experiments proved unequivocally that WR-2721 protected mice against fatal bacteremia associated with TBI.

TABLE 9

PROTECTIVE EFFECT OF WR-2721 ON *PSEUDOMONAS*-INFECTED MICE* AFTER 600 R TBI

	Percent Cumulative mortality						
	Days after irradiation						
Treatment	7	8	9	10	11	12	20
P. aeruginosa†	0	0	0	0	0	0	0
600 R	0	0	0	0	0	0	0
600 R + *P. aeruginosa*†	55	75	90	90	95	95	95
WR-2721 + 600 R + *P. aeruginosa*†	0	5	10	10	10	10	10

* Groups of 20 mice.
† Mice injected intraperitoneally with ∿ LD_1 *Pseudomonas aeruginosa* 6 days after irradiation.

1028225

Medical Physics and Instrumentation

Information Center for Internal Radiation Dose from Radiopharmaceuticals

The staff (Roger J. Cloutier, Evelyn E. Watson, and Audrey T. Schlafke) of the Information Center for Internal Radiation Dose from Radiopharmaceuticals (work supported in part by the U. S. Food and Drug Administration) continues to collect and analyze data related to calculating radiation dose. The addition of a Teletype terminal to the Medical Division PDP-11 computer has increased the Center's capabilities for performing complex dosimetry calculations.

At the request of the Food and Drug Administration (FDA), the Center reviewed the radiation dosimetry for 30 "well-established" radiopharmaceuticals such as ^{131}I-sodium iodide, ^{133}Xe, and ^{57}Co-labeled vitamin B_{12}. In these dose calculations, the Center made use of tables of effective absorbed energy for 14 commonly used radionuclides (70) and modified equations of the Medical Internal Radiation Dose (MIRD) Committee (11). The dose estimates were incorporated into the sample package inserts provided by FDA to drug manufacturers as guides in preparing their package inserts.

For calculating the radiation dose received by bone and marrow from bone-seeking radionuclides such as ^{45}Ca, the Center has made use of Spiers' technique for determining radiation dose to tissues of bone from incorporated β-emitters (Spiers, F. W., presented at IRPA meeting, Washington, 11 September 1973). This technique, which uses Monte Carlo methods to calculate the dose rates from beta emitters, permits us to calculate the mean absorbed beta dose to the hematopoietic marrow in trabecular bone, to the trabecular surfaces and to the endosteal surfaces of cortical bone.

New data (39) enabled the Center to refine the dose estimates for the gallium isotopes – ^{66}Ga, ^{67}Ga, ^{68}Ga, and ^{72}Ga – and to include the dose to the stomach, small intestine, upper large intestine, and lower large intestine (10).

The computer data bank of bibliographic information on internal dosimetry was increased to 3000 entries this year. The Center published a bibliography of medical uses of technetium-99m (74) from data included in the bank. Although many more entries are needed before the bank is fully effective, we now use the bank in a limited way to search for references, particularly those dealing with more recently introduced radiopharmaceuticals.

During 1973, the Center replied to approximately three requests per week for radiation-dose information. Requests were received from governmental agencies, radiopharmaceutical manufacturers, and nuclear medicine sections of research hospitals. The Center's staff also discussed internal dosimetry problems with several visitors from the United States, Brazil, and Hungary.

Instrumentation and Computer Methods

Precision Scan Recorder

Many nuclear medicine laboratories, including our own, are investigating the possibilities of improving the readability of radionuclide scans by computer manipulation of the raw scan data. Generally, the resulting images are displayed on a cathode-ray tube (CRT) with hard copies of the scan produced by photographing the CRT. Physician acceptance of the CRT photographs is slow for a number of subjective reasons. We believe there would be greater acceptance of the hard copy if they were larger and of better quality. Because interface equipment to produce large recordings is not commercially available for our scanner-computer system, we (A. C. Morris, Jr. and T. R. Barclay) have designed and are constructing such a recorder. Our recorder consists of a light-tight enclosure where 14" × 17" film can be exposed to a computer-controlled matrix of illumination intensities. Our optical source for this purpose is a small, argon-filled lamp, chosen because its purple and near-ultraviolet output is efficiently sensed by standard X-ray film. Light from this source is tightly collimated to form a beam 0.1" × 0.1" square, directed to expose only a small, single area of the film. We drive this light over the film in an X-Y motion that is precisely controlled by stepping motors, coupled to a ball-bushing apparatus that achieves a positional accuracy of less than ±0.001 in. in both axes of motion. The original boustrophedonic movement of an area scanner is duplicated (often in a reduced form) by the action of our recorder's light source. The film is exposed to the light-flash duration corresponding to the number of counts in each element of the enhanced scan record. When all elements on one scan line are exposed, our source moves down to the next line and the process is repeated. Such a line-by-line exposure continues down the whole length of film to produce an accurate map of intensities, precisely located, resulting in a developed image that is free of overlaps, gaps, and other artifactual variations in density that are all too common in ordinary scan records.

The argon light source we use in this recorder is one that requires an external source of illumination for prompt ignition. Our solution to this problem was to stimulate the argon bulb with two light emitting diodes (LEDs). These LEDs are positioned at 90° to the bulb-to-film channel so the LED's light cannot expose the film directly, Fig. 7. Also, the standard X-ray film is practically insensitive to the red-wavelength light coming from the LEDs we selected. A schematic diagram of the electronic circuit is shown in Fig. 8.

Construction of the various parts of this recording system is nearing completion. The argon light source has been completely built, tested, and now operates reliably. Construction of the light-tight enclosure, special film cassette, and mechanical-drive assembly is 85% complete. Our schedule calls for constructing electronic controls, interface circuits, and computer programs concurrent with the completion and testing of the light-tight enclosure and source-driving mechanism.

X-Ray Beam Monitor

Several of the Division's radiation-effect studies require exposure rates greater than 50 R/min that need to be monitored during each exposure. Suitable commercial monitors are available; however, they are relatively expensive and provide features that we do not

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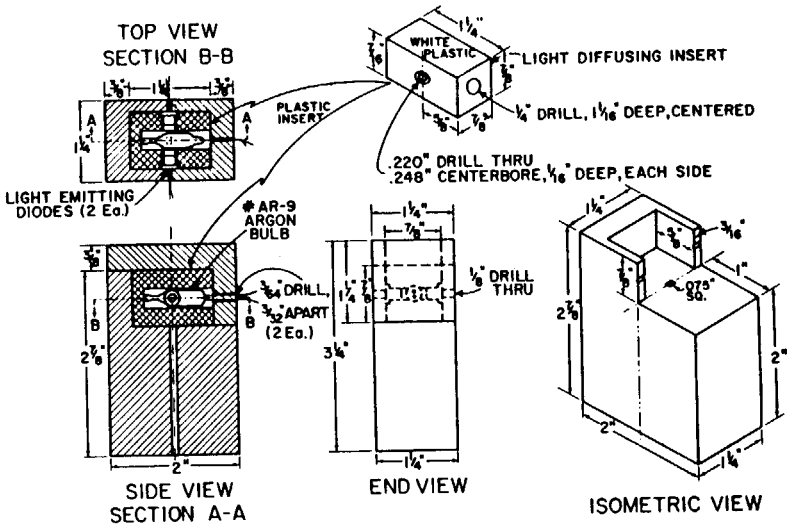


Fig. 7 Light source and collimator for the precision scan recorder. Light-diffusing insert is made of white nylon. Remainder of housing is made from a block of black nylon. Diffused light from the argon bulb passes down the 2-in. long, 0.075-in. square channel to expose the standard X-ray film below.

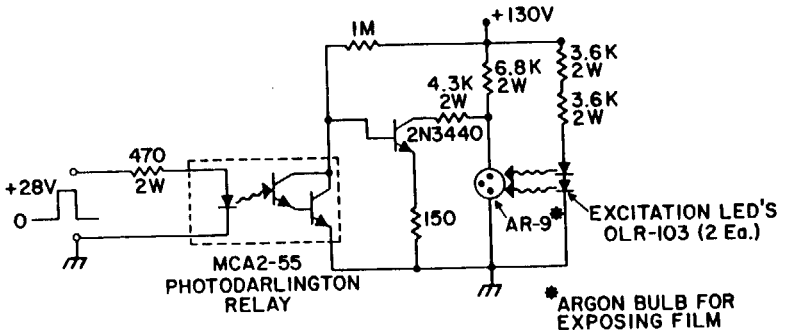


Fig. 8 Schematic diagram of electronic circuit for driving the argon light source in the precision scan recorder. The two light-emitting diodes (LEDs) are used in this circuit to ensure a prompt ignition of the AR-9 bulb. Red-light wavelength from these two diodes does not expose the film—the near-ultraviolet and purple light from the AR-9, as pulsed from the computer, produces a rapid film exposure.

re, ire. To meet our need we (A. C. Morris, Jr., W. T. Pope, J. E. McCarter) designed and built the low-cost monitor shown in Fig. 9. The monitor uses a small X-ray intensifying screen as the radiation detector. The screen is optically coupled to a self-generating silicon solar cell. The solar cell produces only a few microvolts, as it is forced to operate in its current mode by connecting it to a current-to-voltage operational amplifier (Fig. 10). The amplifier's voltage output is highly linear and is given by the expression $V_{out} = I_{in} \times R_f$, where I_{in} is the input current and R_f is the feedback resistance. The operational amplifier's output voltage is used to drive a 100-μamp meter.

Because the input leakage current must be minimized, we selected a Burr-Brown 3317/14 operational amplifier. This amplifier has an input impedance exceeding 10^{11} ohms which causes essentially all of the solar cell's current to flow through the feedback resistor, R_f.

After the detector is mounted inside the collimator of the radiation device, the coarse and fine gain controls are adjusted to give a suitable meter reading. The fine gain and zero controls are 10-turn potentiometers located on the front panel.

Parts used for this meter are readily available and cost less than $250. Assembly of the unit is straightforward and noncritical. In operation, the meter is stable in its response and an exposure-rate change of 1% produces a noticeable change in meter reading.

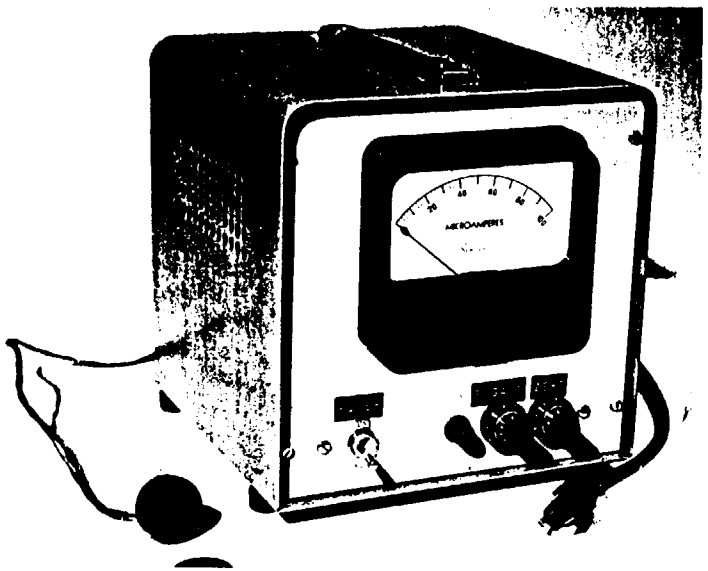


Fig. 9 Completed X-ray-beam monitor. The silicon solar-cell and its associated X-ray intensifying screen are shown in the left foreground. In operation, this screen is attached to the front of the solar cell with black electrical tape to make it light-tight. The detector assembly is then mounted at a standard position in front of the X-ray tube for checking day-to-day radiation-output levels.

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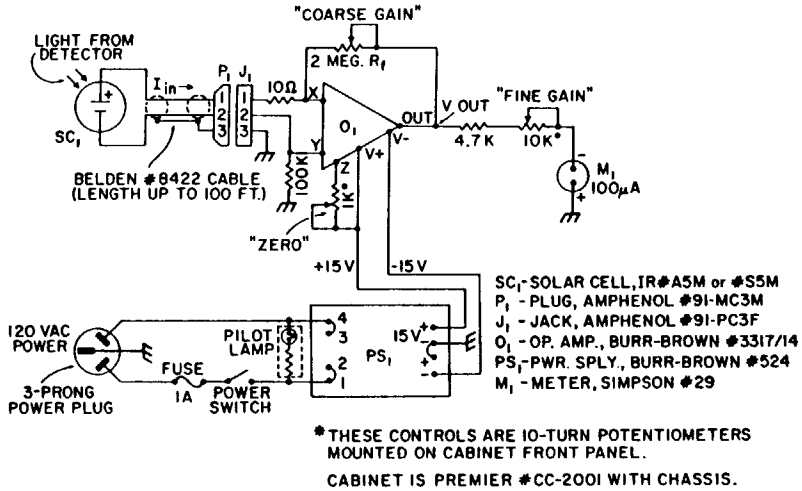


Fig. 10 Circuit of the X-ray beam monitor. Light from small intensifying-screen detector is converted to an input current by the solar cell. The operational amplifier changes this current to a voltage that can be measured by the meter. †Resistance value for the amplifier's feedback potentiometer, R_f, should be selected to give a full-scale reading of the meter, with R_f set to half-value, at the level of radiation measured.

Automation of Gallium-67 Assay

The Medical Division receives ^{67}Ga from Oak Ridge National Laboratory (ORNL) each week and processes it into a radiopharmaceutical for distribution to institutions participating in the cooperative ^{67}Ga localization study. Before the radiopharmaceutical can be shipped, it must be assayed in a manner that is traceable to NBS standards. To complete the assay, four different samples must be counted: duplicate ^{67}Ga samples being assayed, a ^{67}Ga sample assayed the previous week, and an NBS ^{22}Na standard. The assay technique was computerized to shorten the assay time, to eliminate mathematical errors, and to perform checks that would detect malfunctions of the assay equipment or errors in placement of samples during counting. In addition to assaying the ^{67}Ga in mCi/ml, the technique also determines the amount of ^{66}Ga contaminant present.

To shorten the assay time, we (T. R. Barclay) modified the Teletype drive section of our multichannel analyzer so that count data could be transferred directly to our PDP-11 computer. This required altering the logic circuits to provide commas between channels.

Because of limited computer memory, we (Evelyn E. Watson, Roger J. Cloutier) wrote two computer programs in BASIC to complete the assay. The first program accepts the data from the analyzer, sums the counts in the required energy interval, and prints these totals. When the counts from the four samples have been processed, the computer punches a paper tape that is used for INPUT values into the next program. The second program accepts the INPUT, corrects the NBS sodium-22 (^{22}Na) and ^{67}Ga results of the

previous week for decay and then compares the new results with the corrected counts. If they differ by more than ±3% the program signals that the system is not functioning properly. If the differences are less than ±3%, the computer calculates the activity of ^{67}Ga and ^{66}Ga in each new sample at the time of the count and corrects the assay for decay to the time the material will be received by the reseachers. The computer also calculates the percent difference between the ORAU assay and the assay supplied by ORNL. The final computer printout is shown in Fig. 11.

```
GA-67 SAMPLE DIFFERS -.4708699 % FROM PREV. COUNT
NA-22 STD DIFFERS  .5122137E-1 % FROM PREV. COUNT
RATIO .30/.51 COUNTS= .3048284

TIME & DATE OF COUNT?        1330,12,14,73
TIME & DATE OF DOSE ASSAY?   0800,12,17,73
ORNL RESULT (MCI/CC)?        3.18
```

GALLIUM-67 ASSAY

	TOTAL COUNTS	ACTIVITY AT TIME OF COUNT	ACTIVITY AT TIME OF ASSAY	% DIFF. FROM ORNL
GA-67-# 1	60458	5.75	3.18	.13
GA-66-# 1	0	0	<.001	
GA-67-# 2	60089	5.71	3.16	-.49
GA-66-# 2	0	0	<.001	
AVE. GA-67			3.17429	-.18

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ORAU MED PHYSICS
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Fig. 11 Computer printout of gallium-assay results.

Applied Radiobiology

Report on Continuing Efforts to Measure Physiological Responses in the Total-Body Irradiated Human

During the past year we continued our studies relating to TBI and performance decrement in man (Robert C. Ricks, Ann Sipe, and C. C. Lushbaugh). Specific efforts were concentrated on measurement of expired CO_2 levels, changes in the serum levels of creatine phosphokinase (CPK), and the influence of preirradiation exercise training on the induction of physiological responses by TBI.

Correlation of expired CO_2 concentration to pulmonary impedance variance were previously demonstrated (ORAU-122, p 141). Briefly, an increase in pulmonary impedance (during controlled exercise stress) was accompanied by a concomitant increase

1028228

in expired CO_2 concentration. Further, this increase in CO_2 can be shown to correlate with the workload in a direct manner. Using a modified NASA stress test and bicycle ergometer, we found CO_2 concentration increases as workload increases. The test conditions utilize alternating work/rest cycles of two minutes' duration and stepped workloads. Typical results are shown in Fig. 12. In parallel with the CO_2 changes generated by this type of physiologic test using stepped workloads, pulmonary impedance increases in a linear fashion. This linear pulmonary impedance increase is evident until the test subject nears his maximal work capacity when a decreasing slope factor develops. Figure 12 also demonstrates the correlation of pulmonary impedance and cardiac rate changes during stepped exercise.

During the past year two irradiated patients were studied under controlled exercise conditions to determine whether radiation induces additional changes in expired CO_2 concentrations. Some results of this study are illustrated in Figs. 13 and 14. Both patients received 250 R in the LETBI irradiator (15 R/hr, 30 R/day) and demonstrated significant increases in CO_2 production during repetitive ergometric stress (50 watts, 4-min duration) over periods of time up to 60 days. One patient (Fig. 13) showed, after 120 R were accumulated, significant increases in CO_2 concentration when measured during controlled exercise testing. This effect continued for about 2 weeks postirradiation. Another patient (Fig. 14) had a similar response but the increase in CO_2 production reached a peak 3 days postirradiation and rapidly returned to preirradiation levels. In

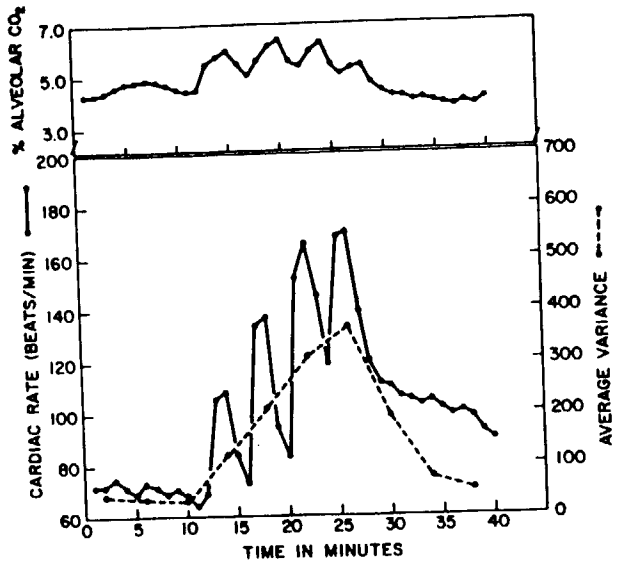


Fig. 12 Composite graph illustrating the effects of a stepwise increase in exercise workload in pulmonary-impedance variance, cardiac rate, and alveolar CO_2 concentration. Test subject was normal male aged 32 years. Exercise period consisted of alternate 2-min work/rest cycles at 50-, 100-, 150-, and 200-watt workloads, respectively, as indicated by peaks in cardiac rate.

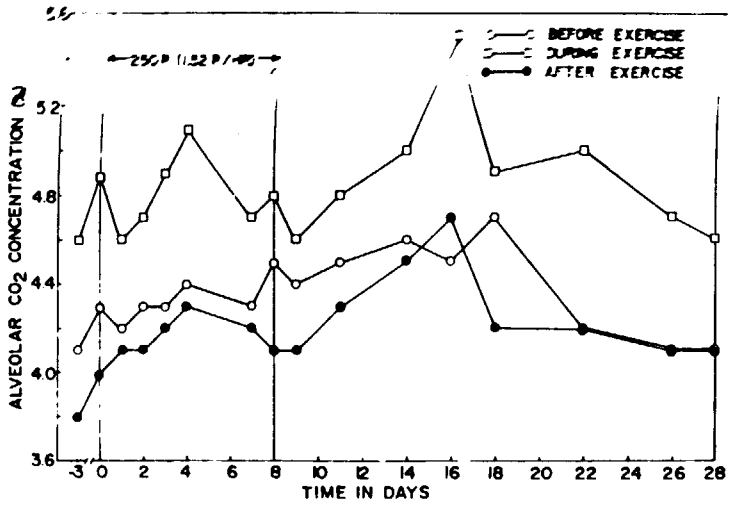


Fig. 13 Alveolar CO_2 levels measured before, during, and after TBI and controlled exercise stress in a patient exposed to 210 R. The after-exercise CO_2 values were averaged over a 25-min postexercise period.

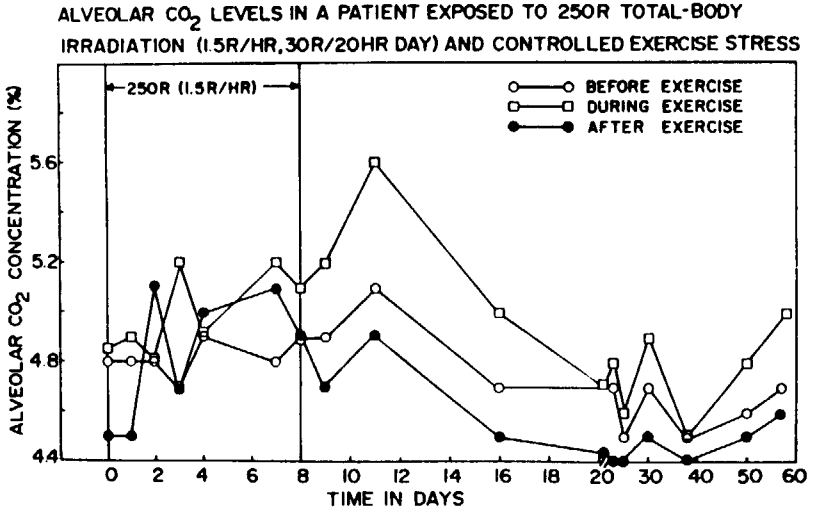


Fig. 14 Alveolar CO_2 levels measured before, during, and after TBI and controlled exercise stress in a patient exposed to 210 R. The after-exercise CO_2 values were averaged over a 25-min postexercise period.

1028229

both patients the expired CO_2 concentration prior to exercise stress (basal level) seemed to predict the type of response seen during and for a 20-min period following exercise although a 20% rise in CO_2 occurred during exercise above basal values of expired CO_2. The basal CO_2 levels themselves rose ~25% and 12% respectively in these patients exposed to 250 R TBI. These results suggest that the metabolic level of the irradiated patient may be altered soon after irradiation therapy begins. This change affects the patient's response to, and recovery from, submaximal exercise stress loads. In addition, the threshold for this change in basal CO_2, concentration (metabolic rate?) may not be produced by radiation alone but by radiation and some time-related low-level exercise stress. This preliminary conclusion stems from data obtained in studying an irradiated, nonexercise-stressed patient (Fig. 15) where only about a 5% increase in CO_2 level was found to occur during irradiation stress alone. These results indicate also that alterations in alveolar CO_2 during exercise in the irradiated patient may be a sensitive means of demonstrating radiation-induced performance decrement. Oxygen consumption rate, however, is an even more precise indicator of the energy cost of exercise, and so we plan to incorporate simultaneous measurement of O_2 consumption rates in our studies early in 1974. Such O_2 consumption curves will be used to measure the energy costs of well-defined work levels and provide further insight into the underlying mechanisms affecting muscular work in irradiated man. By power-spectral analysis of pulmonary impedance and O_2/CO_2 studies, we plan to test the hypothesis that pulmonary alteration (previously described in ORAU-113, -116, -122) can be reversible or irreversible. Increased pulmonary impedance variance could result from increased pulmonary ventilation without any significant change occurring in CO_2 consumption rates. We need to know whether radiation-induced changes in pulmonary impedance, seen as pulmonary impedance baseline shifts, are always reversible. Conceivably, irreversible anatomical or biochemical changes could cause permanent baseline alteration in pulmonary impedance. At the same time, however, radiation-induced systemic changes could be either reversible (transient enzyme blockage, CPK adenosine triphosphate ATP , etc.) or irreversible (muscular atrophy, collagen, deposition, decreased muscular contractibility). Such

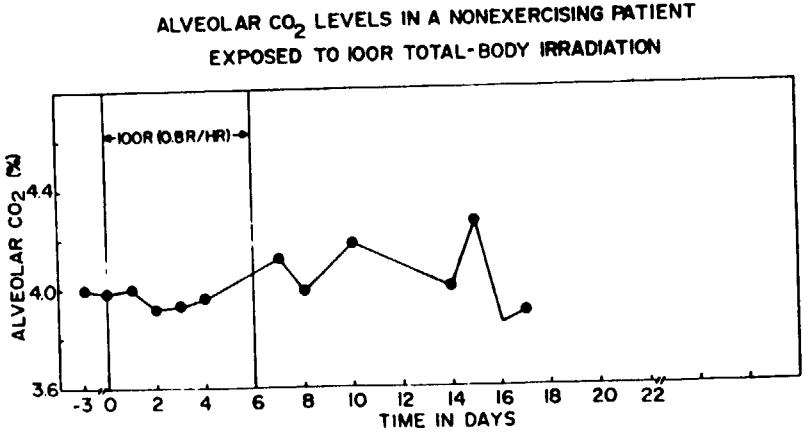


Fig. 15 Representative alveolar CO_2 levels averaged over a 30-min period in a nonexercise-stressed patient exposed to 100 R TBI.

changes we believe would result not only in pulmonary impedance variance shifts but also in altered (increased) O_2 consumption rates that could be demonstrated by our presently well-defined, controlled exercise protocols.

Previously we reported significant increases in circulating levels of CPK in unexercised patients who received total-body exposures up to 150 R and in normal (unirradiated) volunteers subjected to controlled exercise stress. During the past year CPK data were obtained on six additional patients irradiated with exposures up to 150 R. Two-thirds of them demonstrated significant rises in CPK during irradiation therapy that were comparable to data reported in the current literature for CPK response to exercise and in muscular dystrophy. In one of two patients who received 250 R at 1.5 R/hr, CPK levels rose significantly. This patient developed increased CPK after initiation of irradiation therapy (Fig. 16) similar to that seen in patients receiving smaller (100 to 150 R) total-body exposures. A similar rise in serum CPK was noted in a patient who received an average bone-marrow dose of 500 rads prior to bone-marrow transplantation (Fig. 17). Both patients who received these higher doses demonstrated a secondary rise in enzyme level 15 to 25 days postirradiation. This secondary peak has not been previously noted except in some patients receiving 150 R fractionated therapy in the METBI facility. Creatine phosphokinase studies in laboratory mice (Fig. 18) which received 450 R (3.35 R/min) also revealed a secondary peak in CPK when it measured over a 2-week period. The initial increase of CPK in the irradiated mouse is similar to that seen in man; a 2- to 3-fold increase within 2 days following irradiation.

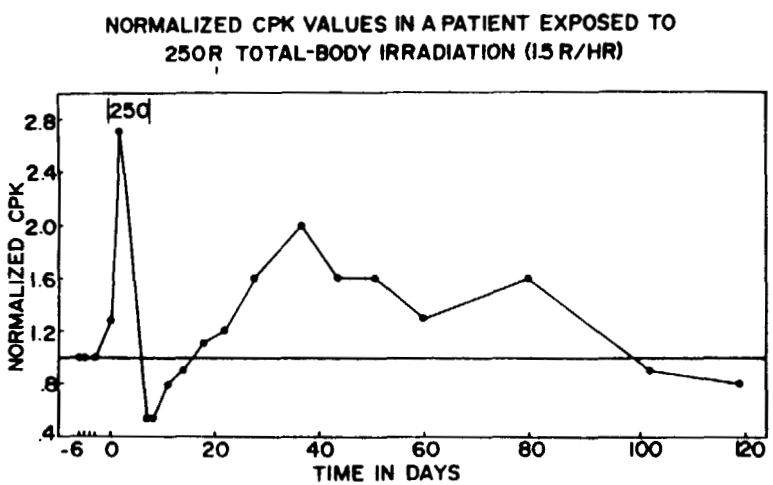


Fig. 16 CPK values obtained from a patient exposed to 250 R TBI.

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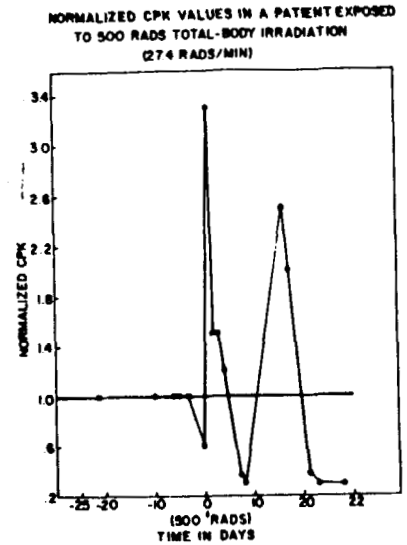


Fig. 17 CPK values obtained from a patient exposed to 500 rads prior to bone-marrow transplant.

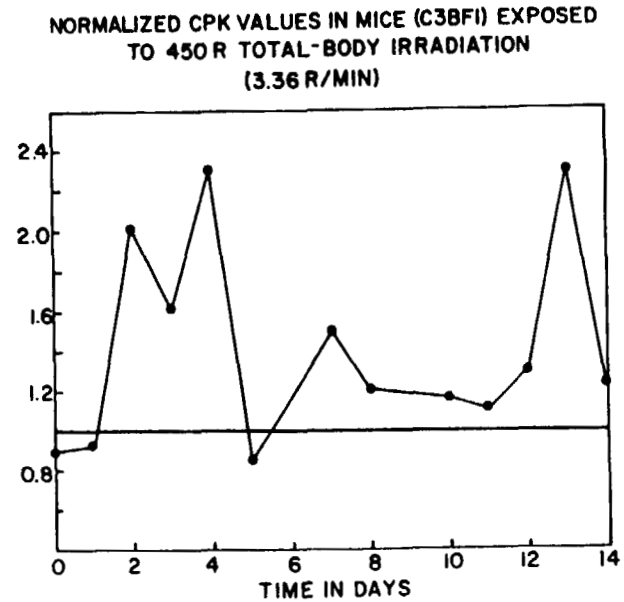


Fig. 18 CPK values obtained from C3BF1 mice exposed to 450 R TBI.

Effect of Exercise Preconditioning on the Response of Trained Ponies to Controlled Exercise Stress and TBI

We have previously demonstrated (ORAU-122, pp 33-34) that power-spectral analysis of pulmonary impedance can be used to measure human pulmonary changes during controlled ergometric stress. When measured during exercise stress, pulmonary impedance gradually decreases over a 1-month test period to ~1/3 its initial value in normal volunteers participating in controlled, repetitive exercise protocols. A similar response has been noted in irradiated patients participating in controlled exercise studies, but the gradual reduction in pulmonary impedance appears to be interrupted by irradiation in a dose-rate-related manner. Patients receiving radiation therapy, but no exercise stress, demonstrate little day-to-day variation in pulmonary impedance when measured daily over 2 weeks and total exposures up to 250 R. The possibility exists, however, that leukemic patients respond to exercise stress in a manner different from normal, nonleukemic volunteers. All participants in these studies were of average physical condition (no regular physical training).

Many physiologic studies in the past involving man have demonstrated that in the event of unusual environmental stress (e.g., zero G, total bed rest) the exercise-conditioned man demonstrates a greater loss of exercise tolerance (more deconditioning) than the untrained individual. This phenomenon has been seen in NASA astronauts who demonstrated various levels of severe deconditioning upon return to earth's gravity following extended manned-spacecraft missions.

A collaborative study at UT-AEC Comparative Animal Research Laboratory (CARL) (Robert C. Ricks, Dan Brown*, Ann Stipe, C. C. Lushbaugh) was undertaken to determine in a large experimental animal the effect of preconditioning exercises on the expected changes in pulmonary impedance waveform. Irradiated and nonirradiated Shetland ponies subjected to controlled exercise stress were used. These 12 ponies had been studied over a 6-year period beginning in 1966 to determine the effect of a prompt radiation exposure upon exercise tolerance and were scheduled now to be killed. In the previous study, six ponies received 650 R TBI exposures of gamma radiation and six served as unirradiated controls; their pulmonary and cardiac rates and rectal temperature were measured and used to evaluate physiologic response to exercise and temperature stress. The results showed only that irradiated ponies did not dissipate body temperature normally when worked in hot environments (>85°) and no other well-defined physiologic effects of irradiation could be demonstrated.

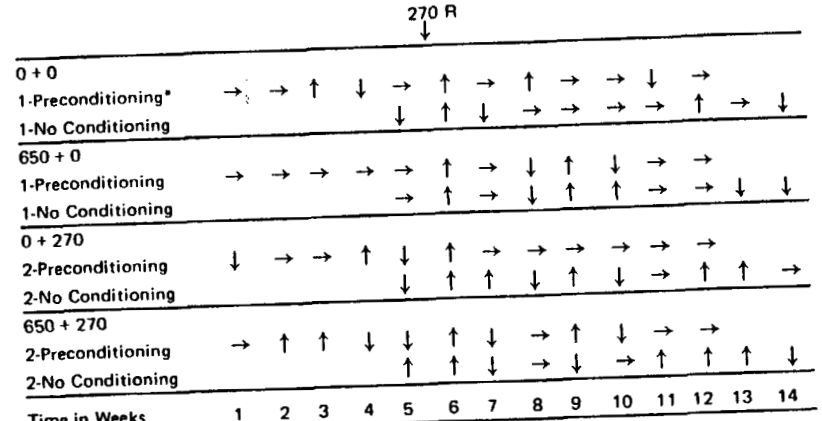
In the current study we exposed eight of these ponies to 270 R at 30 R/day. The ponies were paired according to the following scheme, where the first number indicates prior irradiation and the second number the exposure received in the current study: 0 + 0, 650 + 0, 0 + 270, 650 + 270. In addition, six of the 12 ponies were exercise conditioned for 6 weeks prior to irradiation (current study), the other six exercised only one week prior to irradiation. The exercise consisted of pulling a brake-loaded cart and is described later. Creatine phosphokinase data from these ponies are shown in Table 10 and, while quite variable, demonstrate again (as seen in our human studies) that irradiation and/or exercise stress result in increases in CPK levels. Animals that had received 650 R previously (with or without additional 250 R or no additional irradiation or exercise conditioning) demonstrated the greatest change in CPK response, suggesting that some permanent biochemical lesion had resulted. The possible significance of this change is discussed later in relation to changes in pulmonary impedance variance in this group of ponies.

*UT-AEC Comparative Animal Research Laboratory

1028231

TABLE 10

CREATINE PHOSPHOKINASE (CPK) LEVELS IN PONIES EXPOSED TO 270 R TOTAL-BODY GAMMA IRRADIATION



* Number of animals in each respective treatment.

† Symbol interpretation.

↓ = < ½ previous week's level

→ = steady state

↑ = > 2 X previous week's level

To measure the effects of exercise conditioning prior to irradiation and its effect on performance decrement, two of four teams of ponies were preconditioned 6 weeks prior to irradiation (270 R); the other two teams exercised under controlled conditions only 1 week prior to irradiation. One team of controls was exercise conditioned, the other was not.

Controlled exercise testing was accomplished using matched, trained teams of ponies accustomed to pulling a brake-loaded cart around a circular track. Each pony worked against a workload equal to a resistance of 3/4 horsepower for 30 min each human work-day (M-F) for constant periods of time. In the preconditioned group of ponies, the total work period studied was 5 weeks prior to and 6 weeks after irradiation; in the nonconditioned ponies, it was 1 week prior to and 6 weeks after irradiation.

In all ponies that participated in this study, the pulmonary-impedance variance data were similar to that seen in the normal man when subjected to controlled exercise testing. There was a general trend for the pulmonary-impedance variance to decrease (or remain relatively constant) over the conditioning period. In preconditioned ponies this gradual reduction in pulmonary-impedance variance was interrupted by the onset of TBI (30 R/15-hr day), and was reflected by an increase in pulmonary-impedance variance. This increased variance in irradiated, preconditioned ponies was evident for 3 to 4 weeks post-

irradiation after which the variance values began to decrease. However, a similar shift in the pulmonary-impedance variance was noted in a preconditioned control pony that was forced to stand in its stall for 16 hrs/day to simulate the exercise restriction of the ponies during their irradiation. This result suggests that the mechanism for irradiation-induced fatigue may be similar to that physiologic response induced by the prevention of supine rest (16-hr duration) in the pony.

When compared to preconditioned ponies, pulmonary-impedance variance of nonpreconditioned ponies did not appear to be as severely affected by TBI. Indeed, nonpreconditioned ponies did not, as a group, demonstrate an increased pulmonary-impedance variance postirradiation, with the exception of one animal that showed significantly increased variance during the 9-day irradiation period. As in the preconditioned control pony, nonpreconditioned controls demonstrated increased pulmonary-impedance variance in response to standing 16 hrs/day.

No definite correlations were found in pulmonary-impedance variance data of these ponies that would indicate that a permanent physiologic lesion was present as a result of the 650 R exposure 5 years prior to the current study (270 R). The results do, however, suggest that exercise preconditioning may lower the radiation threshold for increased fatigability. This influence of preconditioning may be important in light of the demanding preflight conditioning and the phenomenon of postflight cardiovascular deconditioning noted in NASA manned-space-flight personnel.

At the end of the study all ponies were killed and necropsied (C. C. Lushbaugh, R. C. Ricks, J. L. West*, and G. Humason) to permit evaluation of the extent of any vascular radiation effects. Specimens of lungs, kidneys, testes, and skin were studied histologically for this purpose. Lung and kidney were chosen to study late radiation effects, and testes to evaluate the levels of acute damage and subsequent repair of the effects of the two exposures. The skin was studied to determine whether Brown's observation that irradiated ponies were relatively heat intolerant during exercise might be related to damage induced in sweat-gland structure.

Objectivity was obtained in this histologic study by coding all slide preparations so that individual ponies and their irradiation histories were not identifiable. After all the histologic appraisals were finished, a diagnostic interpretation was made as to whether or not the changes seen were related to any irradiation, 650 R only, 270 R only, 3 months or 6 weeks prior to necropsy, and 270 R in combination with the previous 650 R exposure. This appraisal and its interpretation were then decoded and compared with the actual histories of the ponies. The results of this study, in summary, were as follows.

Changes seen in pulmonary alveolar-wall collagen and glomerular and arteriolar vasculature did not correlate with either the degree of change in individual physiologic response to exercise or with the irradiation history. In the one pony in which the histologic changes in lung and kidney were extreme and obviously due to radiation, the animal could not be tested physiologically because he was lame. Remarkably, only three instances of unquestionable pulmonary and late renal vascular effects related to the irradiation experience of the animal were found. In the kidneys of most ponies the relation of vascular changes to irradiation was obscured by the presence of changes typical of intercurrent or chronic interstitial nephritis or chronic pyelonephritis. In the lungs, the unfortunate presence of chronic bronchiolar inflammation and peribronchial lymphoid infiltration in most ponies made evaluation of minimal degrees of alveolar sclerosis impossible.

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1028232

The histologic appraisal of the changes in the testes, however, correlated directly with the irradiation histories. The ponies that had received only 650 R exposure had prominent foci of regeneration of testicular tubular germinal epithelium scattered among severely atrophic tubules. Those that had received a recent second exposure of 270 R, in addition to the 650 R, had lost these regenerative foci, while those with no previous exposure and only a recent 270 R exposure showed germinal epithelial atrophy without accompanying tubular sclerosis. The unirradiated ponies had histologically normal testes. Of purely histologic interest, one pony had an adenomatous focal epithelial change that could be interpreted as being neoplastic, and several others had such an extreme focal increase in interstitial cells (Leydig) that doubt was cast on the validity for this species of the widely held radiopathological concept that the histologic increase in these cells after irradiation is apparent and not real. This increase in Leydig cells was most extreme in those animals that received the smaller more recent exposures.

Although the sections of skin were all taken from the same anatomical locus in all animals, the number of sweat glands in them was highly variable and did not correlate with radiation history.

The negative results of this histologic study strengthened our belief that the changes we have been finding in the exercise tolerance of irradiated men reflect reversible metabolic rather than irreversible anatomical changes.

Role of the Spleen in Continuous TBI in Mice

Our studies (T. R. Stokes and C. C. Lushbaugh) on humn hematologic responses to low-dose-rate irradiation have been paralleled by experimental studies in the LETBI facility in which several mouse strains are exposed to similar levels of radiation during their entire life times. Preliminary results from studies in progress have been reported in previous ORAU progress reports. These have shown that at the 1.5 R/hr rate previously used for radiotherapy (LETBI-I) most strains of mice can survive for a significantly major part of their normal life spans. At the new (LETBI-II) rate of 0.8 R/hr, survival is so long (~700 days) that we do not yet know how much life-shortening is produced in the various strains. Our data suggest so far that at some rate between these two dose rates, the mechanism changes from hematopoietic damage that results in a fatal anemia and pancytopenia to some other more easily reparable marrow damage that reveals itself terminally as a myeloproliferation in which excessive hematopoietic cell numbers are produced as in human chronic leukemias. In both syndromes, the spleen, as well as the marrow, seems to be a target organ. Purposeful cellular responses by it seem necessary for survival at exposure rates of 1.5 R/hr and greater rates but until this year our evidence to prove splenic response was important was largely based on inferences drawn from studies of changes in peripheral blood cell numbers and histopathologic studies of the spleens and marrow of animals killed at various times during their irradiation.

Because the rodent spleen has a tremendous capacity to increase its hematopoietic cell output, its role in the survival of mice and rats irradiated acutely (single large exposures) has been extensively studied. Although it was thought that removal of the spleen would hinder recovery of the blood-forming system, several investigators have shown that

splenectomy not only does not reduce the acute $LD_{50/30}$ of rodents, but in some studies has been radioprotective. This is apparently due to the removal of the source of a splenic factor that inhibits bone-marrow mitotic activity necessary for recovery.

The importance of the spleen to survival of exposures to continuous irradiation has not been previously determined in mice. Several studies have been made elsewhere on adult rats given 50 to 85 R/day continuously up to total exposures of 4000 R. As in our mice, intact irradiated rats develop a massive splenic erythroid hyperplasia and marrow cellularity becomes progressively reduced. Because the postirradiation hematopoietic recovery patterns of intact and splenetomized rats are similar, the role of the spleen in tolerance to prolonged continuous irradiation was not definable. In another similar rat study, a difference was found in the recovery patterns of the mononuclears and platelets. The platelets response seemed to be improved by splenectomy and resulted in a greater mean-after-survival time when irradiation was at 84 rads/day. This increased survival was attributed to enhanced maintenance of the platelet counts in the splenectomized rats.

In our studies on the survival mechanisms of C57BL/6 male mice under continuous irradiation, sufficient numbers of splenectomized mice have now been irradiated at 0.8 R-hr, 1.6 R/hr, and 3.2 R/hr for statistically significant results. Daily exposures have been at 18.5, 37.0, and 70.0 R/day, respectively. Splenectomies were performed when the mice were ~120 days old and irradiation started 30 days after surgery. In addition to mortality experiments where all animals are exposed until death, serial-sacrifice studies were performed on mice irradiated at 1.6 and 3.2 R/hr. These studies included measurement of ^{59}Fe-ferrokinetics, WBC and RBC counts, hematocrit, and hemoglobin. Platelet counts and WBC differentials were not made. Results of the mortality experiments are shown in Fig. 19. At the highest exposure rate (3.2 R/hr) there was no different mean-after-survival (MAS) between splenectomized (MAS ~58 days) and intact irradiated mice (MAS ~58 days also) of similar age. A significant difference in survival was seen during the 1.6 R/hr

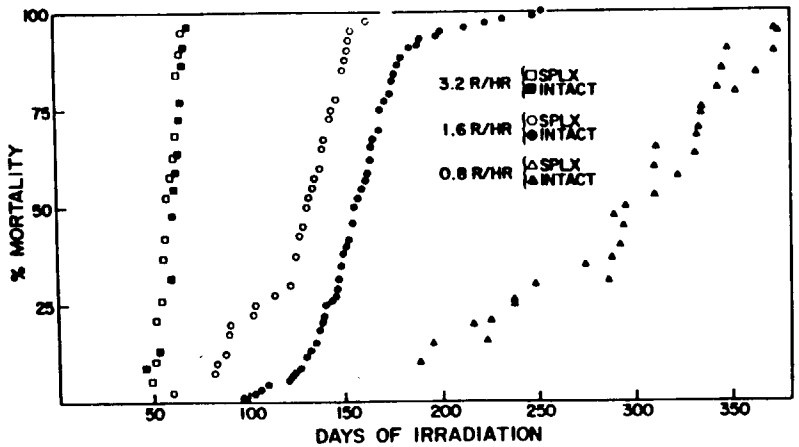


Fig. 19 Cumulative percent mortality of splenectomized and intact C57BL/6 male mice irradiated continuously (~23 hr/day) at 0.8 R/hr, 1.6 R/hr or 3.2 R/hr.

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exposures. The MAS (128 days) of the splenectomized mice was only 83% of that (155 days) of intact irradiated controls. Repetition of this experiment has confirmed this depression in survival rate. The MAS of the splenectomized mice in the second study was only 79% of that of intact mice. At our lowest exposure rate (0.8 R/hr) we found no significant difference in the survival times of intact (MAS ~293 days) and splenectomized mice (MAS 298 days).

Thus, the radioprotective effect described after splenectomy and acute irradiation in mice and after splenectomy and continuous irradiation in rats was not found in our study using continuously irradiated C57BL/6 mice. To the contrary, splenectomy was detrimental to those irradiated at the 1.6 R/hr rate, which is the rate that produces the greatest splenic erythroblastic response. We are analyzing the histologic data to explain the inconsistency of the detrimental effect of splenectomy on the tolerance of mice for continous radiation. Terminal histopathology of the mice in the mortality experiment done this year at 1.6 R/hr consists of an almost complete depletion of all hematopoietic elements in marrow of the splenectomized mice. Since the marrows of the intact mice contain myeloid and erythroid foci and the spleens of these mice often have significant hematopoietic foci, it is possible that the spleen is needed to reseed the marrow. The results obtained in the serial sacrifice experiments showed that the alterations of the peripheral RBC and WBC populations of the splenectomized mice coincide, both in time and magnitude, with those of their analogous intact irradiated controls at both 1.6 and 3.2 R/hr, so we are unable to quantitate any effect of splenectomy on hematopoietic regenerative capacity in usual hematologic terms. We do not have data on platelet levels in these mice but our marrow preparations are being studied for differences in megakaryocyte numbers since Lamerton has proposed that the increased longevity of his splenectomized rats was due to better platelet production. Perhaps the reverse may be true for mice and the dose rate at which this system is most critical is at ~1.6 R/hr (37 R/day).

Radiation Immunology and Marrow Transplantation

Therapeutic Allogeneic Bone-Marrow Transplantation

Transplantation of allogeneic bone-marrow was attempted in a fourth patient as treatment of end-stage acute myelogenous leukemia (N. Gengozian, C. L. Edwards, Helen Vodopick, and K. F. Hübner). The immunosuppression regimen used to prepare the recipient was that described in ORAU-113 subsequently amended with approval of the ORAU/ORNL Committee on Human Studies, with an increased whole-body radiation dose of 500 rads (694 R midline, in air dose).

The patient, a 54-year-old man, found to have acute myelogenous leukemia in October 1971, was treated initially at ORAU with combinations of chemotherapeutic drugs. After a prolonged induction phase his leukemia eventually went into remission in January 1972. This remission was maintained with methotrexate until March 1973, when there was a significant drop in his platelet count and the number of blasts in his bone marrow was found to be 30%. He was then readmitted and induction chemotherapy was restarted while preparations were being made for the marrow transplant.

The patient's only brother volunteered to serve as a marrow donor. HL-A typing indicated an "A" match, although the mixed lymphocyte culture test for histocompatibility showed an equivocal patient-vs-donor reactivity and a slightly greater donor-vs-patient reaction. While continuing the chemotherapy (vincristine, prednisone, cyclophosphamide, and cytosine arabinoside) the patient was transferred to the laminar-flow clean rooms, prepared with prophylactic antibiotics, and started on the immunosuppressive regimen. He received 500 mg of horse antihuman thymocyte globulin (H-AHTGG) intravenously daily for 8 days. Although each dose was filtered through a 0.22 micron pore-size filter and his skin test for sensitivity to horse serum had been negative, with each infusion of H-AHTGG he experienced severe fever and chills, and after the eighth infusion he developed urticaria. On day 9 (April 9, 1973) he was irradiated at the UT-AEC CARL Variable-Dose-Rate Irradiation Facility (VDRIF), for an exposure of 694 R (500 rads) at 38 R/min. During the radiation he experienced no ill effects, but within 40 min he had a severe chill, his temperature went up to 39°C, he became flushed, and his pulse rate increased to 184/min with electrocardiographic evidence of a rapid atrial fibrillation. This was controlled with intravenous digoxin and by 3 hr postirradiation his condition had stabilized. He experienced no nausea or vomiting after the radiation, perhaps because of the prophylactic chlorpromazine given prior to the radiation.

On the next day (April 10, 1973) he was given 12.07×10^9 bone-marrow cells from his brother. The total volume was 478 ml of marrow suspended in 552 ml of heparinized tissue-culture fluid (TC-199). Within 24 hr, a macular erythema developed over the trunk and extremities, along with a slight generalized edema of the skin and subcutaneous tissues. This was followed by diarrhea approximately 48 hr after the marrow infusion.

Methotrexate 10 mg/m^2 body surface was given intravenously on days 2, 4, 7, and 11, and then weekly, in an effort to avoid serious graft-versus-host (GvH) complications. Nevertheless the dermatitis, diarrhea, and low-grade fever persisted and on April 19 (9 days after infusion of marrow) a skin biopsy showed changes compatible with GvH disease. The skin gradually took on a texture of sandpaper with confluent petechiae appearing over the chest, abdomen, and proximal extremities (Fig. 20). The rash and diarrhea temporarily improved after prednisone (Fig. 21) was started, but later it recurred much more severely as the generalized maculopapular eruption progressed to a generalized erythroderma and then exfoliation (Fig. 22).

The platelet count, which had been suppressed to levels of less than 10,000/mm^3 prior to the radiation, never recovered. Likewise, there was never a reticulocytosis after the transplant and donor-type red cells were never detected above background levels, clearly indicating a failure to achieve an erythrocyte graft. The white blood cell (WBC) count did gradually increase after about 30 days posttransplant, but this increase was comprised chiefly of lymphocytes. A macrophage inhibition test in the presence of mumps antigen suggested that these lymphocytes were from the donor, as the recipient lymphocytes showed no macrophage inhibition in the presence of mumps antigen prior to the transplant.

The patient's general condition gradually deteriorated with hypoproteinemia and inanition. He developed a spontaneous pneumothorax requiring intubation to expand the lung. Despite re-expansion of the lung, his respiratory problems increased until his death on June 16, 67 days after infusion of marrow cells. In addition to the generalized exfoliative dermatitis, autopsy revealed bilateral patchy bronchopneumonia, a pulmonary abscess, diffuse cerebral edema, but no evidence of recurrence of the leukemia.

This case differed from the three earlier cases in significant ways (Table 11). Neither

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Fig. 20 Marrow-transplant patient showing skin changes of GvH on 14th day. The picture shows confluent petechiae over the pectoralis region of the chest and deltoid area of the forearms with a wider distribution of erythematous macules and petechiae.



Fig. 21 Marrow-transplant patient at day 22 showing the temporary improvement but persistent maculopapular eruption.

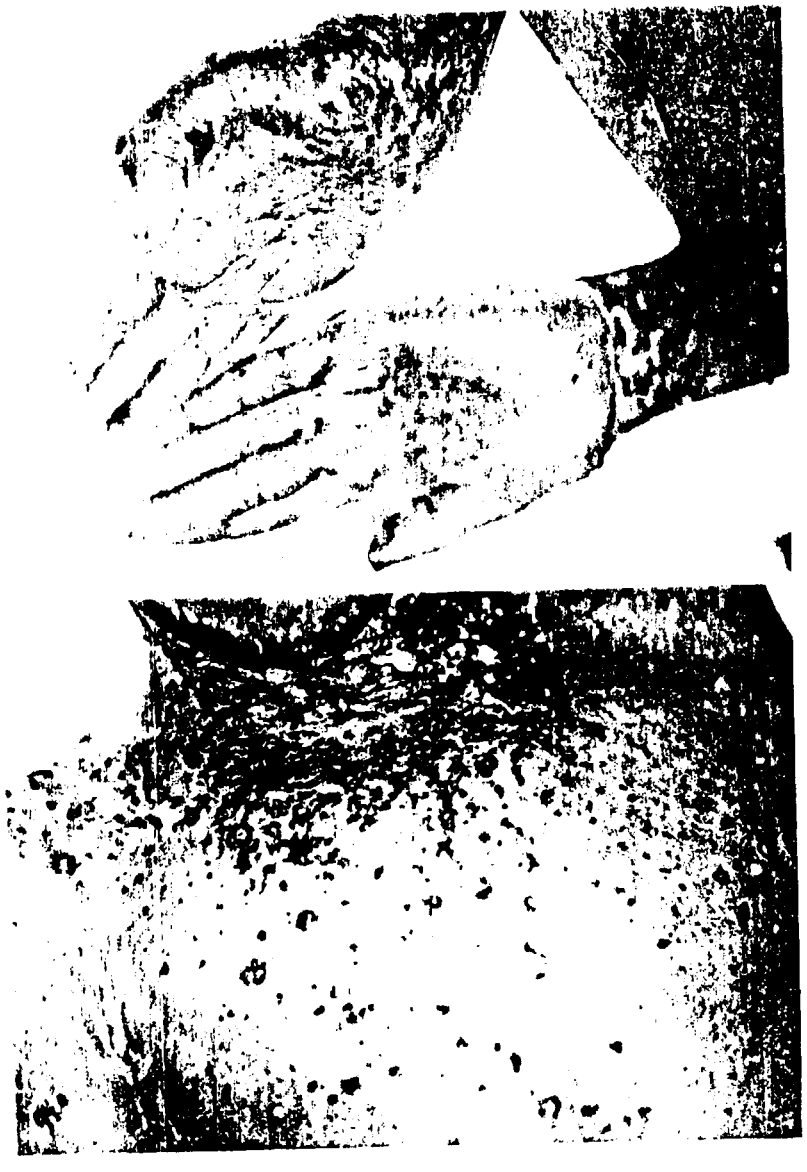


Fig. 22 Marrow-transplant patient on day 56:

(a.) Hands of the marrow-transplant patient showing exfoliation.

(b.) Chest showing diffuse erythema, exfoliation, and encrusted serum that had exuded from the denuded areas.

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TABLE 11

	No. 1	No. 2	No. 3	No. 4
Age	52	53	41	54
Sex	M	F	M	M
Diagnosis	AML	AML	AML	AML
Previous remission	7 mo.	3 mo.	No	14 mo.
Blasts in blood at time of transplant	No	No	Yes	No
Antilymphocyte globulin	500 mg/d H-AHTGG	500 mg/d G-AHLG†	500 mg/d H-AHTGG	500 mg/d H-AHTGG*
Radiation dose	370 rads	370 rads	370 rads	500 rads
Steroids during infusion	Yes	Yes	Yes	No
Donor–Sex	M	M	M	M
Donor–Age	40	55	34	52
H-LA Match	A	A	D	A
MLC donor-vs-recipient	Neg	Neg	+	±
MLC recipient-vs-donor	+	Neg	±	±
Number of marrow cells infused	18×10^9	15×10^9	18×10^9	12×10^9
MTX after infusion	40 mg p.o. day 2, 30 mg day 4, 7, 11§	40 mg p.o. day 2, 4, 20 mg day 7, 11	35 mg p.o. day 2, 4 7 and 11	19 mg i.v. on day 2, 4, 7, 11‡
RBC graft	Yes	Yes	No	No
RBC marker	Yes	Yes	Yes	Yes
Granulocyte graft	Yes	Yes	No	No
Lymphocyte graft: T-cells	?	No	?	Yes
B-cells	Transient	Yes	No	?
Platelet graft	Yes	Yes	No	No
Survival posttransplant (days)	122	92	60	67
Leukemia at death	No	Yes	Yes	No

* Horse antihuman thymocyte gamma globulin.
† Goat antihuman lymphoblast globulin.
‡ 10 mg/m^2 meter body-surface area.
§ 0.5 mg/kg body weight.

of the previous patients showed significant GvH reaction nor evidence of a lymphocyte (T-cell) graft. This patient received 694 R total-body irradiation compared to 500 R for each of the other patients. Smaller doses of methotrexate were used for immunosuppression after the transplant in this patient. He received 10 mg/m^2 of body surface (19 mg) intravenously instead of 0.5 mg/kg (35 mg) orally on days 2, 4, 7, and 11. In this patient we failed to achieve either an erythrocyte or granulocyte graft such as was achieved in the earlier two patients with "A" matched donors. The higher radiation dose was expected to result in greater immunosuppression of the patient and presumably would have increased the chance of getting a permanent hematopoietic graft. Thus, our failure to get a successful graft would seem to be due to the GvH reaction. Why this patient had such a severe GvH reaction while the others had none is not clear. The smaller dose of methotrexate may have been inadequate to prevent GvH disease. Whether this was a critical factor and a larger dose would have prevented the GvH reaction, or even significantly ameliorated it, remains uncertain. Also, what effect the prednisone the other patients were receiving at the time of the transplant may have had on suppressing GvH or preventing a lymphocyte graft is not certain. A question raised by the previous three patients in whom we saw no evidence for GvH disease or even a lymphocyte graft was whether this immunosuppression regimen prevented T-cell grafts and therefore also prevented GvH disease. This patient's course clearly shows that that is not the case.

Variation in T- And B-Cell Deficiency in Radiation Chimeras

Previously we (38) had demonstrated that immunologically incompetent spleen cells from long-term, allogeneic radiation chimeras ($CD2F_1$ bone marrow had been given intravenously to lethally X-irradiated $C3BF_1$ hosts to produce CD chimeras) could be reconstituted in vitro by T cells taken from the thymus of normal host-type mice (allogeneic to donor-type spleen cells of the chimera) but could not be reconstituted by T cells from the thymus of normal donor-type mice (syngeneic to spleen cells of the chimera), or by bone-marrow (B) cells. This finding showed that these chimeras are deficient primarily in the T-cell population and suggested that reconstitution was mediated through an allogeneic effect. If syngeneic cooperation between T cells from the thymus of normal mice (NTC) and the B cells of the radiation chimera cannot take place it is possible that a reason for this lack of cooperation is that the supplied donor-type NTC (syngeneic to B cells of the chimera) may become involved in the maintenance of tolerance in these chimeras. However, it has been well documented by other investigators that T cells cannot cooperate with syngeneic B cells in vitro unless the T cells have been previously activated with specific antigen in vivo. Therefore, our current investigation (P. Urso and N. Gengozian) was directed toward achieving cooperation between donor-type (syngeneic to donor-type cells of the chimera) T cells that had been activated by sheep red blood cells (SRBC) in vivo and the B cells of the chimera. In addition, if reconstitution by an allogeneic effect is characteristic in chimeras, immune competence should also be restored to incompetent spleen cells from radiation chimeras in which $C3BF_1$ bone marrow is given to lethally X-irradiated $CD2F_1$ hosts (C3 chimeras) by T cells from host-type mice ($CD2F_1$) provided that T- but not B-cell deficiency exists in the donor-type ($C3BF_1$) lymphocytes of this combination.

Activated T cells (ATC) were prepared by injecting intravenously immediately after irradiation 30 to 140 $\times 10^6$ thymus cells mixed with 7.5 $\times 10^7$ SRBC into lethally X-irradiated (950 R) syngeneic recipients. Seven or 8 days later 1 to 10 $\times 10^6$ cells from the spleens of these mice were cultured in vitro with 7 to 10 $\times 10^6$ spleen cells from CD or C3 chimeras, in the presence of SRBC and the plaque (anti-SRBC)-forming cell (PFC) response was determined 4 or 5 days after culture. Table 12 shows the results obtained. As can be seen, allogeneic ATC increased the PFC response 17-fold in spleen cells from 11 out of 12 CD chimeras; on the other hand, syngeneic ATC induced a response from only three out of 13 chimeras, and the response was much weaker (a 3-1/2-fold increase). The effect of ATC in C3 chimeras was strikingly different from that observed in CD chimeras. Thus, allogeneic ATC induced an increase (7- to 10-fold) in PFC from only two out of 16 C3 chimeras while syngeneic ATC induced a similar increase from only three out of 13 chimeras. These results confirm our earlier finding that CD chimeras are deficient primarily in the T-cell population.

Since the PFC response of C3 chimeras could not be appreciably improved by ATC, the cellular defect probably lies in other interacting cells needed for antibody response against SRBC. Therefore, the functional capacity of B cells of C3 chimeras was tested by culturing spleen cells from these chimeras with SRBC and with 1 to 10 $\times 10^6$ B cells obtained from spleens of adult mice previously thymectomized, lethally X-irradiated, and given 40 $\times 10^6$ syngeneic bone-marrow cells intravenously. These B cells were used 1 to 5 weeks after bone-marrow transfer. As can be seen in Table 13, allogeneic B cells induced an increase (6-fold) in PFC from only four out of 19 CD chimeras, while in C3 chimeras allogeneic B cells induced an increase (22-fold) in PFC from eight out of 19 mice.

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TABLE 12

PLAQUE-FORMING CELL (PFC)* RESPONSE OF SPLEEN CELLS FROM ALLOGENEIC CHIMERAS CULTURED IN VITRO WITH ALLOGENEIC OR SYNGENEIC ACTIVATED T CELLS (ATC)†

		Responders			Nonresponders		
Chimera	Type of ATC	No. of mice	$\bar{X}$ PFC	$\bar{X}$ PFC of chimera cells + SRBC only	No. of mice	$\bar{X}$ PFC	$\bar{X}$ PFC of chimera cells + SRBC only
$CD2F_1 \rightarrow$ $C3BF_1$	Allo.	11	1072 ±113	63 ±16	1	72 ±22	67 ±19
	Syn.	3	166 ± 42	47 ±16	10	35 ± 5	80 ±19
$C3BF_1 \rightarrow$ $CD2F_1$	Allo.	2	175 ±19	18 ± 8	8	42 ±14	33 ±10
	Syn.	3	153 ±65	21 ± 9	10	26 ± 5	28 ± 7

* Values are recorded as the number of PFC/10^6 culture cells. Mean ($\bar{X}$) PFC values include standard errors.

† Symbol abbreviations:
Allo. = allogeneic; Syn. = syngeneic; SRBC = sheep red blood cells.

TABLE 13

PLAQUE-FORMING CELL (PFC)* RESPONSE OF SPLEEN CELLS FROM ALLOGENEIC CHIMERAS CULTURED IN VITRO WITH ALLOGENEIC OR SYNGENEIC B CELLS†

		Responders			Nonresponders		
Chimera	Type of ATC	No. of mice	$\bar{X}$ PFC	$\bar{X}$ PFC of chimera cells + SRBC only	No. of mice	$\bar{X}$ PFC	$\bar{X}$ PFC of chimera cells + SRBC only
$CD2F_1 \rightarrow$ $C3BF_1$	Allo.	4	142 ±29	24 ±6	15	60 ±7	102 ±14
	Syn.	0	–	–	17	29 ±5	29 ± 5
$C3BF_1 \rightarrow$ $CD2F_1$	Allo.	8	346 ±106	15 ±4	11	28 ±7	42 ±13
	Syn.	0	–	–	15	6 ±1	30 ± 8

* Values are recorded as the number of PFC/10^6 culture cells. Mean ($\bar{X}$) PFC values include standard errors.

† Symbol abbreviations:
Allo. = allogeneic; Syn. = syngeneic; SRBC = sheep red blood cells.

Syngeneic B cells were ineffective in both types of chimeras. These results show that, in addition to being severely deficient in T cells, CD chimeras are also slightly deficient in B cells; C3 chimeras, on the other hand, appear to be deficient in both T and B cells, but mostly in B cells.

Our data suggest, therefore, that spleen cells from CD chimeras contain primarily functional B cells but few T cells, while those from some C3 chimeras contain functional T cells but few B cells. To test the capacity for T-cell function of these chimeras, cells from their spleens were exposed to specific antitheta (Θ) antiserum and cultured together in the presence of SRBC in vitro. Anti-Θ in the presence of guinea pig complement is known to

specifi. , destroy T cells which bear the Θ antigen. Figure 23 shows that mixtures of untreated spleen cells from these chimeras induced a 27-fold increase in the PFC response, which was completely abrogated with anti-Θ-treated spleen cells from C3 chimeras but not with treated spleen cells from CD chimeras. Thus, spleen cells from C3 chimeras do indeed contain functional T cells. Since our data (Table 13) indicated that C3 chimeras were mostly B-cell deficient, spleen cells from CD chimeras were tested for T-cell function in an allogeneic system where B cells but not T cells are present. Spleen cells from CD chimeras were cultured with spleen cells from normal $C3BF_1$ mice after the normal spleen cells had been treated with anti-Θ. Figure 24 shows that spleen cells of CD chimeras do indeed contain some functional T cells. Mixtures of spleen cells from CD chimeras and normal $C3BF_1$ mice after treatment with anti-Θ induced a 3-1/2-fold increase in PFC by the treated $C3BF_1$ cells.

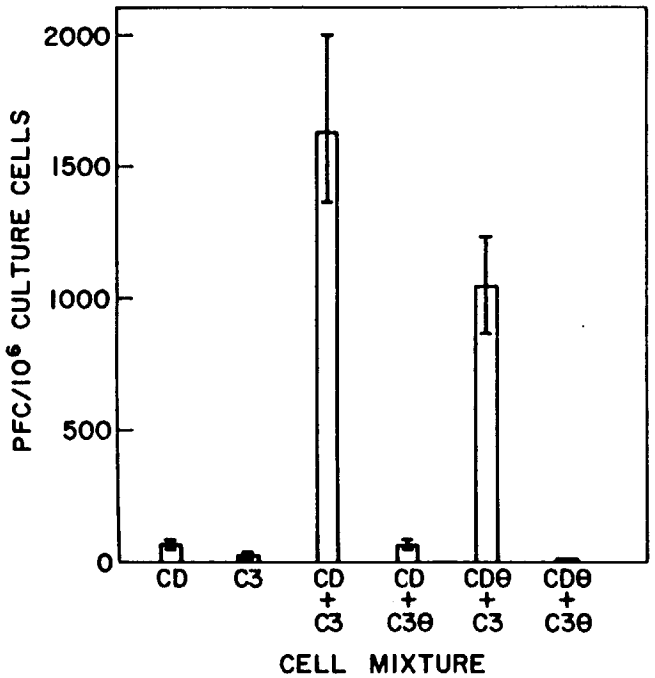


Fig. 23 Antibody response by mixtures of allogeneic chimera spleen cells in vitro.

CD = Spleen cells from $CD2F_1 \rightarrow C3BF_1$ chimeras.

C3 = Spleen cells from $C3BF_1 \rightarrow CD2F_1$ chimeras.

θ refers to treatment of spleen cells with anti-θ antiserum before culturing.

Total number of cells cultured in any one mixture equalled 8×10^6. When allogeneic cells were cultured together the amount cultured for each type was 4×10^6.

Vertical lines indicate standard errors.

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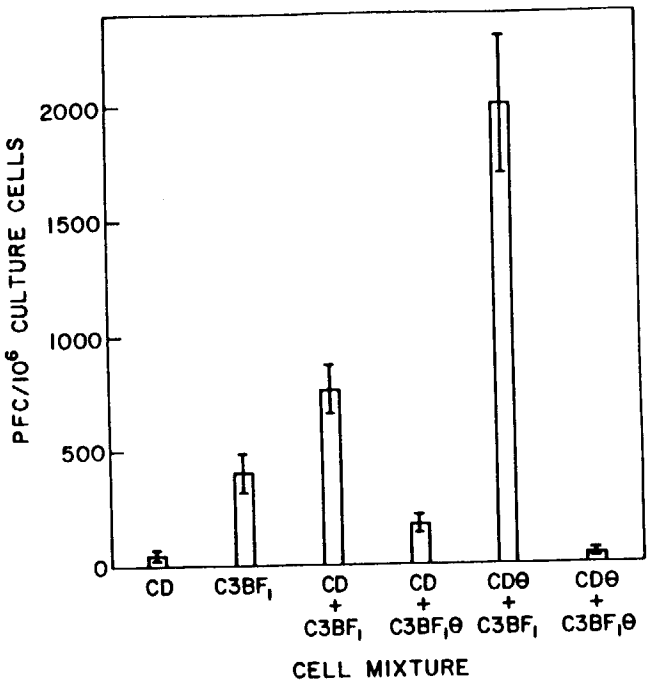


Fig. 24 Antibody response by $CD2F_1 \rightarrow C3BF_1$ chimera spleen cells mixed with normal $C3BF_1$ spleen cells.

CD = Spleen cells from $CD2F_1 \rightarrow C3BF_1$ chimeras.

θ refers to treatment of spleen cells with anti-θ antiserum before culturing.

Total number of cells cultured in any one mixture equalled 8×10^6. When allogeneic cells were cultured together, the amount cultured for each type was 4×10^6.

Vertical lines indicate standard errors.

The data presented here clearly show that, in assessing the status of immunocompetent cells in allogeneic chimeras, the degree of deficiency in T and B cells will vary. Thus, for example, CD chimeras are primarily deficient in T cells but have functional B cells while C3 chimeras are deficient mostly in B cells. The reasons for these cellular defects are unknown, but one possibility is that the severity of GvH reaction dictates the degree of deficiency in both cell populations. The frequency of secondary disease, as measured by cumulative mortality, was greater in B-deficient C3 chimeras (90% of the animals died) than in the T-deficient CD chimeras (50% died). Also, in a previous study using different allogeneic radiation chimeras, the ability to form humoral antibody to a variety of particulate antigens appeared to be related to the degree of secondary disease experienced by each animal prior to antigenic challenge (13).

The data in this report support our contention that restoration of the antibody response in the allogeneic chimera is mediated by an allogeneic effect since the PFC response in both types of chimeras was appreciably enhanced by allogeneic T or B cells but not by syngeneic cells. The failure of syngeneic activated T cells (and B cells) to cooperate effectively with the B cells (or T cells) of the chimera lends credence to our suggestion that exogenous or endogenous syngeneic cells become involved in a feedback mechanism that develops in these animals for the purpose of maintaining tolerance.

Effect of Antilymphocyte Serum Pretreatment on Immunologic Reconstitution of Irradiated Animals

The use of antilymphocyte or antithymocyte serum (ALS, ATS) as a specific depressant of immunologic activity has been reported in a variety of experimental situations. A more recent application of the immunosuppressive qualities of ATS has been realized in the preparation of recipients for transplantation of allogeneic bone marrow, a procedure we have exploited in performing clinical transplants in leukemic patients (E. Allen and N. Gengozian). As reported previously, the use of ATS in conjunction with total-body irradiation has permitted engraftment of foreign bone marrow after exposure of patients to sub- or midlethal levels of irradiation of approximately 375 rads, which is in contrast to the dose of 1000 rads used by other investigators in the absence of any ATS pretreatment (14). In addition to its adjunctive effect in immunosuppression of the recipient, we and others have observed a reduced incidence of a GvH reaction and a slow and often incomplete repopulation of donor lymphocytes in the ATS-treated recipients. How these observations related to the fact that the recipients had been pretreated with ATS has been obscure, because of the frequent inability to detect any circulating ATS in the serum prior to cell infusion. One hypothesis offered to explain the reduced GvH reaction in ATS-treated recipients has been that the ATS covers potential antigenic sites of the recipient and thereby prevents the donor cells from reacting against the host. Since all the foregoing observations have been made in allogeneic grafting situations, we felt the problem could best be approached through a syngeneic-cell-transfer study that would eliminate any GvH activity and allow for an assessment of immunocompetence of the grafted cells against an innocuous antigen.

The experimental design was as follows: Mice were given four daily intraperitoneal injections of rabbit antimouse thymocyte globulin (RAMTG), and then, after varying periods of time, they were exposed to 950 R and injected with snygeneic spleen cells obtained from mice that had been presensitized to sheep red blood cells (SRBC). The effect of any antimouse thymocyte globulin, free in the circulation or bound to cells of the recipient, on the immunologic capacity of the infused syngeneic mouse cells could then be measured by an appropriate assay, which in our model was the detection of antibody plaque-forming cells (PFC) in the spleen of the recipient.

Before performing any spleen-cell transfers it was necessary to determine the rate at which the RAMTG was cleared from the circulation of mice that had received four daily injections totaling 1 ml. Mice so treated were bled at daily intervals and the serum tested for cytotoxic activity against normal mouse thymocytes. Figure 25 shows that 2 days after the last injection, the cytotoxicity was approximately 74%, but within 4 days this dropped to 15% and on day 7 no cytotoxic activity could be detected in the serum of the recipients.

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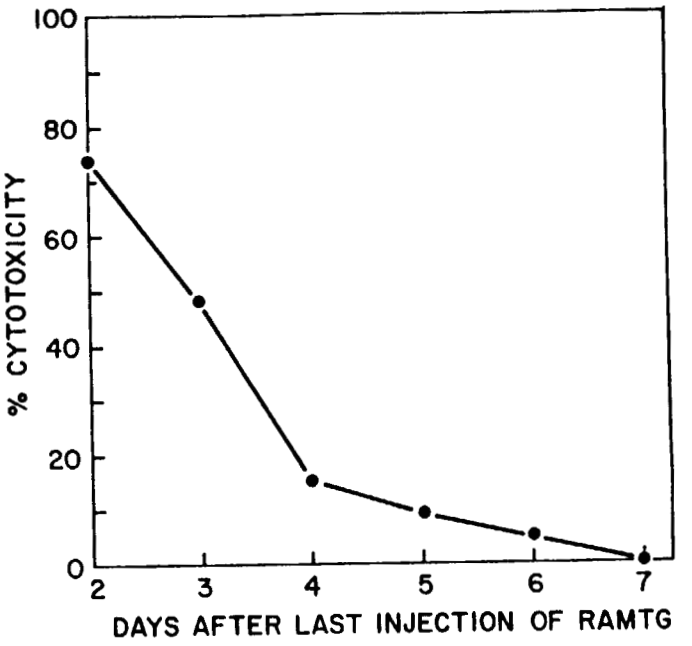


Fig. 25 Rate of clearance of rabbit antimouse thymocyte globulin (RAMTG) in mice as measured by percent cytotoxic antibody to mouse thymocytes after four injections.

To determine the direct and indirect effects of ATG on the transplanted cells, animals receiving RAMTG were tested on two different schedules, the first at a time when large quantities of cytotoxic antibody were still detectable in the recipient, and the second at a time when there was no cytotoxic activity remaining. Therefore, for the first study, the target spleen cells were given to 950 R recipients 2 days after the last injection of RAMTG and the PFC capacity of the spleen measured 6 days later. Controls included mice pretreated with normal rabbit globulin (NRG) or saline. To facilitate analysis of the PFC data, the number of PFC obtained in the saline-treated group was taken as 100% and the values obtained with the NRG and RAMTG-treated groups were taken as fractions of this. Figure 26 shows the PFC assay of these animals when assayed 6 days after transfer. There was a striking reduction in both the 19S and 7S PFC response per spleen in the RAMTG-treated group when compared with the saline-treated controls. The NRG-treated group also was depressed, but not to the extent of the RAMTG animals. The number of 19S PFC per 1×10^6 spleen cells also was reduced; however, the reduction in the number of 7S PFC per 1×10^6 cells was not conclusive because of the large standard error. Analysis of the cellularity and weights of the spleens of the treated animals revealed no significant differences among the groups, suggesting that cellular destruction was not the cause of the marked reduction in PFC found in experimental animals.

A second group of RAMTG-treated recipients was lethally irradiated and injected with syngeneic spleen cells 7 days after completion of RAMTG, a time when no cytotoxic

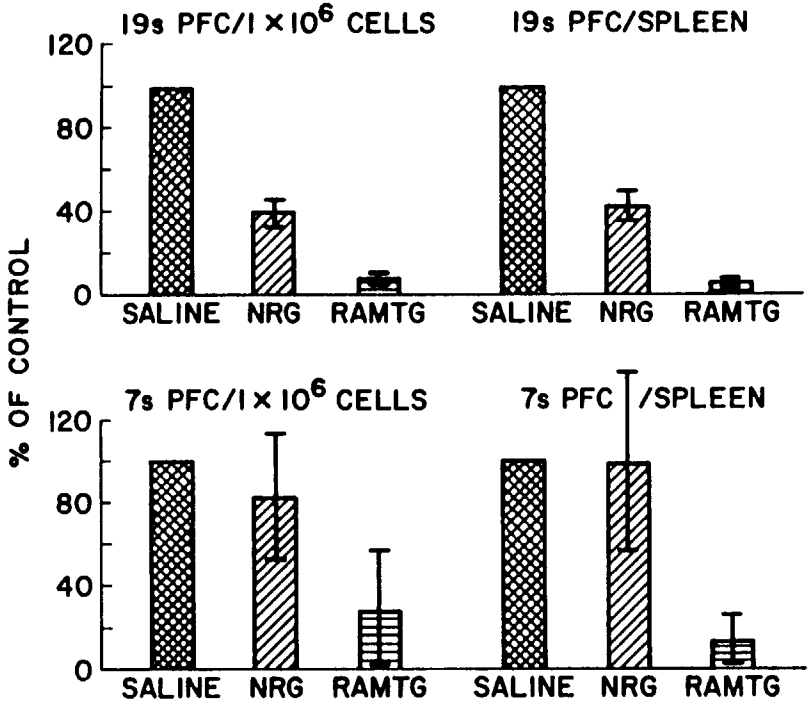


Fig. 26 PFC activity of presensitized spleen cells transferred to saline, normal rabbit globulin (NRG) or rabbit antimouse thymocyte globulin (RAMTG) pretreated recipients. Cells injected 2 days after last dose of RAMTG.

activity could be detected in the serum. When assayed for PFC, both the 19S and 7S responses of the RAMTG pretreated animals were still reduced considerably, relative to the controls, although some recovery is suggested when comparison is made to the data in Fig. 27. Note that, in contrast to the previous experiment, significant suppression was not observed in the NRG-pretreated group in these experiments; indeed, the responses were slightly greater than the saline controls. Similar nonspecific depressing and enhancing effects of NRG have also been noted by others; these effects vary with the time interval between NRG treatment and antigenic challenge. As observed in the first study, spleen weight and cellularity did not differ significantly among the control and experimental groups.

Having demonstrated that ATG pretreatment of mice could indeed affect immunologically committed cells, even when the latter were administered at a time when no lymphocyte toxic factors were detectable, we were next interested in determining the immunologic reconstitution of ATG-pretreated, lethally irradiated animals given normal

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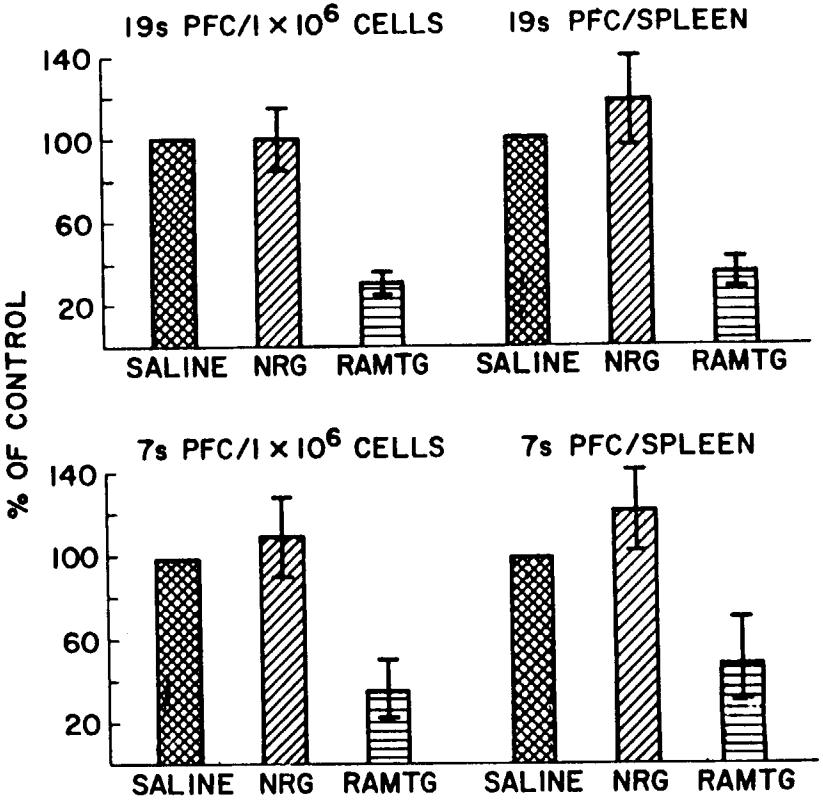


Fig. 27 PFC activity of presensitized spleen cells transferred to saline, normal rabbit globulin (NRG) or rabbit antimouse thymocyte globulin (RAMTG) pretreated recipients. Cells injected 7 days after last dose of RAMTG.

syngeneic bone marrow for protection from radiation death. The bone marrow was given 1 day after 950 R exposure and 7 days after the last injection of RAMTG, a time when no cytotoxic factors were present in the serum. Control mice were pretreated with saline or NRG prior to X-irradiation and bone-marrow treatment. Mice were then allowed to recover for periods of 30 to 65 days after irradiation and marrow injection. At these times, groups of mice were injected intraperitoneally with SRBC and the PFC response assayed 6 days later. The results shown in Table 14 reveal that immunologic recovery was incomplete when the experimental animals were challenged at 30 days, but that at day 65 their response was comparable to that obtained with the saline or NRG-treated controls.

The results of this study provided definitive proof that administration of ATG prior to lymphocyte cell infusion can adversely affect the immunologic reconstitution of animals, even though all apparent cytotoxic factors had been eliminated from the circulation at the time the cells were injected. The mechanism of this effect, however, is not yet known and can only be speculative at this time. For example, although cytotoxic factors

TABLE 14

ANTIBODY FORMATION IN ISOGENEIC RADIATION CHIMERAS THAT HAD BEEN PRETREATED WITH ANTITHYMOCYTE GLOBULIN ANTISERUM

Treatment †	Day of immunization postgrafting: 30	65
	19S Antibody-forming cells/spleen	
Saline	68,387	93,550
Normal rabbit globulin	74,243	110,465
Rabbit antimouse thymocyte globulin	27,299	111,250

† Indicates treatment of mice prior to recovery 950 R and isogeneic bone marrow (see text).

were not present in the circulation, attachment of significant amounts of the ATG to the cell surface could have affected their homing or proliferative capacities; alternatively, binding of the ATG to lymphocytes present in the recipient's spleen could have allowed complexing and therefore inactivation of the infused cells homing to this organ. Answers to these two possible mechanisms are possible and are currently being investigated.

Experimental Hematology

Studies on Human Granulocytic Chalone

Since our previous report on granulocytic chalone (ORAU-122, pp. 62-63) we (Helen Vodopick and Elizabeth Rupp) have tried to find an inhibitory substance extracted from human granulocytes. Chalone, according to previous criteria, should be: (1) an inhibitor of cell mitosis, (2) tissue specific but not species specific, (3) reversible in action, and (4) noncytotoxic. In our initial studies we used rat granulocytes as a source of chalone. An extract made from rat peritoneal granulocytes and fractionated by gel filtration (Sephadex G-25) was tested against a suspension of rat marrow. During short-term cultures, the incorporation of tritiated thymidine into marrow cells capable of synthesizing DNA was used to measure the degree of inhibition produced by the fractionated extract. Fractions having inhibitory properties caused a 40 to 60% decrease in the incorporation of tritiated thymidine with no evidence of cellular damage when compared with control samples.

In subsequent experiments, we were surprised to find a high salt concentration in these fractions which possessed inhibitory properties. We were able to show that this salt concentration caused at least half of the reduction in incorporation of tritiated thymidine.

Because we were unable to separate the salts from the chalone fractions when Sephadex G-25 was used, we chose to use Sephadex G-50 for better resolution and separation. Both salts and amino acids can be excluded from the fraction containing inhibitory material. To further identify the approximate molecular weight of these fractions, we use bacitracin, a polypeptide with a molecular weight of 1450, and Cytochrom C, molecular weight 13,000, as markers for the elution pattern of fractions obtained from our extract.

1028240

After fractionation, the samples are concentrated by lyophilization. Although the eluent, ammonium bicarbonate, is extremely volatile, a small residue is still present after one lyophilization. Therefore, all samples are resuspended in water and relyophilized before testing these fractions against rat-marrow suspension.

We have attempted to find an inhibitory substance produced by normal human granulocytes. Granulocytes are obtained from whole blood anticoagulated with heparin (10 units/cc of blood). After initial sedimendation of red blood cells by gravity, the leukocyte-rich supernatant plasma is centrifuged to concentrate leukocytes and to remove platelets. In another centrifuge tube the leukocyte concentrate is layered over a density gradient of Hypaque-Ficoll which is then centrifuged for 15 min at 400 g. The granulocyte button is recovered, washed, and then disrupted by sonication. In order to remove all particulate material, the disrupted cells are centrifuged at 89,000 g for 45 min. The supernatant material is fractionated by means of gel filtration as described previously. These fractions are then tested individually for their ability to inhibit tritiated thymidine incorporation into rat-marrow suspension. Suspensions incubated with inhibitory fractions incorporated 20 to 25% less labeled thymidine than control samples did.

We have found three separate fractions which caused reduced thymidine incorporation: the first fraction eluted after the void volume; the second fraction eluted in the area in which bacitracin was recovered; and the third fraction eluted after the entire bed volume had been collected. The first fraction containing the macromolecules may be producing a nonspecific type of inhibition on the marrow cells. The fraction recovered after a bed volume has been eluted contains aromatic amino acids (tyrosine, phenylalanine, tryptophan), which supposedly have been held back on the column. The fraction found in the area in which the bacitracin has been recovered is salt-free and almost amino acid-free. We are now proceeding to determine which of these fractions possesses other properties of chalone activity.

The action of chalone should be reversible. In order to test for this characteristic, we took each rat-marrow suspension which showed reduced incorporation of tritiated thymidine and washed it free of the inhibitory substance obtained from human granulocytes. When these marrow cells were then tested for their ability to incorporate tritiated thymidine, all samples incorporated amounts of labeled thymidine equal to control samples. Thus, we showed that no permanent damage occurred in these cells previously inhibited by the fraction under investigation. Quantitative chamber counts of cells also remained stable.

Although granulocytic chalone of animal or human source presumably stops granulocytic precursors at the G-1 phase in the cell cycle, the synthesis of RNA and the metabolism of amino acids by these same cells should not be impaired. We used tritiated uridine to measure RNA synthesis and tritiated leucine to measure amino acid metabolism by those cells which had reduced thymidine incorporation. Reduced incorporation of both the labeled uridine and leucine was found. This finding would suggest that these cells had been put into a metabolic limbo during which more than one cellular function had been halted at least temporarily.

We are currently trying to ferret out which of these three fractions has specific inhibitory activity for granulocytic cells.

RESEARCH IN NUCLEAR MEDICINE

This portion of the Medical Division's mission is to advance the diagnostic and therapeutic applications of radioactive materials, with special emphasis on tumor-localizing agents, why they localize and how better ones can be developed.

Summary of Accomplishments

Experimental work in radiopharmaceutical development has emphasized the higher-atomic-number rare-earth radionuclides which have tumor-localizing propensities. Nuclides of thulium, erbium, and dysprosium have been studied extensively in animals with transplanted tumors. It appears that these nuclides may compare favorably with gallium-67 (^{67}Ga) in tumor-localizing potential. Their distribution is essentially complete by 4 hr after injection, but they concentrate in bone and kidney more than does ^{67}Ga. For best results it will necessary to use high-specific-activity nuclide preparations. Related nuclides that are mainly β-emitters may have some potential value as therapeutic agents. A trial made with a transuranium element, einsteinum-253, (^{253}Es) showed that it too had a significant concentration in animal tumors.

Animal studies have shown the possibilities of an improved marrow-scanning agent with radiogold. Gold-199 is demonstrated as preferable to ^{198}Au because the former gives less radiation dose per useful photon and because its emissions have an energy more suitable for imaging with the gamma camera. Success has been achieved in producing a higher concentration in marrow as compared with that in liver. This has been done by making a colloid of smaller particle size, produced with reducing agents (pyruvic aldehyde and glyoxal) rather than those used earlier in preparing gold colloids. Polyvinylpyrrolidone (PVP) has been substituted for gelatin as a stabilizing agent.

In collaborative work with ORNL, further progress was made in studying the radioprotective drug WR-2721, which may possibly enhance the relative effectiveness of radiotherapy on tumors as compared with normal tissues. In a group of animal tumors studied, the drug showed generally low uptake in the malignant cells, a favorable result in relation to possible therapeutic use. In an effort to determine suitable doses for larger animals and eventually man, toxicity studies have been carried out in rats and dogs. Preliminary results suggest that the doses per unit weight tolerated by mice are toxic to dogs; however, there is some suggestion that the effective doses for WR-2721 may be more related to body surface area than to weight, and if this proves true, radioprotective doses may be much smaller in larger mammals.

Work on the localization of ^{67}Ga in cells has failed as yet to confirm the results of other investigators who have found a large portion of the gallium in soluble cell fractions. Centrifugation studies showed that thulium-167 (^{167}Tm) and indium-111 (^{111}In) have a distribution in cell fractions similar to that of ^{67}Ga. Another tumor-localizing nuclide, bismuth-206 (^{206}Bi), also localizes mainly in cytoplasmic fractions, but in a pattern quite different from that of ^{67}Ga. The macromolecular binding of ^{67}Ga in thymus is similar to that of tumor tissue in contrast to that seen in other normal tissues.

In a clinical study, ^{67}Ga and ^{111}In were compared by giving the two nuclides simultaneously to a small group of patients. Later scans made with energy selection helped to show the relative distribution of the two, and in those instances where surgical material

1028241

was available, a quite satisfactory quantitative differential counting method was worked out. In only one of three patients was a strong tumor uptake shown, and in this one, the ^{67}Ga uptake was far superior to that of ^{111}In.

During the year, the Medical Division's computer facility was considerably expanded in its resources and capabilities. A major change was from a time-sharing to a multiprogramming environment. This alteration in operating system in the IBM 1800, along with other developments such as front-end buffering by the PDP-11, enables the computer team to more fully meet the needs of the investigators.

Radiopharmaceutical Development

Tumor-Localizing Agents

Rare-Earth Radionuclides. (R. L. Hayes, B. L. Byrd, L. C. Washburn*, and J. J. Rafter). Recent reports (Hisada, K. and Ando, A., J. Nucl. Med. 14, 615-617, 1973) have indicated that the higher-atomic-number rare-earth radionuclides show an affinity for nonosseous tumor tissue. We have confirmed these results (91) and anticipate that the clinical usefulness of several of these radionuclides in tumor diagnosis will be investigated in depth at the Medical Division and elsewhere. Prior to clinical trials, we have studied those factors that influence the distribution of these radionuclides in both normal and tumor-bearing animals.

*Presidential Intern

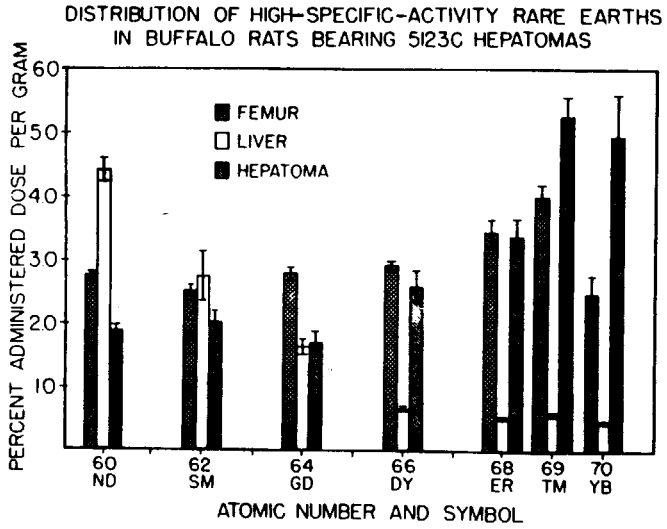


Fig. 28 Concentrations (24 hr) of high-specific activity rare-earth radionuclides in femur, liver, and tumor of male Buffalo rats bearing Morris 5123C hepatomas (groups of four animals). Concentrations were normalized to a body weight of 250 g.

1028242

Figure 28 shows a histogram of the distribution of various high-specific-activity rare-earth radionuclides in liver, bone, and 5123C hepatomas (radionuclides obtained in part from Isotopes Development, Oak Ridge National Laboratory). As has previously been reported, there was a dramatic decrease in the concentration of the rare earths in liver as the atomic number of the nuclides increased. At the same time, the concentration in the 5123C hepatoma increased markedly, thulium showing the highest tumor concentration. Table 15 shows the results of this study in a more detailed form. Also shown in Table 15 are the average results obtained with this model tumor system for ^{67}Ga. The tissue distribution of ^{170}Tm in the normal Buffalo rat through a period of 28 days is shown in Fig. 29.

TABLE 15

TISSUE DISTRIBUTION OF RARE-EARTH ISOTOPES (24 HR) IN RATS BEARING 5123C HEPATOMAS*

Isotope	^{147}Nd	^{153}Sm	^{153}Gd	^{159}Dy	^{171}Er	^{170}Tm	^{169}Yb	^{67}Ga
Atomic number	60	62	64	66	68	69	70	31
Tumor conc. (%/g)	1.9	2.0	1.7	2.6	3.3	4.3	4.9	9.3
Ratio tumor conc. to:								
Liver	0.4	0.7	1.0	4.0	6.7	10.0	11.0	20.0
Spleen	2.2	6.4	2.5	3.4	6.3	7.5	6.3	8.0
Kidney	0.6	0.5	0.7	1.4	2.1	3.0	4.8	17.0
Lung	6.5	6.4	6.5	13.0	19.0	27.0	25.0	48.0
Muscle	100.0	85.0	110.0	160.0	180.0	120.0	150.0	82.0
Femur	0.7	0.8	0.6	0.9	1.0	1.1	2.0	13.0
Marrow	12.0	9.6	7.4	9.4	12.0	15.0	7.6	8.7
Blood	89.0	76.0	53.0	76.0	120.0	230.0	100.0	150.0

* Groups of four animals each.

TABLE 16

RARE-EARTH RADIONUCLIDES FOR TUMOR SCANNING

Isotope	Decay mode	$T_{1/2}$	γ energy (%)	Comments
^{167}Tm	EC	9d	208 KeV (43%)	^{168}Er p, 2n
^{171}Er	β^-	7.5h	$\sim$300 KeV (90%)	σ ^{170}Er = 5.7; ^{171}Tm $T_{1/2}$ = 1.9y
^{157}Dy	EC	8.2h	326 KeV (91%)	σ ^{156}Dy (0.05%) = 3

Of the higher-atomic-number rare-earth radionuclides that might be used for tumor scanning, three are particularly suited for this purpose: ^{167}Tm, erbium-171 (^{171}Er), and dysprosium-157 (^{157}Dy). Their properties are shown in Table 16. Although ^{171}Er and ^{157}Dy would be produced by neutron activation of the stable elements, they could be produced with very high-specific activities (mCi/μg). The relatively short half-lives of both ^{171}Er and ^{157}Dy do not constitute a problem (as they would with short half-lived gallium radioisotopes), since the tissue distribution of the higher-atomic-number rare-earth radionuclides is essentially complete by 4 hr (see Table 17). Should sufficiently high tumor-to-nontumor ratios be obtained in selected patients, consideration might be given to the use of rare-earth radionuclides for internal radiotherapy, since a number of these nuclides are beta emitters with no (or only slight amounts of) associated gamma emissions (lutetium-177 [^{177}Lu], ytterbium-175 [^{175}Yb], and ^{169} Er).

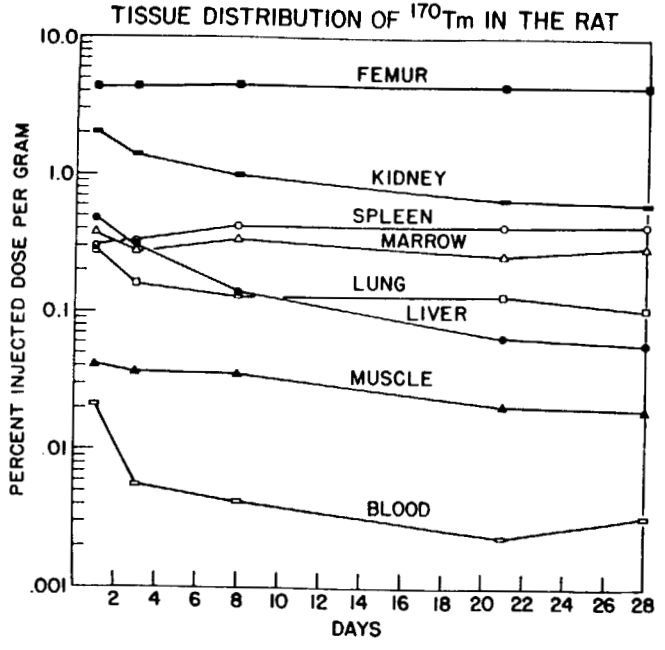


Fig. 29 Concentration of ^{170}Tm in various tissues of the normal male Buffalo rat as a function of time after administration (groups of four animals at each time period). Concentrations were normalized to a body weight of 250 g.

In Table 15 it is apparent that radionuclides of ytterbium, thulium, erbium, and dysprosium compare favorably with ^{67}Ga with respect to the tumor-to-nontumor ratios obtained in rats bearing Morris 5123C hepatomas. A further comparison of ^{67}Ga with a rare-earth radionuclide (^{169}Yb) in other tumor systems is shown in Table 18. Again, as with the 5123C hepatoma, the absolute concentration of ^{67}Ga was better than that of ^{169}Yb in tumor tissue except in Erhlich ascites (EA) cells, where the ^{169}Yb concentration was ~3 times greater than that of ^{67}Ga. Tumor-to-nontumor ratios were, however, approximately the same (except for EA cells). It would thus appear on the basis of these data that rare-earth radionuclides might show as high an affinity for tumor tissue in human subjects as does ^{67}Ga. It is apparent, however, that rare-earth radionuclides concentrate to a much greater extent in bone and kidney than does ^{67}Ga, and this may obscure tumor visualization where sites of tumor are under or adjacent to the skeleton. On the other hand, lesions in the bone (both primary and metastatic) should be as readily visualized with rare-earth radionuclides as they are with ^{67}Ga. Another advantage of the rare-earth radionuclides indicated in Table 16, particularly so for ^{167}Tm, is that their energies are suitable for use with the gamma camera. Visualization of ^{67}Ga with the camera is poor compared to that obtained with rectilinear systems.

An important requirement for the effective use of rare-earth radionuclides for tumor detection will be the use of high-specific-activity preparations. Table 19 shows the rather

1028243

TABLE 17

INFLUENCE OF TIME ON TISSUE DISTRIBUTION OF ^{171}ER AND ^{169}YB IN RATS BEARING 5123C HEPATOMAS

Isotope	^{171}Er			^{169}Yb	
Time	2 hr	4 hr	24 hr	4 hr	24 hr
Tumor conc. (%/g)*	3.3	4.5	3.4†	3.5	4.9†
Ratio tumor conc. to:					
Liver	5.0	5.6	6.7	7.9	11.0
Spleen	5.5	7.9	6.4	4.6	6.3
Kidney	1.3	1.7	2.0	3.4	4.7
Lung	6.3	12.0	19.0	11.0	25.0
Muscle	43.0	87.0	180.0	66.0	160.0
Femur	1.5	1.4	1.0	2.1	2.0
Marrow	7.2	10.1	12.5	5.9	8.0
Blood	6.2	19.0	130.0	9.9	110.0

* Percent administered dose/g normalized to body weight of 250 g.
† Not significantly different from 4-hr value.

TABLE 18

COMPARISON OF THE RELATIVE AFFINITIES OF ^{169}YB AND ^{67}GA (24 HR) FOR VARIOUS TUMOR TISSUES

	7777 Hepatoma		CA-755		P-1798		EA Cells	
	^{169}Yb	^{67}Ga	^{169}Yb	^{67}Ga	^{169}Yb	^{67}Ga	^{169}Yb	^{67}Ga
Tumor conc. (%/g)	3.20	5.80	4.70	7.90	3.10	6.00	4.30	1.50
Ratio tumor conc. to:								
Liver	9.50	7.30	1.50	1.20	0.80	1.20	1.90	0.46
Spleen	6.90	5.00	3.30	2.30	1.90	1.40	3.30	0.63
Kidney	4.10	8.00	0.33	2.40	0.22	1.10	0.40	0.38
Lung	4.00	19.00	2.10	4.00	1.10	1.80	4.10	0.67
Muscle	210.00	34.00	23.00	57.10	5.40	11.00	6.30	1.20
Femur	1.60	4.60	0.30	0.80	0.20	0.70	0.30	0.27
Marrow	5.20	5.10	–	–	–	–		
Blood	190.00	33.0	28.00	4.70	14.00	6.60	14.00	0.62
Fluid	–	–	–	–	–	–	3.60	0.36

extreme effect that the simultaneous administration of stable erbium (at the milligram level) had on the tissue distribution of ^{171}Er in rats bearing 7777 hepatomas. Concentration of ^{171}Er in liver and spleen was greatly increased while that in the tumor was correspondingly lowered. Even at the microgram level of stable erbium administration, there was a rather dramatic increase in the concentration of ^{171}Er in liver and spleen above ~10 μg Er/kg; the tumor concentration was not affected, however. This carrier effect was also seen when stable erbium (at the microgram level) was administered with high-specific-activity ^{170}Tm. It thus will be important to use carrier-free or high-specific-activity preparations of the rare-earth radionuclides in tumor-scanning procedures.

The studies discussed above involved administration of rare-earth radionuclides in the citrate form at a dose level of 1 mg citrate/kg. Table 20 shows the effect of other forms of chelates on the distribution of ^{167}Tm in rats bearing 7777 hepatomas. NTA and HEIDA gave approximately the same distribution patterns as did the citrate while gluconate was somewhat poorer in tumor specificity. HEDTA, a strong chelate, greatly decreased the concentration of ^{167}Tm in tumor tissue and also produced much poorer tumor-to-non-

TABLE 19
EFFECT OF ERBIUM CARRIER ON THE DISTRIBUTION OF ^{171}ER (24 hr) IN THE RAT

Tissue	Carrier (mg/kg)			
	~ C.F.	0.1	0.5	1.0
	Percent administered dose/g			
Liver	0.50	3.40	8.00	10.00
Spleen	0.24	0.76	1.60	4.80
Kidney	1.30	3.00	1.40	1.00
Lung	0.19	0.20	0.33	0.64
Muscle	0.02	0.02	0.02	0.01
Femur	4.50	2.61	1.40	1.10
Marrow	0.26	0.56	1.30	2.00
Blood	0.01	0.03	0.13	0.18
Tumor	2.30	1.60	0.88	0.44

TABLE 20
TISSUE DISTRIBUTION OF ^{167}Tm (24 hr) IN RATS BEARING 7777 HEPATOMAS WHEN ADMINISTERED INTRAVENOUSLY IN VARIOUS FORMS*

Tissue/ratio	Form				
	Gluconate	Citrate	NTA	HEIDA	HEDTA
Tumor (%/g)	2.70	3.20	2.70	2.70	0.17
Ratio tumor conc. to:					
Liver	3.10	7.50	6.90	6.40	2.30
Spleen	3.30	6.10	4.40	4.80	0.44
Kidney	2.60	3.00	3.30	3.40	0.53
Muscle	160	170	140	210	110
Femur	1.10	1.30	1.10	1.10	0.88
Femoral marrow	6.50	8.00	6.70	6.10	4.90
Blood	150	150	140	120	19.0

* 5.2 μM/kg; four animals per group.

tumor ratios (particularly for the spleen, kidney, and blood). A study has been made of the effect produced by an increase in the citrate dosage on ^{167}Tm tissue distribution. No effect on tumor concentration of ^{167}Tm was observed with a citrate dose as high as 20 mg/kg. There was also no change in the tumor-to-nontumor ratios except for slight increases for spleen and blood.

As for ^{67}Ga, the tissue distribution of the higher-atomic-number rare-earth radionuclides (as represented by ^{170}Tm) is affected by both sex (Table 21) and age (Table 22). The differences in tissue distribution produced by differences in sex and age were not as pronounced as those seen with ^{67}Ga; however, the differences were highly significant.

Both ^{67}Ga and ^{111}In tend to localize in inflammatory lesions. It was therefore of interest to determine whether a similar concentration would occur with the rare-earth radionuclides. Table 23 shows that ^{169}Yb did indeed localize to a considerable degree in an abscess produced by a microorganism (*S. aureus* 6339), the abscess having the highest concentration of all tissues assayed except for those of the bone and kidney.

Since the 4f rare-earth elements bear a considerable resemblance in ionic radii and chemical properties to the 5f transuranium elements, we tested the distribution of one of

1028244

the higher-atomic-number transuranium elements in tumor-bearing animals. Einsteinium-253, element 99 (kindly provided by J. E. Bigelow of Oak Ridge National Laboratory), did indeed shown an affinity for tumor tissue (19) with good tumor-to-nontumor ratios obtained in rats bearing the 5123C hepatoma but with only modest ratios in other animal tumor systems.

The Anomalous Tissue Distribution of ^{67}Ga DTPA. (B. L. Byrd and R. L. Hayes). Tissue concentrations of ^{67}Ga administered to tumor-bearing rats in the HEDTA and EDTA chelate forms are unusually low (particularly with EDTA) when compared to

TABLE 21
TISSUE DISTRIBUTION (24 hr) OF ^{170}TM IN THE MALE AND FEMALE FISCHER RAT*

Tissue	Sex		Significance
	Male	Female	P
	Percent administered dose/g†		
Liver	0.74	0.56	0.001–0.01
Spleen	0.57	0.31	<0.001
Kidney	0.62	0.52	0.02
Lung	0.13	0.11	–
Muscle	0.03	0.02	<0.001
Femur	5.00	4.30	<0.001
Marrow	0.33	0.22	–
Blood	0.02	0.01	0.001

* Groups of five animals each.
† Percent administered dose/g normalized to body weight of 250 g.

TABLE 22
EFFECT OF AGE ON THE TISSUE DISTRIBUTION (24 hr) OF ^{170}TM IN THE MALE BUFFALO RAT*

Tissue	Age		Significance	
	2 months	5 months	P	
	Percent administered dose/g†			
Liver	0.36	0.48	< 0.001	
Spleen	0.19	0.29	< 0.001	
Kidney	0.76	2.10	< 0.001	
Lung	0.14	0.29	< 0.001	
Muscle	0.02	0.04	< 0.001	
Femur	4.60	4.30	< 0.05	< 0.02
Marrow	0.26	0.38	< 0.01	< 0.001
Blood	0.01	0.02	< 0.001	
Average weight	190	370		

* Groups of five animals each.
† Percent administered dose/g normalized to body weight of 250 g.

obtained with ^{67}Ga in the citrate form, although the tumor-to-nontumor ratios are approximately the same. This behavior is in keeping with the high stability constants for gallium HEDTA and gallium EDTA chelates (i.e., log K_1 [Ga-EDTA] = 20.3), which in turn would be expected to result in rapid urinary excretions of these chelates. It was, therefore, surprising when preparations of ^{67}Ga DTPA gave tumor and normal tissue concentrations similar to those observed with ^{67}Ga citrate, since the stability constant of gallium-DTPA is reported to be even higher than that of gallium EDTA (log K_1=26.8). Table 24 shows a repeat of this study in which the tissue distribution of ^{67}Ga DTPA was compared with that of ^{67}Ga EDTA and ^{67}Ga citrate in rats bearing RFT tumors (implants were made at the same time in male rats of the same age). The DTPA for this study was purchased from a source other than that used in the original study. An acid DTPA solution of ^{67}Ga was neutralized with sodium hydroxide and then heated in a boiling water bath for ~30 min before administration. A previous in vitro study of the binding of ^{67}Ga by plasma proteins in the presence of various chelating agents had also indicated that DTPA was ineffective in blocking protein binding of ^{72}Ga when compared to that seen with EDTA (Hartman, R. E. and Hayes, R. L., unpublished results). One possible

TABLE 23

TISSUE DISTRIBUTION (24 hr) OF ^{169}YB IN RATS INFECTED WITH *S. aureus* 6339

Tissue	Percent administered dose per gram*	
	Percent	S.E.
Liver	0.47	0.01
Spleen	0.68	0.04
Kidney	1.53	0.04
Lung	0.19	0.01
Muscle	0.03	0.005
Femur	3.60	0.18
Marrow	0.63	0.09
Blood	0.02	0.001
Abscess tissue	1.30	0.17
Abscess fluid	0.59	0.17

* Normalized to body weight of 250 g.

TABLE 24

EFFECT OF DTPA AND EDTA ON THE TISSUE DISTRIBUTION (24 hr) OF ^{67}Ga IN THE FISCHER 344 RAT-BEARING RFT TUMORS*

Tissue	Citrate	EDTA	DTPA
		Percent administered dose/g	
Liver	1.50	0.045	0.90
Spleen	2.80	0.130	1.30
Kidney	0.81	0.089	0.61
Lung	0.30	0.013	0.21
Muscle	0.17	0.001	0.12
Femur	1.30	0.038	0.87
Marrow	1.70	0.039	0.78
Blood	0.18	0.007	0.15
Tumor	2.00	0.055	1.70

* Groups of four male animals each with data normalized to body weight of 250 g.

1028245

explanation for this apparent anomalous behavior of ^{67}Ga in the DTPA system may lie in the kinetics of the dissociation at physiological pH of gallium DPTA on the one hand and that of gallium HEDTA and gallium EDTA on the other. Saito and Tsuchimoto (J. Inorg. Nucl. Chem. 23, 71-79, 1961) have reported that the rate of dissociation of gallium EDTA and gallium HEDTA is exceedingly slow at a pH of 7.0 (as evidenced by the slowness of the rate of exchange between ionic gallium [citrate] and gallium EDTA and HEDTA forms). Thus, even though the equilibrium stability constant of gallium DTPA is higher than that of gallium EDTA and gallium HEDTA, the rate of dissociation of gallium DTPA might be much more rapid at physiological pH leading to higher tissue concentrations of ^{67}Ga. Exchange rate measurements have not been reported on gallium DTPA.

Determination of ^{67}Ga and ^{111}In in the Presence of Each Other. (J. E. Carlton, Evelyn E. Watson, Roger J. Cloutier, and R. L. Hayes). The Medical Division's clinical research program has embarked on a study of the concentration of ^{67}Ga and ^{111}In in patients administered both radionuclides at the same time. Suitable choice of narrow gamma energy bands has made it possible to scan the two nuclides in the presence of each other (see page 77 in Nuclear Medicine Section). Samples of tissue from surgical procedures and autopsies have been assayed by using both a lithium-drifted germanium detector (GeLi) and a well-scintillation counter. Figure 30 shows a spectrum obtained on a representative tissue sample using the GeLi detector. Although this detector system yields ratios of one radionuclide to the other, absolute assays are rendered difficult by geometric considerations. Geometry does not represent as great a problem with well-scintillation counting, but resolution is poor. To overcome this latter problem, it is possible, using narrow energy windows and a set of simultaneous equations for correction for spectrum overlap, to arrive at reasonably accurate assays for both ^{67}Ga and ^{111}In in the presence of each other. Dual channel counting and computer processing of a punched paper-tape readout considerably simplifies the determinations.

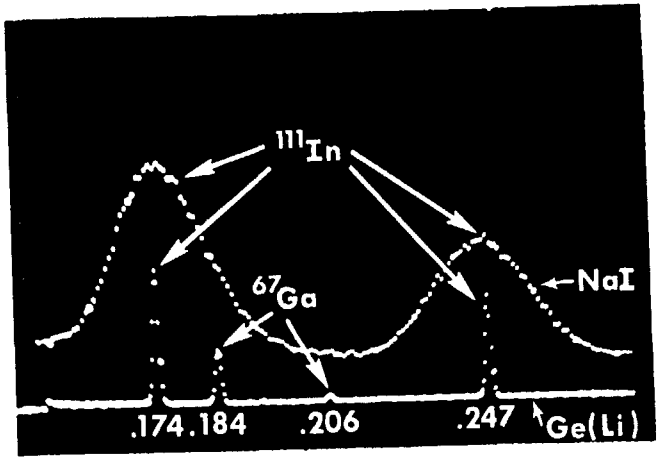


Fig. 30 The figure above compares the spectrum obtained on a representative tissue sample using the Ge(Li) detector with that obtained with a NaI detector.

.ocessing of ^{67}Ga for the Cooperative Project. (J. E. Carlton, Evelyn E. Watson, and J. D. Berger). During the 1973 calendar year a total of 11,600 mCi of ^{67}Ga in 694 shipments was sent to the 15 other institutions participating in the Cooperative Group to Study Localization of Radiopharmaceuticals. The processing is a joint venture of the Medical Division and the Isotopes Division at Oak Ridge National Laboratory. Oak Ridge National Laboratory produces the ^{67}Ga and prepares and packages it in the citrate form. The Medical Division supplies the solutions and packaging materials, tests for pyrogenicity and sterility, and monitors the assay. No reactions of patients to the preparations have been noted by any of the participants in the study to date.

Bone-Marrow Scanning Agents

Our recently renewed studies of radiolabeled colloidal preparations have centered around attempts to formulate a bone-marrow scanning agent of greater utility than those at present available (L. C. Washburn, D. C. Swartzendruber, B. L. Byrd, and R. L. Hayes).

Previous clinical investigations at ORAU of bone-marrow abnormalities have involved the use of commercially supplied colloidal gold labeled with ^{198}Au and ^{99m}Tc sulfur colloid. The ^{198}Au colloid gave excellent scanning results in experimental subjects, but at the same time involved high radiation doses, particularly to the liver, and it was therefore deemed unacceptable for general clinical use. Gold-199 is currently available from Oak Ridge National Laboratory as a by-product of the production of platinum-195 (^{195m}Pt). This radioisotope of gold has a lower gamma energy emission (160 KeV) than ^{198}Au (410 KeV), and it is estimated that its use for scanning would result in only about one-half to one-third the radiation dose produced by ^{198}Au for equal numbers of detected photons. Because of the low-energy gamma associated with the decay of ^{199}Au, camera visualization should be of high quality.

In previous work we have noted the tendency of small-particle colloidal preparations to deposit preferentially in the bone marrow. It thus might be possible to produce a considerable enhancement of deposition in bone marrow without, at the same time, administering an objectionable dose of stable gold, as would be the case with RES blockage. If this result could be obtained, the radiation dose to the liver could be reduced even further. (The normal distribution of commercial colloidal ^{198}Au results in ~90% deposition in the liver.) Our efforts with colloidal gold have involved attempts to find reducing agents that would produce particles in the 2-5 mμ diameter range instead of those of a much larger size (from 20-100 mμ) that are available commercially.

A survey of the literature suggested that glucose in basic solution might be a suitable reducing agent. This method has been used by others to prepare a "seed colloid" for use in the preparation of uniform colloids of larger particle size; the investigators claimed the formation of uniform particles of 5 mμ diameter (Henry, R., Herczeg, C., and Fisher, C. Int. J. Appl. Radiat. Isotopes 2, 136-139, 1957; Douis, M. Minerva Nucleare 7, 448-450, 1963). However, our attempts to use this method gave large particle size ranges, usually about 2 to 20 mμ. In addition, another problem arose through a side reaction. Henry, et al. (Int. J. Appl. Radiat. Isotopes 2, 136-139, 1957) have reported that, in basic solution, glucose is split into pyruvic aldehyde and lactic acid; apparently pyruvic aldehyde is the

effective reducing agent in the glucose reduction of gold. When gelatin wa.
tective colloid in our initial studies of glucose as a reducing agent, there was
action between the gelatin and the pyruvic aldehyde formed, resulting in a solutio.
was very difficult to microfilter. Nevertheless, tissue distribution studies were carried ou.
in rabbits, using the glucose method at levels of 2 mg/kg (2 animals) and 10 mg/kg (1 animal). Only the marrow and liver showed significant uptake (all other organs had less than 10% of the concentration of the marrow and liver). At 2 mg/kg the marrow-to-liver ratios (M/L) were 1.1 and 1.3 and at 10 mg/kg the M/L was 2.2. These results, though far from ideal, were encouraging enough to warrant further study. (In previous studies using ascorbic acid as the reducing agent [commercial method], 50 mg/kg was required to achieve an M/L ratio of 1.4.)

Since pyruvic aldehyde is apparently the effective reducing agent in the glucose method, we tried pyruvic aldehyde itself as a reducing agent. This gave a maximum particle diameter, as measured by electron microscopy, of 8 to 9 mμ. Table 25 shows the tissue distribution in rabbits of colloidal ^{199}Au made by the pyruvic aldehyde method after

TABLE 25

TISSUE DISTRIBUTION OF ^{199}Au-COLLOID (PYRUVIC ALDEHYDE-GELATIN METHOD) IN THE RABBIT AFTER I.V. ADMINISTRATION: EFFECT OF CONCENTRATION*

Specimen	Percent administered dose/g	
	2 mg Au/kg†	10 mg Au/kg‡
Marrow	1.81	2.41
Liver	0.94	0.81
Spleen	0.74	0.71
Kidney	0.0063	0.0081
Lung	0.011	0.0089
Muscle	0.00024	0.00035
Blood	0.0020	0.0053
Urine	0.00089	0.0017

* Two rabbits per group; normalized to 2.5 kg rabbit
† Two hours postinjection
‡ Three hours postinjection

the intravenous injection of 2 mg/kg and 10 mg/kg. The blood disappearance half-times were 15 min for the 2 mg/kg and 50 min for the 10 mg/kg dose. Although the particle sizes were obviously smaller than in the glucose method, the problem of the side reaction between the aldehyde and gelatin persisted. Other workers, however, have reported (Caro, R. A., Nicolini, J. O., and Radicella, R., Int. J. Appl. Radiat. Isotopes 19, 547-550, 1968) that the biological behavior of radiogold colloids stabilized with polyvinylpyrrolidone (PVP) is very similar to those stabilized with gelatin. We have found no stabilizer side reaction using either glucose or pyruvic aldehyde and PVP. When glyoxal, an aldehyde similar to pyruvic aldehyde, was used as the reducing agent and PVP as the protective colloid, again no side reaction was observed and an even smaller particle preparation was obtained. The maximum particle diameter observed in electron micrographs was 6 mμ. At

1028246

2 mg/kg and 10 mg/kg, the spleen was the organ of greatest uptake, as seen in Table 26. Marrow-to-liver ratios were also somewhat decreased. It is thought that these differences in distribution are probably due to the nature of the stabilizer. However, in another preliminary experiment involving a single rabbit, where the preparation of the colloid was altered by decreasing the initial ionic gold concentration, a considerable increase in bone-marrow specificity was achieved with a greatly reduced dose of stable gold (0.1 mg Au/kg). The spleen concentration still exceeded the liver concentration, but the M/L ratio was 4.6. The blood disappearance half-time was, however, 6.4 hr. Further studies with glyoxal-produced gold colloids are in progress.

TABLE 26

TISSUE DISTRIBUTION OF ^{199}Au-COLLOID (GLYOXAL-PVP METHOD) IN THE RABBIT AFTER I.V. ADMINISTRATION: EFFECT OF CONCENTRATION*

Specimen	Percent administered dose/g†	
	2 mg Au/kg	10 mg Au/kg
Marrow	0.86	1.47
Liver	0.72	0.57
Spleen	2.57	3.63
Kidney	0.0039	0.0055
Lung	0.026	0.012
Muscle	0.0007	0.0013
Blood	0.0062	0.0072
Urine	0.0033	0.0028

* Two animals per group; two hr postinjection
† Normalized to 2.5 kg rabbit

Studies of the Radioprotective Drug WR-2721

Preirradiation injection of the radioprotective drug S-2-[3-aminopropylamino] ethylphosphorothioic acid (WR-2721) preferentially protects the normal, as opposed to the malignant, tissues of tumor-bearing mice from radiation injury. The major factor contributing to this difference in response appears to be the relatively poor absorption of the drug by the tumor (see ORAU-122). WR-2721 is thus a promising agent for increasing the efficiency of radiation therapy of cancer, either by reducing the undesirable effects resulting from normal tissue injury or by allowing higher doses of radiation, with greater antitumor effect, without increasing the injury to normal tissues. A study of the feasibility of the clinical use of WR-2721 has continued (L. C. Washburn, J. J. Rafter, J. E. Carlton, R. L. Hayes, C. L. Edwards, and J. M. Yuhas*).

In order to determine whether deficient drug absorption was characteristic of solid tumors and to extend the study of the absorption patterns to the problems which might be encountered in clinical radiotherapy, normal and tumor tissue concentrations of ^{35}S-labeled WR-2721 were determined 30 min after injection of graded doses of the drug (1, 50, 100, and 300 mg/kg) in two species (mice and rats) bearing four different solid tumors

*Biology Division, Oak Ridge National Laboratory.

1028247

(P-1798 lymphosarcoma and CA-755 adenocarcinoma in mice and the RFT tumor and Morris 7777 hepatoma in rats). It was demonstrated that: (1) three of the four tumors examined showed deficient absorption of the drug, but the Morris 7777 hepatoma absorbed WR-2721 as readily as did the host's normal tissues (see Figs. 31 and 32); (2) the relative concentration of the drug in normal and tumor tissues was independent of the injected dose between 50 and 300 mg/kg; (3) the injection of tracer levels of WR-2721 (1 mg/kg) holds promise as a method for identifying those tumors which show a deficient absorption, but not to quantitate the degree of the deficiency; and (4) the relationship between the injected dose and tissue concentration, after correction for species differences in surface area, was species-independent for lungs, small intestine, and spleen, but dependent for liver and kidney (see Figs 33 and 34). This final observation may hold the key to the clinical use of the drug since, if the surface area factor is valid, a much smaller dose per kilo in larger animals would give tissue concentrations similar to those seen with much greater doses in small animals. For example, one might obtain the same concentration of WR-2721 in the human lung, small intestine, and spleen by injecting only one-tenth the amount per body weight of WR-2721 required for such concentrations in the mouse. This could help to alleviate some of the serious toxicity problems described below. In order to test this surface area theory in lower animals, we are at present comparing tissue concentrations of WR-2721 in species ranging from the mouse through the dog, following the injection of the same "mg per unit surface area" dose.

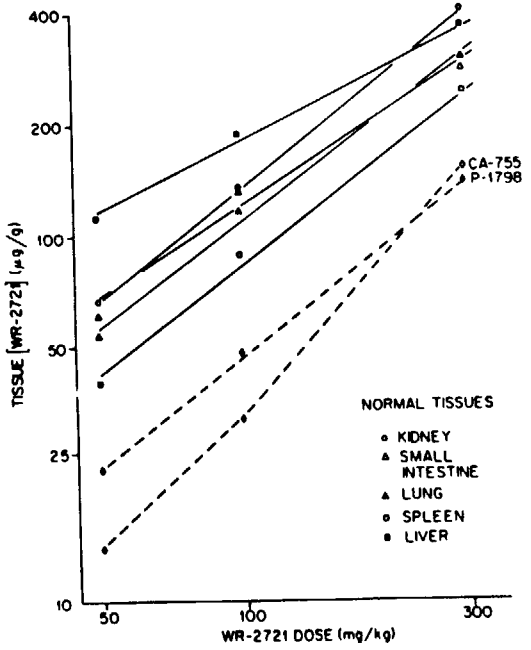


Fig. 31 Tissue concentration of WR-2721 in mice bearing two different transplanted tumors as a function of WR-2721 dose in mg/kg.

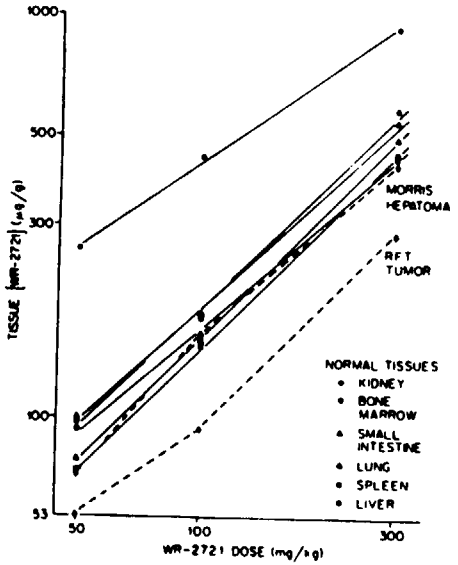


Fig. 32 Tissue concentration of WR-2721 in rats bearing two different transplanted tumors as a function of WR-2721 dose in mg/kg.

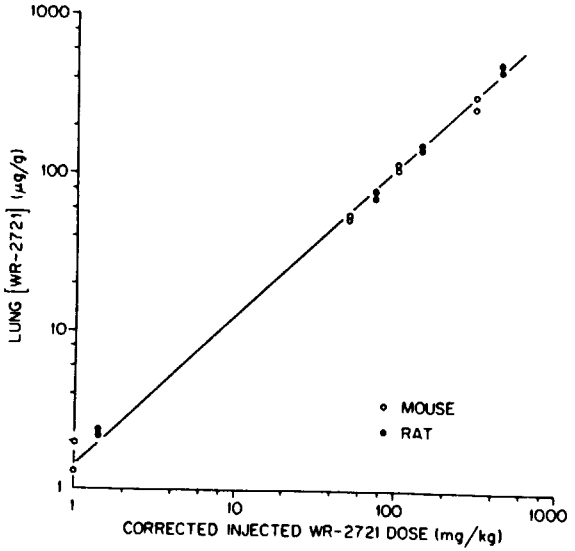


Fig. 33 Concentration of WR-2721 in the lungs of mice and rats as a function of drug dose with correction for species differences in surface area.

1028248

In order to obtain approval from FDA for the investigation of WR-2721 in humans, detailed intravenous toxicity studies are required. Extensive acute and subacute studies have now been completed in rats, along with a limited subacute study in mongrel dogs (L. C. Washburn, J. J. Rafter, and R. L. Hayes). A more extensive subacute study in pure-bred beagles is planned. The acute I.V. toxicity of WR-2721 was tested in male and female COBS rats using six rats of each sex at each of five dosage levels: 329, 379, 435, 500, and 575 mg/kg. In all the rat studies, a 5% (isotonic) aqueous solution of WR-2721 was administered via the tail vein. The acute LD_{50} value based on pooling the animals of both sexes was 490 mg/kg. A discernable sex difference was also noted. Male rats had an LD_{50} of 449 mg/kg, while that for female rats was 537 mg/kg.

In a subacute intravenous study of rats, ten COBS rats of each sex (20 total) were administered daily doses of 5% aqueous WR-2721 for 15 days at each of three dosage levels: 100, 150, and 200 mg/kg. The animals were then observed for 15 days following the last dose. Weight loss was measured as a percentage of the initial weight.

At 100 mg/kg, no drug-related deaths were observed. There appeared to be a steady weight loss through eight daily doses (800 mg/kg accumulative), with the female rats having a more pronounced weight loss (19.4% of body weight versus 10.6% for the male). After the eighth daily dose, the weights tended to become stabilized, or even to increase slightly, through the remaining doses (1500 mg/kg accumulative).

At a daily dose level of 200 mg/kg, no animals survived the entire planned dosage schedule. All rats lived through five daily doses (1000 mg/kg accumulative), and all were

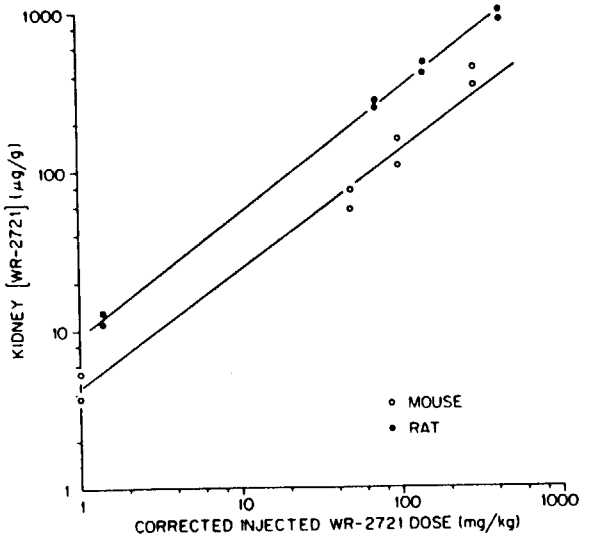


Fig. 34 Concentration of WR-2721 in the kidneys of mice and rats as a function of drug dose with correction for species differences in surface area.

dead after eight daily doses (1600 mg/kg accumulative). Weight loss was pronounced (25.6% for females and 29.1% for males), even though the planned 15-day dosage schedule was not carried out.

At a daily dose level of 150 mg/kg, two of twenty animals survived. No deaths were observed through eight daily doses (1200 mg/kg accumulative). The 18 observed deaths were spread out over the remainder of the injection schedule. Weight loss averaged 27% for the females and 30.5% for the males. Thus, the maximum tolerated daily dose of WR-2721 for the rat appears to be approximately 100 mg/kg.

Prior to the study of rats, a brief intravenous toxicity study was performed in four mongrel dogs. The protocol called for daily I.V. administration for 15 days of a 5% aqueous solution of WR-2721 at a dosage level of 100 mg/kg, along with complete hematology and clinical chemistry data both prior to and during the dosage schedule. Only one animal of four, a female collie-type animal, survived the 15-day experiment. One animal died following four daily doses, another following six daily doses, and still another was sacrificed in a moribund condition following 13 daily doses. All animals experienced emesis (vomited mucous-like substance within 10 min of injection), mydriasis, and ataxia. The symptoms generally persisted for about 2 hr after drug administration. There was a noted loss of appetite and extreme weight loss; the surviving animal had lost 27.2% of its initial weight by the end of the 15-day dosage schedule. Within 1 week after the end of drug administration, the animal regained its appetite and began gaining weight. Thirty days after the last dose, the animal was sacrificed and autopsied, as was the animal which was sacrificed in a moribund condition following 13 daily doses. Except for emaciation in the moribund animal, neither animal showed any drug-related gross or histopathological changes (Bill M. Nelson).

Subcellular Studies of Tumor-Localizing Agents

Association of ^{67}Ga with Tumor Tissue Cell Sap. (D. H. Brown, B. L. Byrd, J. E. Carlton and R. L. Hayes). Previously we have reported on a number of different tumors that were essentially alike with respect to their ^{67}Ga subcellular binding sites, i.e., ^{67}Ga was found mainly in lysosomes (5) and in a very small granule fraction not yet fully characterized (86 and ORAU-122). A number of investigators have reported that, in a variety of tumors, other than those we examined, ^{67}Ga activity found in nuclear fractions obtained from tumor homogenates by some investigators is probably artifactual. Others have found high levels of ^{67}Ga in the soluble cytoplasmic phase of the cell. We have found similar results for the following tumors: BW5147 mouse lymphatic leukemia, B-16 mouse melanoma, human Hodgkin's tumor, and Ehrlich ascites tumor cells. In each instance the tissue was extremely difficult to homogenize, usually because of its fibrous nature. Thus far we have been unable to homogenize these tumors without losing much of the ^{67}Ga content in the debris resulting from the procedure; consequently, we can make only a qualitative evaluation of the ^{67}Ga-binding sites. If the ^{67}Ga is initially associated with the soluble phase of such tumor cells and is not found there as a result of the cell disruption procedure, certain key particle enzymes should not be found in high concentration in the "soluble" zone obtained by the various isolation procedures. The lysosomal enzyme, acid phosphatase, is a reliable marker for testing this hypothesis. Assays of the subcellular fractions of a homogenate of Ehrlich ascites tumor cells are shown in Fig. 35. Since such a

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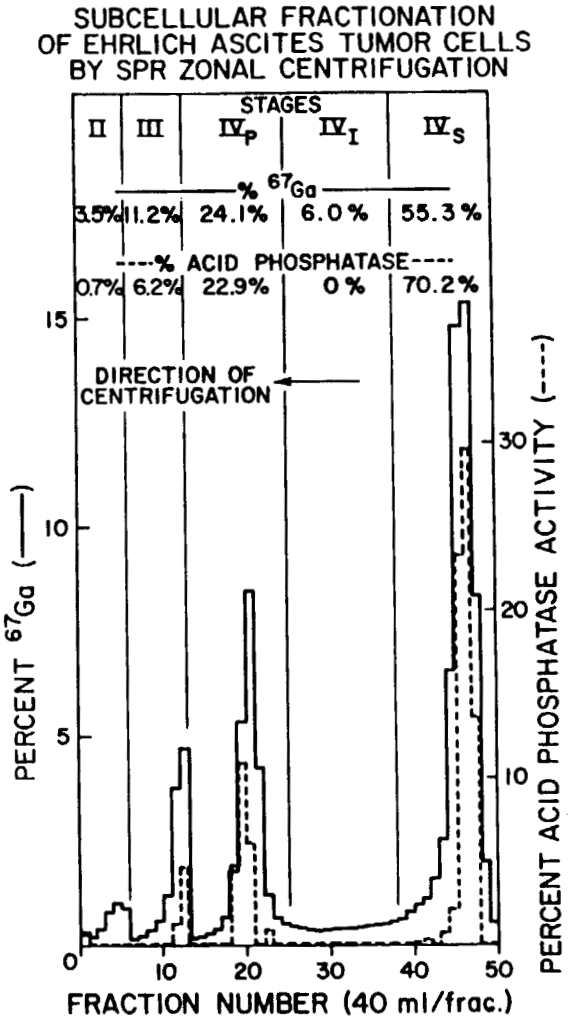


Fig. 35 Ehrlich ascites tumor-cell subcellular fractionation by SPR zonal centrifugation (Ref. 4). The percent values for ^{67}Ga and acid phosphatase at the top of the figure show the distribution of these activities in the separate zones.

large percentage of the acid phosphatase present was ound in the soluble zone (IVs), we conclude that many of the subcellular particles were probably damaged in the isolation procedure (homogenization plus separation). This does not preclude, however, the possibility that the ^{67}Ga was actually in the soluble phase of the intact tumor cells. Before one can conclude that it was not, an isolation procedure must be devised that yields intact

the subcellular particles coupled with recovery of most of the ^{67}Ga in the isolated particle fractions.

Subcellular Distribution of ^{167}Tm, ^{111}In, and ^{206}Bi in Tumor Tissue. (D. H. Brown, J. E. Carlton, and R. L. Hayes). Our subcellular studies also include the investigation of tumor-localizing radionuclides other than ^{67}Ga. Subcellular distribution profiles utilizing our sequential-product-recovery (SPR) zonal-centrifugation procedure (4) have been obtained for ^{167}Tm, ^{111}In, and ^{206}Bi. Table 27 shows a comparison of these radionuclides with ^{67}Ga in the 5123C hepatoma. The subcellular uptake profiles of ^{167}Tm and ^{111}In are similar to that of ^{67}Ga. The majority of the radioactivity was found in stage IV_p, the small particle zone obtained by SPR zonal centrifugation of the tumor homogenates. Bismuth-206 is quite different: it shows a more even distribution in stage II (mitochondria), stage III (lysosomes), and stage IV_p, but like ^{67}Ga and ^{111}In, ^{206}Bi is not appreciably associated with the nuclei (stage I). Other tumor-localizing radionuclides will be characterized by this procedure in an attempt to correlate tumor type with similarities and differences in subcellular binding profiles.

TABLE 27

COMPARISON OF THE SUBCELLULAR DISTRIBUTION OF ^{206}Bi, ^{167}Tm, AND ^{111}In WITH THAT OF ^{67}Ga IN THE MORRIS 5123C HEPATOMA

	Stage*	Stages†				
	I	II	III	IV_p	IV_i	IV_s
^{206}Bi	11.4	25.2	16.5	31.1	8.3	18.9
^{167}Tm	7.9	7.0	16.8	54.3	4.7	17.1
^{111}In	5.0	5.0	23.0	53.6	5.5	12.9
^{67}Ga	2.1	3.8	27.6	53.3	5.6	9.7

* Percent of total counts put in SZ-14 zonal rotor.
† Percent of total counts put in B-29 rotor for SPR zonal-centrifugation procedure.

Purification of Small ^{67}Ga-Binding Granules. (D. H. Brown, J. E. Carlton, B. L. Byrd, and R. L. Hayes). Several approaches have been used to characterize the small granule fraction (stage IV_p particles) that bind ^{67}Ga. The stage IV_p vesicles obtained by SPR zonal centrifugation are not a homogenous population of particles. They probably consist of Golgi, plasma membrane, and endoplasmic reticulum vesicles. In addition, the high acid phosphatase activity found in this zone (stage IV_p) indicates that extremely small lysosomes (possibly primary lysosomes) could be in this fraction.

If we assume the densities for Golgi, plasma membrances (PM), endoplasmic reticulum (ER), and lysosomes (isolated in sucrose gradients) are respectively 1.06-1.09, 1.08-1.13, 1.125-1.18, and 1.22, then these organelles should separate to some degree in sucrose gradients. Isopycnic banding of the stage IV_p particles should provide clues to the nature of the ^{67}Ga-binding vesicles. From the results of such an experiment shown in Fig. 36, we see that the vesicles containing the ^{67}Ga have a median density of 1.20. We are, therefore, forced to exclude both Golgi and PM vesicles as the particles binding ^{67}Ga. However, we cannot exclude the ER vesicles, since the peak of the profile for NAD diaphorase (an ER enzyme) is clearly in the zone of the ^{67}Ga peak. In a like manner, the

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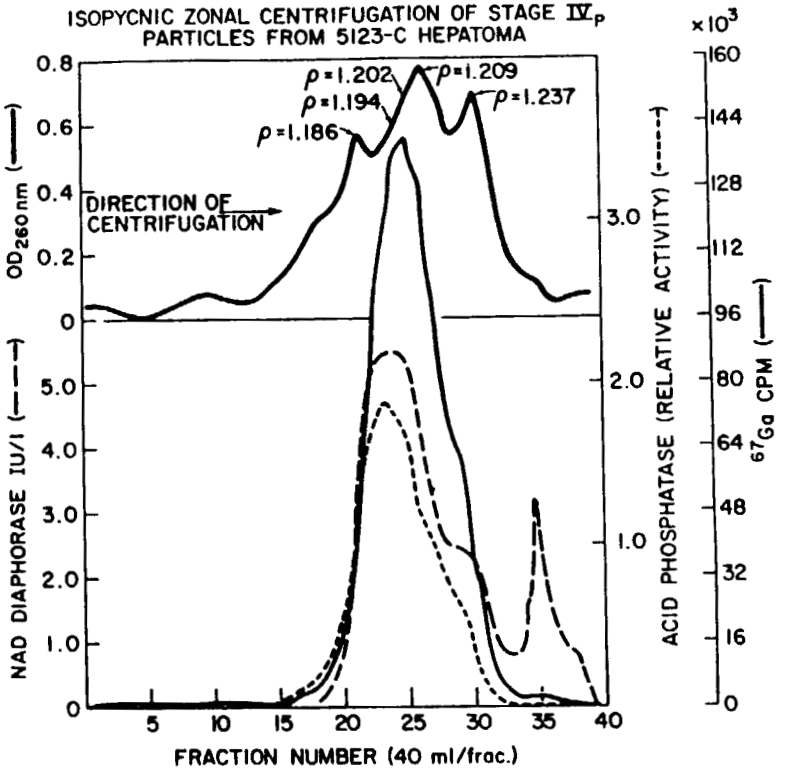


Fig. 36 Isopycnic zonal centrifugation of Stage IV_p particles from a 5123C hepatoma. SPR zonal centrifugation was performed in the normal manner except that the last stage (IV_p) was not pumped out of the B-XXIX rotor. The material remaining in and near the starting zone (600 ml) was removed by pumping 55% sucrose in at the edge of the rotor. The stage IV_p particle zone was then moved out in the rotor to 6.3 cm by pumping water in at the core. The rotor was then accelerated to 28,000 rpm and held there for 20 hr or $6.4 \times 10^{11}\ \omega^2 t$.

peak of the acid phosphatase activity lies in this zone, although it is somewhat skewed toward the lighter density particles (peak at ρ =1.186). These data are somewhat surprising, since we would have expected any lysosomal enzymes to be in a region of more dense particles (ρ=1.22). The acid phosphatase activity may be either a nonspecific one rising from the ER vesicles or an acid phosphatase isozyme which has recently been described for ER (Lin, C. W. and Fishman, W. J., J. Histochem. Cytochem. 20, 487-498, 1972). Obviously, greater resolution of the stage IV_p ^{67}Ga-binding vesicles must be achieved before we can precisely define their density and enzyme character. (Other types of lysosomal specific enzymes would also be helpful in clearing up the question of the lysosomal nature of the particles.)

We have also found that a zonal centrifugation procedure used for liver tissue to

...rate smooth (SER) and rough (RER) endoplasmic reticulum did not resolve the ^{67}Ga-binding vesicles from the SER or RER, if indeed they are different from ER vesicles. In fact, the procedure, when used on hepatoma tissue, did not separate SER from RER. The specific conditions of the procedure caused all the vesicles, the RER and SER, etc., to band isopycnically in the same zone (ρ=1.206). The vesicles derived from the tumor tissue are apparently more susceptible to ionic imbalance and/or osmotic effects because of the presence of 15 mM CsCl which was dispersed throughout the sucrose gradient. Therefore, we cannot exclude the possibility that the vesicles may be SER, RER, lysosomes, or some other particles not yet described.

Macromolecular Complexes of ^{67}Ga, ^{111}In, and ^{206}Bi in Extracts of Tumor and Normal Tissue. *(R. L. Hayes and J. E. Carlton).* Further extension of our work on the characteristics of the macromolecular agents that bind ^{67}Ga in aqueous extracts of normal and tumor tissue (18) indicates that the gel-filtration profile of tumor tissue (increased ^{67}Ga associated with a 5-4×10^4 molecular weight [MW] component) varies from that of not only liver and spleen but also from that of kidney, lung, and lymph nodes (Table 28). On the other hand, normal thymus showed a profile similar to that of tumor with greatly enhanced ^{67}Ga concentration in the 5-4×10^4 MW region (41%). Furthermore, this 5-4×10^4 ^{67}Ga peak in the thymus of rats bearing transplanted 5123C hepatoma and RFT tumors was considerably reduced (Table 29).

TABLE 28

ASSOCIATION OF ^{67}Ga WITH INTERMEDIATE ZONES OF GEL-FILTRATION PROFILES OF AQUEOUS EXTRACTS OF VARIOUS TISSUES

Tissue	Percent ^{67}Ga in intermediate zones of gel-filtration profiles	
	1.2-1.0 x 10^5 MW zone	5.0-4.0 x 10^4 MW zone
Tumor*	18	41
Liver†	10	13
Spleen	12	13
Kidney	4	4
Lymph nodes‡	17	16
Lung	14	13
Thymus	9	41

* Values are average of RFT and 5123C rat and P-1798 mouse tumors (Ref. 18).
† Values are average of livers from Fischer and Buffalo rats and a BALB/c x DBA/2F$_1$ mouse (Ref. 18).
‡ Mesenteric lymph nodes from Buffalo rats; all other normal tissues are from Fischer rats.

Interestingly enough, ^{111}In, another chemically related radionuclide with tumor-localizing properties, also showed a high concentration (55%) in the 5-4×10^4 gel-filtration profile region of extracts of normal rat thymus. We have previously observed that, like ^{67}Ga, ^{111}In has a high concentration in this peak in 5123C hepatoma extracts (43%) but, again like ^{67}Ga, it is not prominent in that of normal liver (18). Extracts of RFT tumor also show ^{111}In associated with a 5-4×10^4 peak (45%). These observations on the similarity of the macromolecular associations of ^{67}Ga and ^{111}In in the thymus and in tumor tissue may have immunological implications. The concentration of ^{67}Ga in the 5-4×10^4 MW fraction from an extract of abscess tissue (*S. aureus* 6339-induced) was intermediate between that of tumor and normal tissue (excluding thymus), and this observation might also have an immunological basis.

1028251

TABLE 29

ASSOCIATION OF ^{67}Ga WITH INTERMEDIATE GEL-FILTRATION PROFILES FROM AQUEOUS EXTRACTS OF RAT THYMUS

Tissue	Percent ^{67}Ga in intermediate zone of gel-filtration profiles	
	1.2-1.0 x 10^4 MW peak	5.0-4.0 x 10^4 MW peak
Fischer Rats		
Thymus (normal)	9	41
Thymus (in animal with RFT tumor)	10	26
Buffalo Rats		
Thymus (normal)	21	35
Thymus (in animal with 5123C hepatoma)	28	23

A comparison of the extractabilities and general properties of the ^{67}Ga-, ^{111}In- and ^{206}Bi-macromolecular complexes found in both normal and tumor tissue is now in progress. Like ^{67}Ga and ^{111}In, ^{206}Bi also has tumor-localizing properties. The behavior of ^{111}In is similar to that of ^{67}Ga when organelle-disruptive treatments are used on tissue samples; 75-90% of the ^{111}In present in the intact tissue is released in a nonultrafilterable form into the "soluble" supernatant fraction after ultracentrifugation. Bismuth-206 activity is released to the extent of only 50-65% by this type of treatment. Like ^{67}Ga, the macromolecular complexes of both ^{111}In and ^{206}Bi are stable on standing at 5° C (1 week) as judged by lack of ultrafiltration of activity. Contrary to the results obtained with ^{67}Ga, both ^{111}In and ^{206}Bi macromolecular complexes are stable to heat (60° C for 2 hr) and alkaline conditions (to pH 10.0), again as judged by lack of ultrafiltration of radioactivity. The buffer agents mes, tris, bis tris, barbital-acetate, pipes, and imidazole do not affect the integrity of the ^{111}In and ^{206}Bi macromolecular complexes. As with ^{67}Ga, bis tris propane causes a major breakdown of the ^{111}In and ^{206}Bi complexes. The cellular and subcellular tissue destinations of ^{111}In and ^{67}Ga thus appear to be similar in many respects. The distribution of ^{206}Bi will require further investigation before any conclusions can be reached.

Clinical Investigations in Nuclear Medicine

Whole-Body Counting and Thyroid Uptake

The Medical Division uses three whole-body counters of different sensitivities to measure the retention of radionuclides in the body. These are the high-level counter, linear scanner, and low-level counter. Since each system differs in physical characteristics such as crystal size, number of crystals, geometry, and electronics, it is important to know that measurements made with the different counters are in agreement.

During this past year, we (*W. D. Gibbs and H. D. Hodges*) compared the responses of the linear scanner and the low-level counter by measuring 31 patients who contained iodine-131 (^{131}I) within the overlapping ranges of both detectors. Analysis of the 76

matched pairs of data gave a correlation of 0.99. The linear relationship between counters can be expressed as:

$$R_{LLWBC} = 0.998\ R_{LS} + 1.77$$

where R_{LLWBC} is the total-body retention measured with the low-level whole-body counter and R_{LS} is the retention measured with the linear scanner. Both counters were calibrated by the optimum window technique developed earlier at ORAU.

Several laboratories, including our own, have suggested the use of whole-body counting as a measure of thyroid uptake. This technique would result in considerable radiation-dose reduction because only 50 nanocuries of ^{131}I would need to be administered In the 1969 Research Report, (ORAU-110) we presented data that showed a good correlation between the 48-hr whole-body retention and the thyroid uptake of patients. Since that report, we have collected additional data. Table 30 shows the correlation between 48-hr whole-body retention and the 4-, 24-, and 48-hr thyroid uptake. Correlation is good for both the earlier and recent data, although best for the 48-hr thyroid uptake. The change in intercepts is suspected to be the result of a change in the stable iodine intake.

TABLE 30

CORRELATION BETWEEN 48-hr WHOLE-BODY RETENTION AND THYROID UPTAKE

Thyroid uptake measurement at	Coefficient of correlation	
	1965-1969	1970-1973
4 hr	0.882	0.883
24 hr	0.954	0.972
48 hr	0.962	0.977

The relationship of thyroid uptake to whole-body retention can be represented by $R=aT + b$, where R = whole-body retention, T = thyroid uptake, a = slope, and b = intercept. Analysis of the pre- and post-1970 data by regression analysis shows that the slopes are essentially identical; however, there does appear to be a real difference in the intercepts at 24 and 48 hr, Table 31.

TABLE 31

REGRESSION ANALYSIS OF WHOLE-BODY RETENTION AND THYROID UPTAKE

	Slope	Intercept
4 hr		
1965-1969	1.17	17.8
1970-1973	1.21	14.6
24 hr		
1965-1969	1.00	10.9
1970-1973	1.05	6.21
48 hr		
1965-1969	1.07	9.45
1970-1973	1.09	4.60

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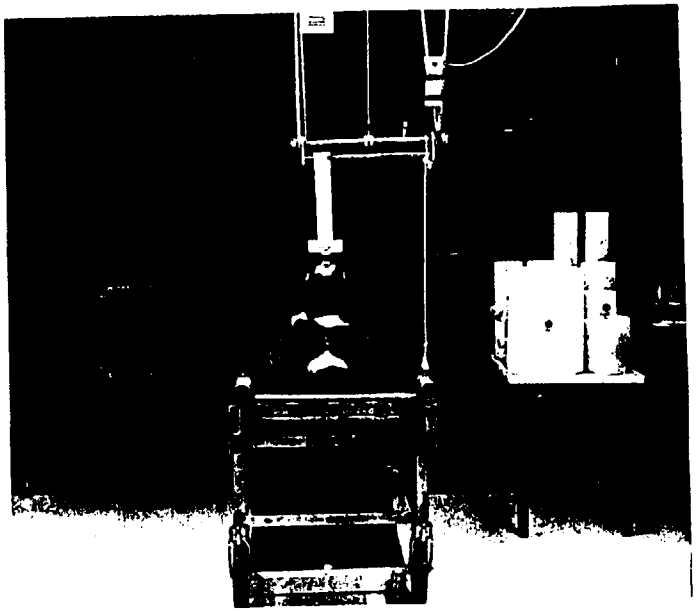


Fig. 37 Thyroid-uptake and whole-body-counting systems.

Although thyroid uptake measured by whole-body counting may be in slight error in patients with impaired kidney function, these patients can be measured by the standard thyroid-uptake procedure. Whole-body retention measurements do not suffer from possible errors associated with thyroid uptake, such as misplaced or inadequate "B" filter or abnormal extensions of functioning thyroid.

To simplify making thyroid uptake and whole-body count almost simultaneously, we have suspended our thyroid-uptake system directly above the whole-body counter stretcher (Fig. 37). The hinged support allows both vertical and lateral movement of the detector system used for thyroid-uptake measurements. After counting the thyroid, the detector is raised and the patient is rolled into the low-level whole-body counter for the retention measurement. Because both measurements are made together, none of the activity can be lost by excretion between measurements.

Indium^{111} and ^{67}Ga for Tumor Scanning

Although ^{67}Ga scanning is of proven value for detecting tumors in a number of clinical situations, its applicability is limited by 1) the amount of ^{67}Ga retained in the bowel and the difficulty in distinguishing this from activity in abdominal lesions: 2) its nonspecificity for tumors, e. g., uptake in inflammatory lesions; and 3) failure of ^{67}Ga to localize in many tumors. We are therefore looking for other radionuclides and pharmaceuticals that may prove to be more effective tumor-localizing agents that can be used to supplement ^{67}Ga scans in tumors that fail to take up ^{67}Ga.

ong the nuclides reported by others as having tumor-localizing properties is ^{111}In. Scans made with the shorter half-lived ^{113m}In in 1968 indicated that indium probably would be less effective than ^{67}Ga, although, because of the short half-life, we were unable to carry out the study for more than 3 to 5 hr instead of the 48 hr recommended for ^{111}In and ^{67}Ga. Hayes, et al., have looked at ^{114m}In and ^{111}In in a number of animal tumor models and have concluded that better target-to-nontarget ratios were obtained with ^{67}Ga (60). Since then, reports have appeared in the literature alleging tumor detection with ^{111}In superior to that seen with ^{67}Ga. Some superiority can, we know, be attributed to its decay scheme and its gamma emissions of nearly ideal energy for scanning. A study is now in progress (C. L. Edwards, G. A. Andrews, R. L. Hayes, W. D. Gibbs, J. E. Carlton) involving simultaneous direct comparisons in patients of the distribution of ^{111}In chloride with that of ^{67}Ga simultaneously and also assaying blood, urine, and tissues for both nuclides.

Indium-111 decays by electron capture with a T 1/2 of 2.8 days giving off two gamma rays of 173 KeV (89%) and 247 KeV (94%) (Fig. 38). On the other hand, the gamma-ray spectrum for ^{67}Ga is complex. When the two spectra are superimposed, it is possible to see that the 247-KeV photopeak of the ^{111}In may be separated from the 296-KeV photopeak of ^{67}Ga by using very narrow spectrometer windows (Fig. 39). Phantom studies have been made with small sources of the two nuclides using dual spectrometer settings of 285 to 305

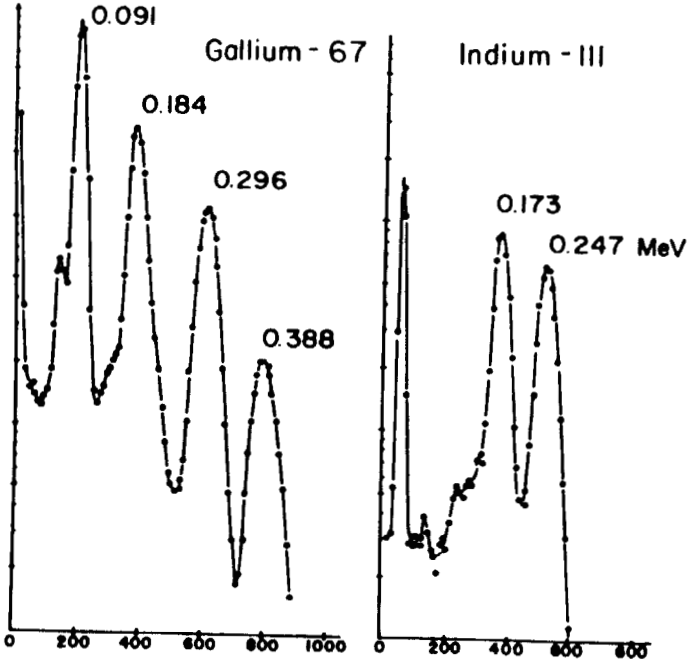


Fig. 38 Spectra of gamma emissions from gallium-67 and indium-111.

1028253

KeV for ^{67}Ga and 233 to 253 KeV for ^{111}In; one scan thus shows mainly the ^{67}Ga distribution and the other that of ^{111}In. So that both scans would have approximately the same information density, and the count rate for any tissue assay would be similar for both nuclides, it was necessary to administer approximately four times as much ^{67}Ga as ^{111}In. That this would be true can be seen from the relative abundance of the 247 KeV photon per millicurie of ^{111}In (94%) compared to that of the 296-KeV photon of the ^{67}Ga (17%).

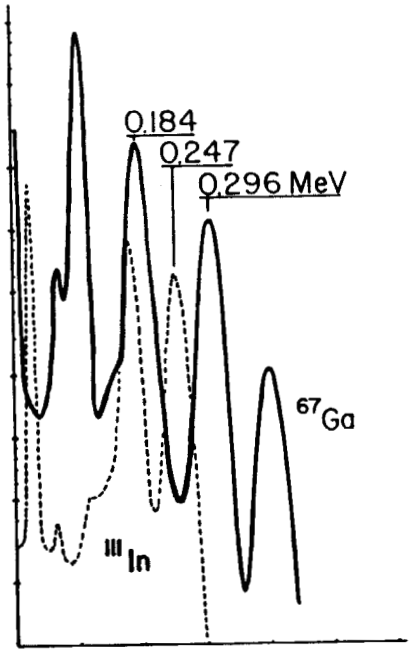


Fig. 39 Gamma-ray spectra of ^{111}In (shaded) superimposed on spectra of ^{67}Ga showing the separation of the 0.247 KeV ^{111}In photopeak from that of the 0.296 KeV ^{67}Ga photopeak.

Six patients were given ^{111}In and ^{67}Ga simultaneously for studies this year (0.011 millicurie per kg and 0.045 millicurie per kg, respectively). Patient 4, a 48-year-old man with disseminated malignant lymphoma and fatal marrow failure, died 18 hr after the injection. Assays of his various organs were obtained for both nuclides (Table 32). These values showed a higher concentration of ^{111}In in terms of percent of administered dose per kg for all tissues except kidney and pancreas. This may be in part due to a greater intestinal excretion of the ^{67}Ga. The two patients on whom we had urinary excretion data both showed a greater excretion of ^{67}Ga dose than ^{111}In, particularly during the first 24 hr. Likewise, the ^{111}In blood levels at the time of scanning (48 hr after the dose) were higher than that of ^{67}Ga in two patients on whom we had this data.

Excised tumor tissue from three patients (2, 3, and 5) and biopsied tissue containing

TABLE 32

TISSUE CONCENTRATIONS OF SIMULTANEOUSLY ADMINISTERED ^{67}Ga AND ^{111}In IN ONE PATIENT AT AUTOPSY

Tissue or organ	Earlier reported ^{67}Ga series*	^{67}Ga %/kg†	^{111}In %/kg†	Ga/In‡
Spleen	0.4 –10.2	1.91	3.50	0.55
Kidney cort.	0.7 – 8.4	18.29	15.42	1.19
Adrenal	0.6 –11.5	3.18	6.57	0.48
Marrow	0.7 – 9.9	2.17	3.23	0.67
Liver	0.6 – 5.2	2.17	3.92	0.55
Bone	0.04– 3.5	1.91	2.24	0.85
Lymph nodes	0.2 – 6.6	2.70	3.39	0.80
Pituitary	1.3 – 1.6	1.48	3.23	0.66
Lung	0.3 – 3.0	1.99	3.58	0.56
Prostate	0.5 – 3.2	1.38	1.59	0.87
Skin	0.3 – 2.6	1.83	2.23	0.82
Thyroid	0.1 – 2.2	2.54	2.86	0.89
Pancreas	0.3 – 1.3	1.70	1.70	1.00
Testes	0.9 – 1.1	1.91	2.65	0.72
Bladder	0.2 – 2.6	1.80	2.23	0.81
Diaphragm	0.2 – 1.3	0.91	1.17	0.78
Heart	0.1 – 1.1	0.96	2.01	0.48
Muscle	0.03– 1.1	0.49	0.56	0.88
Brain	0.0 – 0.5	0.06	0.18	0.33
Whole blood	–	1.84	4.84	0.38

* Nelson, B. et al, J Nucl Med, 13:92, 1972.
† Percent of injected dose/kg body weight corrected to the standard 70 kg man.
‡ Based on the percent of the dose/g of tissue, not on mCi/g.

The administered doses were 3.34 mCi ^{67}Ga and 0.83 mCi ^{111}In.

no tumor but obtained as part of a staging laparotomy for Hodgkin's disease in patient 6 were assayed for both nuclides. The tumor assay values are shown in Table 33. Only in the adenocarcinoma was the concentration of either nuclide significantly greater than the concentrations in normal tissues shown in Table 34.

Scans were made at 48 hr on five of the patients receiving both isotopes. In two patients with malignant lymphocytic lymphoma, tumors were not seen on the scans.

TABLE 33

TUMOR CONCENTRATIONS OF SIMULTANEOUSLY ADMINISTERED ^{67}Ga AND ^{111}In

Patient	^{67}Ga*	^{111}In*	$^{67}Ga/^{111}In$*
No. 2 Malignant lymphoma, poorly differentiated, lymphocytic	0.2	0.8	0.25
No. 3 Poorly differentiated adenocarcinoma, probably primary in pancreas	21.9	5.02	4.4
No. 5 Malignant lymphoma, well-differentiated type	0.7	1.8	0.4

* Percent dose/kg of tumor corrected to the standard 70 kg man.

1028254

TABLE 34

TISSUE CONCENTRATIONS OF SIMULTANEOUSLY ADMINISTERED ^{67}Ga AND ^{111}In IN ONE PATIENT WITH HODGKIN'S DISEASE

Tissue	^{67}Ga*	^{111}In*	$^{67}Ga/^{111}In$*
Liver	6.2	9.1	0.7
Spleen	4.8	9.8	0.5
Lymph nodes	1.5	3.0	0.5
Whole blood	0.7	1.5	0.5

*Percent dose/kg corrected to the standard 70 kg man.

mediastinal Hodgkin's tumors of patient 6 were seen somewhat better on the ^{67}Ga than the ^{111}In scans (Fig. 40). Two adenocarcinomas were much more apparent on the ^{67}Ga scans (Fig. 41 and 42) than on ^{111}In scans.

These preliminary studies show the feasibility of simultaneous scanning for these two nuclides as well as radioassay of tissues to permit direct comparison of their tumor-localizing properties in humans. While in these patients ^{67}Ga showed a much greater tumor affinity, generalizations regarding the relative merits of the two radionuclides must await further comparative studies.

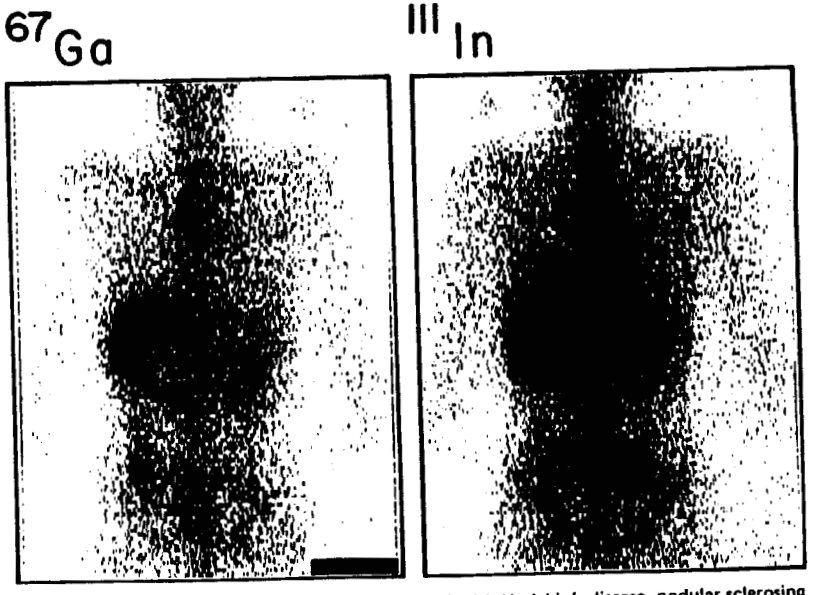


Fig. 40 Torso scans of a 31-year-old man (patient 6) with Hodgkin's disease, nodular sclerosing type, showing the ^{67}Ga distribution (left) and the ^{111}In distribution (right) made simultaneously. The tumor is located in the superior mediastinum. Small reference sources of ^{67}Ga and ^{111}In are included in the scans over the left and right shoulders respectively.

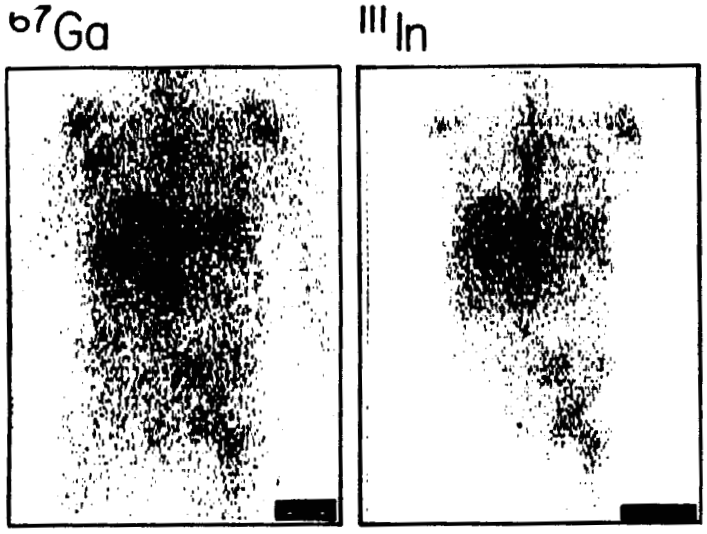


Fig. 41 Simultaneous torso scans of a 55-year-old woman (patient 1) with mediastinal poorly-differentiated adenocarcinoma, metastatic from the breast, showing the uptake of ^{67}Ga in tumor of the right axilla, the spine, and epigastrium. There is relatively little uptake of ^{111}In by the tumor.

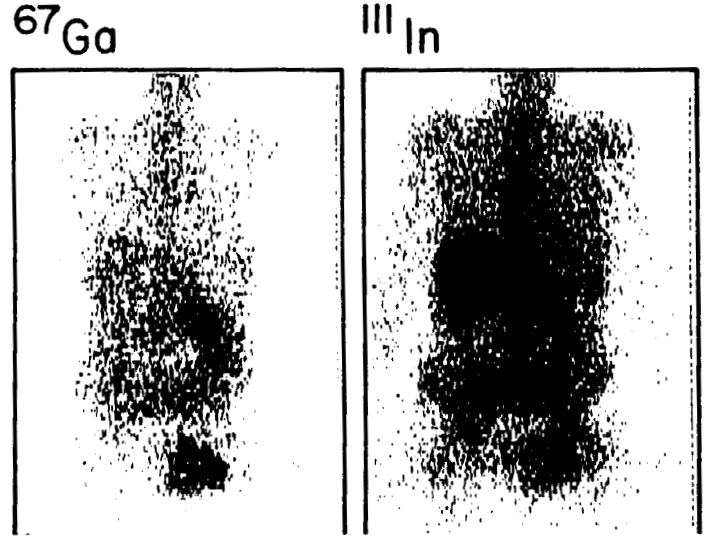


Fig. 42 Simultaneous torso scans of a 71-year-old woman (patient 3) with poorly-differentiated adenocarcinoma of the pancreas or stomach, metastatic to the left femoral region. Both nuclides are concentrated in the tumor although the ^{67}Ga uptake is much higher (Table 33).

1028255

The Cooperative Group to Study Localization of Radiopharmaceuticals

This cooperative group was established in 1970 under the sponsorship of ORAU. It arose because of the need to establish fairly promptly the relative merits of groups of radiopharmaceuticals that may be used for the same clinical purposes. In the absence of such a cooperative effort, reports from different individual institutions tend to give conflicting results, and it may be impossible to determine whether the differences are a real indication of the value of the pharmaceutical or are a misleading result of small sample size, patient selection, details of preparation of the radiopharmaceutical, variations in scan performance or interpretation, or some other factor peculiar to one institution. The cooperative group attempts to standardize variables as much as possible, and, because of the large size of the patient populations available, can obtain statistically significant results fairly promptly.

The experience of the group has been one of remarkably good interaction and cooperation. Members attend meetings, often on weekends, at considerable sacrifice of time. Problems of standardization and consistency have been greater than originally anticipated, but much progress has been made in meeting them. Members have found the group effort useful beyond attempting to fill the stated objectives; the interactions tend to provide information exchange and insight into, for example scan interpretation – values applying to nuclear medicine in general.

The initial focus of the group has been on tumor-localizing radiopharmaceuticals, and the initial preparation studied has been ^{67}Ga citrate. The objectives have been (1) to make an adequate assessment of the usefulness of ^{67}Ga and (2) to have a bank of data on it against which other competing nuclides could be tested for their relative merits. In this effort, we are seeking mainly a means of detecting soft tissue tumors that are ordinarily not found by specific nuclear medical procedures previously developed for liver, brain, bone, etc. (However, the ^{67}Ga does show some usefulness in these sites as well as those soft tissue areas ordinarily not subject to assessment by other methods.) As a part of this study, the Medical Division has shipped out many millicuries of ^{67}Ga, made at Oak Ridge National Laboratory and processed for clinical use here. This has been a new experience for us; previously processing of radiopharmaceuticals had been restricted to some of our own needs. Over 3,000 studies have been done at the 17 cooperating institutions and more than two-thirds of these have already been reported in a standard format and are in the Medical Division's computer data bank. This work has been financed to a large extent by local resources at the participating institutions; they have supplied all local costs except the isotope itself. The costs for the ^{67}Ga, the meetings, and the central data service at ORAU have been paid by the AEC and by two grants, one from the National Cancer Institute and one from the American Cancer Society. The latter two grants will run out shortly after the end of 1973; renewals have been requested.

The group's current objectives are to complete the reporting of the gallium data and then to start a comparative study with ^{111}In which may be used either as the chloride or as a tag from the anticancer drug bleomycin.

The group has been organized in a democratic fashion. Robert Greenlaw played a large role in initiating it and is now its chairman. Ralph Kniseley, as secretary, provided much of the leadership at ORAU, serving as secretary until his leave of absence, which began in August 1973. With his departure, Gould Andrews has taken on these duties with the

help of Lowell Edwards. The rest of the current Executive Committee includes: Charles Boyd, A. B. Brill, Frank DeLand (Vice Chairman), Robert Greenlaw (Chairman), Thomas Haynie, David Teates, and Gerald Johnston (ex-officio).

A considerable amount of information about protocols and data collection techniques is given in last year's Research Report (ORAU-122). There are also two special ORAU reports on procedures:

Kniseley, R. M. *Coding index for tumor scanning.* USAEC Report ORAU-119, Oak Ridge Associated Universities, 1973.

Kniseley, R. M. *Cooperative group to study localization of radiopharmaceuticals.* USAEC Report ORAU-118, Oak Ridge Associated Universities, 1973.

Three preliminary papers have been accepted for publication in the *Journal of Nuclear Medicine*. They represent only a portion of the data collected; they are based on untreated cases and do not include any of the many follow-up studies or the additional untreated cases studied after the preparation of the manuscripts. These papers are as follows:

1. Johnson, G., Benua, R. S., Teates, C.D., Edwards, C. L., and Kniseley, R. M. *^{67}Ga citrate imaging in untreated Hodgkin's disease:* Preliminary Report of Cooperative Group.

   Abstract: In 151 untreated patients with Hodgkin's disease approximately 90% had one or more positive sites demonstrated on scan. Of histologically proven or clinically evident sites of tumor known by means other than scanning, 73% were positive. A "false-positive" rate of less than 5% was discovered. Of 249 lesions proven histologically or obviously apparent by other means, 21% were first detected by ^{67}Ga, although the sequence of procedures in the patient's workup may have some influence on this number. The percentage of positive scans is highest in the neck and chest. Gallium-67 imaging appears to have a place in staging Hodgkin's disease but a negative scan does not exclude the presence of disease. In comparison with lymphangiography it appears to be slightly more sensitive, but each of these procedures found lesions missed by the other.

2. Greenlaw, R. H., Weinstein, M. B., Brill, A. B., McBain, J. K., Murphy, Linell, and Kniseley, R. M. *^{67}Ga citrate imaging in untreated malignant lymphoma:* Preliminary Report of Cooperative Group.

   Abstract: In 167 cases of previous untreated malignant lymphoma (other than Hodgkin's disease) scanned with ^{67}Ga, approximately 78% had one or more positive sites demonstrated on scan. Of histologically proven sites, 51% had a positive scan and with those sites apparent or suspected but not biopsied, 54% were positive. Thus, a negative scan does not exclude the presence of disease. A low "false-positive" rate was encountered; if a lesion is demonstrated by scan, it is highly likely that disease is present. Disease, unsuspected or believed absent, was first known at the time of scan in 28 sites. An additional 42 positive scan sites as yet unconfirmed by other evidence may also represent lesions discovered de novo by ^{67}Ga. The percent of positive scans is highest in the thorax. Histiocytic types, including mixed cell types, of lymphoma have higher rates of positive scans than lymphocytic types. Overall, the rate of positive scans is higher in Hodgkin's disease than in other lymphomas.

3. DeLand, F.H., Sauerbrunn, B. J. L., Boyd, C., Wilkinson, R. H., Jr., Friedman, B. I., Moinuddin, M., Preston, D. F., and Kniseley, R. M. *^{67}Ga citrate imaging in untreated primary lung cancer*: Preliminary Report of Cooperative Group.

   Abstract: In 172 untreated patients with primary lung cancer, approximately 84% had one or more sites demonstrated on scanning with ^{67}Ga; 80% of individual lesions histologically verified had positive scans. It is occasionally possible to detect rather small lesions and, conversely, on occasion to miss some large lesions (5 cm or larger). Negative scans were obtained in 16% of lesions histologically proven. In the present analyses, rates of detection differ somewhat according to histologic type, but not strikingly. With further developments in tumor-localizing agents and instruments, the agent may eventually be useful in the initial diagnostic workup of suspected lung carcinoma in certain clinical situations. As a single screening scan procedure, it should prove useful in assessing the extent of disease before surgery or other type of therapy.

At the present time a number of additional manuscripts are under preparation by members of the group.

As a part of the cooperative group effort, a pathology review committee has been set up to look at histopathology of lymphomas and Hodgkin's disease in an effort to assure uniform classification.

Lymphoma Pathology Panel

As part of the project for evaluating ^{67}Ga for tumor scanning, the Cooperative Group to Study Localization of Radiopharmaceuticals (CGSLR) set up a panel to review histologically the study cases classed as malignant lymphoma. Correlations between ^{67}Ga localization and tumor histology are of interest for all tumors, but malignant lymphoma, which includes Hodgkin's disease and lymphatic leukemias, is of special concern because of previous findings that ^{67}Ga concentrated in histiocytes and that different types of lymphoma vary greatly in ability to concentrate ^{67}Ga. Modern lymphoma classifications, particularly for Hodgkin's disease, have considerable advantage over older schemes and can be used to rank the neoplasms by prognosis, whether related to the grade of malignancy or to host resistance.

The operational plan of the pathology panel (Ralph M. Kniseley, C. C. Lushbaugh, and Bill Nelson) was devised at the suggestion of the National Cancer Institute, in con-

1028256

sultation with pathologists experienced in similar lymphoma pathology panels. Physicians in CGSLR were asked to send histologic material and a copy of the original pathology report of their study cases of malignant lymphoma to the ORAU Medical Division. Here they were registered and prepared for pathology review. The material received by January 1, 1974 from patients scanned with ^{67}Ga at the 17 cooperating nuclear medicine facilities consists of 207 acquisitions. Each of these acquisitions usually included multiple slides or paraffin blocks (or both) from operative procedures. In some cases more than one diagnostic surgical procedure had been done. First, all of the slides were examined independently by two ORAU pathologists without knowledge of previous diagnoses or clinical information. These independent reports were then compared with the original diagnosis and pathology report. When a disagreement resulted, the slides were reviewed by the ORAU pathologists and if not resolvable, the concurrence of two of the three opinions was the diagnosis considered a consensus, which was then coded for computer file and subsequent comparison with results of scans. When the three opinions were at odds or when the ORAU diagnosis conflicted significantly with the referral diagnosis, the slides were reviewed by John Shively, College of Medicine (Memphis). In the few cases where his opinion did not produce a majority, C. W. Berard (NIH, Bethesda) made the final diagnosis.

Acquisition of specimens from all cases is incomplete and so the use of these revised diagnoses with the results of ^{67}Ga scans would be premature. An evaluation of the results of the Lymphoma Pathology Panel review up to now showed that six (3%) of the 207 acquisitions were not considered to be malignant lymphomas. Material received so far from three of these six patients is not diagnostic of neoplasm; the other three patients were considered to have a neoplasm other than malignant lymphoma.

In 105 (50.5%) of the 207 acquisitions, the diagnosis reached by the panel differed from the original. As expected, the biggest source of disagreement arose from the Panel's use of a diagnostic scheme slightly modified from the *Manual of Tumor Nomenclature and Coding* of the American Cancer Society, while many of the contributing pathologists retain older classifications that do not include a breakdown of Hodgkin's disease into subtypes, do not specify cellular components of so-called lymphosarcoma, or do not distinguish between the nodular and diffuse patterns. In those cases represented by two or more acquisitions the diagnosis almost invariably remained the same. Only one case showed the well-known transition from the nodular to the diffuse type of lymphoma and this occurred during a period of 7 years. The quality of the preparations was sometimes a problem and this was especially noticed in classifying the non-Hodgkin's lymphomas by cell type. A common source of controversy was found in distinguishing lymphocytic from histiocytic neoplasms: Abnormal histiocytes are usually seen in a lymphocytic lymphoma; histiocytic lymphomas often included lymphocytic components, some of which were poorly-differentiated. In part because of a postulated role of histiocytes in ^{67}Ga localization, we retained the "mixed-type" category even though Lukes argues that the prognosis is essentially that of a histiocytic lymphoma, and regards "significant numbers" of abnormal histiocytes as indicative of the histiocytic type of malignant lymphoma, assuming no evidence for Hodgkin's disease.

The occasional difficulty of distinguishing Hodgkin's disease from another lymphoma, in particular the distinction of the more malignant varieties, was not a serious problem in this review. The three acquisitions coded as the rare lymphocyte-depleted type of Hodgkin's disease (formerly classified as Hodgkin's sarcoma) were considered Hodgkin's disease by all pathologists, while only two (5%) of the 41 acquisitions coded as

1028257

the histiocytic type of malignant lymphoma (formerly "reticulum cell sarcoma") were called Hodgkin's disease by contributing pathologists.

Within the classification of Hodgkin's disease (84 acquisitions) the Panel diagnoses were often more specific than those of the contributor. In many cases, the contributing diagnosis was "Hodgkin's granuloma" or merely "Hodgkin's disease," and these were fairly consistently reclassified by the ORAU panelists as either the nodular sclerosing (37 acquisitions) or mixed-cell type (27 acquisitions).

At present, less than half of the lymphoma cases under study by CGSLR have been reviewed histologically by this pathology panel, or by Drs. Berard and Lieberman who are reviewing independently at their own institutions. The fact that we reclassified half of the cases we reviewed for the sake of obtaining uniformity in diagnostic terms suggests that a similar result would be seen in cases not yet reviewed. Whether or not this procedure is worth the effort will, however, be determined retrospectively when the group determining gallium localization by scanning procedures determine whether the type of lymphoma has an effect on gallium localization. If no correlation is found, our review of all the cases may not be needed, and the modern lymphoma classification will have been shown to have limited usefulness in this particular clinical context. It may well be that a quantitation of certain cell types (as has been shown to improve the prognostication of the Rye Classification of Hodgkin's Disease) would show a better correlation with gallium uptake. Even crude estimations of numbers of lymphocytes and histiocytes might be worthwhile in this respect, and could be done by the ORAU panel on slides retained in the registry.

Computer Methods

Computer Support Systems

The computer group functions primarily as a core facility to research areas. Because of limited personnel and equipment, all computing resources must adhere to priority scheduling; however, the facility and staff are made available to other ORAU divisions when priorities permit. At present, the group is providing service to the Manpower Development Division and the University Programs Office.

In October of this year, an additional 8k of core was added to the IBM 1800. This increased the core size to 32k, and enabled us to complete conversion from the Time Sharing Executive (TSX) to the Multiprogramming Executive (MPX). The conversion from TSX to MPX has been costly in many ways, but we needed to run under MPX to support more disk space (2311 drive) and to gain flexibility by partitioning core. Until we obtained the additional core, we ran under two operating systems. The primary conversion cost has been programmer time since much effort has been spent on converting operating systems. Some Fortran programs had to be reworked for MPX and assembler programs using input/output functions had to be rewritten. The low-exposure-rate total-body irradiator data acquisition program was the last major program converted to MPX in late 1973 and we no longer run under two executive systems.

With the additional core, our immediate horizons have been greatly lifted, and we are now in the final stages of developing a system which will have direct impact on several areas of Medical Division research. The design of this system has been completed and three development areas are nearing completion with final testing scheduled for early 1974. The system, which integrates the facilities of the IBM 1800 and the PDP-11, will

greatly increase utilization of both computers.

The PDP-11 will run BASIC, handle buffering for multiple terminals and collect data from the Ohio Nuclear Scanner. Three teletype terminals are in the offing with sites to be located in the Hematology Laboratory, Medical Records, and at the Ohio Nuclear Scanner. The system has been designed for expansion, and additional terminals may easily be added. The immediate limitation in the PDP-11 will be core size.

The IBM 1800 will collect data, maintain limited on-line files for retrieval, and dump data to magnetic tape for permanent storage. The most immediate limitation in the IBM 1800 will be mass storage space.

The system has been divided into three areas for synchronous development. The first objective was to set up communications between the PDP-11 and the IBM 1800 (M. S. Hansard and T. E. Akin). This has been done, although minor modifications are yet to be completed. The second aim was to file the data by means of process programs in the IBM 1800 (T. E. Akin). Here the most important factors are generality and expandibility. The third objective was to dump data, purge files when necessary and keep backup available in case of failure (J. S. Ishee). These three steps will be completed early in 1974 and it is expected that the system will be immediately expanded by more terminals and more capability on existing terminals for such activities as retrieval.

During the last half of 1973, utilization of the computer averaged 250 hours per month which is a 40% increase over the first half of the year. The most significant increase was due to usage of a BASIC terminal connected to the PDP-11. Use of this terminal increased from a total of 6 hr in July to greater than 50 hr in December. One of the teletype terminals that will become operational during the first quarter of 1974 will capture laboratory data at its source for inclusion in an interactive data bank and the second, in our Medical Records Office, will capture other data and serve as a data recovery unit.

A brief retrospective look at our computer operations illustrates best how this central facility has extended the research capabilities of ORAU. The highly successful studies for the National Aeronautics and Space Administration were possible because of our utilization of the ORAU inhouse computer and a larger Oak Ridge National Laboratory computer. The significant ^{67}Ga cooperative studies with 17 participating institutions was undertaken with confidence in our ability to collect, store, and analyze large data banks. This work is currently actively progressing. The same resource is making a necessary and useful contribution to the AEC-FDA-sponsored Internal Dosimetry of Radiopharmaceuticals Information Center.

During 1973, we developed a computer program for making valuable contributions to the analysis of data collected in clinical research. The program (J. S. Ishee) is a generalized plotting program that uses the hematology and chemistry data for input (Fig. 43). A typical use of this program has been to generate graphs of laboratory test results on selected groups of patients with different types of treatment for chronic granulocytic leukemia.

During the year, a number of research areas have been aided by technical personnel writing their own analysis programs using the PDP-11 BASIC language. This type of utilization will increase considerably when additional BASIC terminals are installed.

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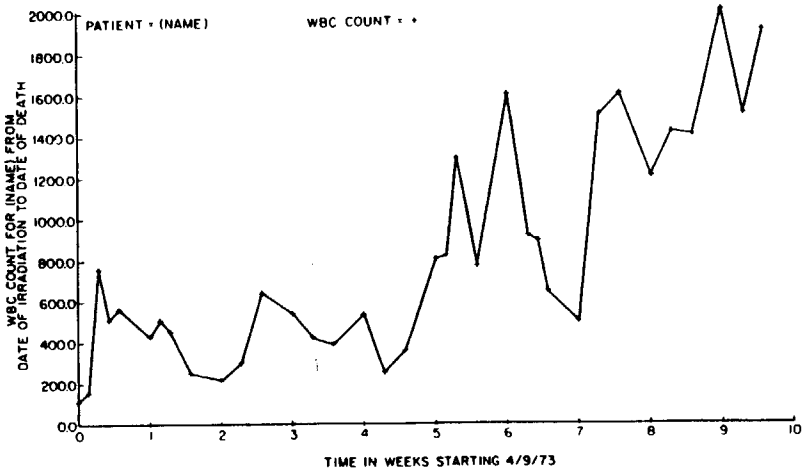


Fig. 43 White blood cell count of a bone-marrow transplant patient from the date of irradiation until the date of death. This graph was made from hematology results stored on magnetic tape and plotted by an IBM 1627 plotter.

BASIC STUDIES IN RADIATION, NEOPLASIA, IMMUNOLOGIC RESPONSES, AND METABOLISM

These investigations arise from our primary missions in cancer and radiation effects. They take advantage of opportunities that arise in our particular institutional arrangement from the talents of investigators on our staff, the opportunity for close clinical cooperation within the Division, and the unusual opportunities for collaboration with the Oak Ridge National Laboratory and the University of Tennessee, Knoxville. Some of this basic work arises to fill a need disclosed by the findings in clinical research programs; thus the clinical group may be either a provider of opportunity for, or a seeker of help from, the scientists doing fundamental laboratory research.

Summary of Accomplishments

In a continuing project focused on biochemical pathways that are prominent in cancer cells, the biological chemistry group detected several new enzymatic activities that permit interconversions and alternate biosynthetic routes for key intermediates in the metabolism of the ether-linked lipids that are characteristically elevated in cancer cells. Three significant results in this area are: (1) the enzyme catalyzing the unique alkyl-acyl displacement reaction (the first step in ether lipid biosynthesis) is not very selective for the alcohol; (2) the desaturase that catalyzes the conversion of an alkyl lipid to a plasmalogen is similar, but not identical, to the desaturases that insert double bonds into fatty acids; and (3) a catabolic pathway (lysophospholipase D) has been detected in mammalian cells that appears to provide an important mechanism for the redistribution of glycerolipid moieties.

For the first time, data have been obtained that suggest a possible function for the ether lipids. The close association of essential fatty acids with ether lipids, apparent from earlier studies of tumor lipids, has led to experiments that clearly show that the ether-linked glycerolipids can serve as a reservoir for polyunsaturated fatty acids in animals that are fed diets deficient in essential fatty acids.

As the enzymatic reactions responsible for the metabolism of the ether lipids are becoming better understood, it has been possible to initiate an investigation of the regulatory mechanisms and control points involved. During the past year a system was developed and characterized that serves as a useful model for evaluating the regulatory features that make tumor cells different from normal cells with respect to their ether and ester contents. In this system, L-M fibroblasts can be manipulated so that the levels of ether lipids are varied according to the growth conditions.

A new direction in the biochemistry program is the pulmonary surfactant project, which has an emphasis on cancer and irradiation. Results obtained during 1973 have shown how the unusual surfactant molecule is synthesized by an enzyme preparation from alveolar type II cells of a lung adenoma.

In the marmoset research program a study aimed at identifying leukocyte antigens led unexpectedly to development of an 'autoimmune-like' disease phenomenon

1028259

...nbling idiopathic thrombocytopenic purpura (ITP). Isoimmunization of marmosets with white blood cell-platelet preparations has consistently resulted in thrombocytopenia and variable anemia, often leading to death of the animal. The finding of antibody-to-donor platelets in the immunized recipients suggests this system to be analogous to ITP in man, pathogenically considered to be an autoimmune process. Surprisingly, a search of the literature has revealed the lack of a suitable animal model for this disease, and further investigation of the marmoset seems warranted, particularly in view of the twinning-chimerism phenomenon which will permit a study of the cellular immune aspects of the problem. In colony husbandry, an analysis of data collected over a five-year period has shown that the parturition time of marmosets can be predicted with a fair degree of accuracy. Using an external palpation technique which permits measurement of the enlarging uterus during pregnancy, we have found that, in over 60% of detected pregnancies the parturition time of full-term live twins can be estimated to within ±5 days. This technique therefore provides us with a fairly reliable measure of the stage of gestation of a pregnant marmoset, which should be extremely helpful in future studies that will be concerned with manipulation of the fetal and neonatal immunologic systems of the marmoset. Collection of autopsy data has continued to yield useful information. This year a study was made of the development of teeth in relation to age of colony-bred animals and showed that dentition can be used as a reliable indicator of age. This data will have application to newly imported young animals, the ages of which until now could only be guessed at or roughly estimated. An additional case of colon cancer in an *S. oedipus* marmoset was found this year, bringing to eight the number of spontaneous colonic cancers detected. This cancer has yet to be found in the *S. fuscicollis* species of marmosets, the primary animal maintained in our colony. Whether this represents a species difference in sensitivity to carcinogenic agents in our colony environment or to factors indigenous to the native habitats of these two species is yet to be resolved.

In the ultrastructural anatomy program further studies on ^{67}Ga in tumor-bearing animals showed that the initial uptake of the gallium was not a good quantitative indicator of the mass of tumor present and that after it was taken up by the cancer cells, its rate of excretion was not a reliable indicator of tumor-cell death. Studies on ^{67}Ga uptake in peritoneal cells of normal mice showed uptake in monocytic cells, but the relative uptake in various types of cells indicated that the mechanism was not one of simple phagocytosis. Another study brought further elucidation of the previously reported fact that after total-body irradiation the retention of ^{67}Ga is decreased: there is a particularly rapid fall in the blood levels of the nuclide in the irradiated animal.

During the past year the cytogenetics program has completed a massive statistical analysis of the effect of oral contraceptives on chromosomes. The results are too complex to summarize; in general, oral contraceptive users averaged more chromosome breaks in lymphocyte cultures than did non-users. The large bank of data on normals showed important differences among persons and in a given person at different times of study. It seems likely that the possible range of normals has been too narrowly interpreted in the past by investigators who used normals deliberately selected for a low incidence of chromosome breaks. The study included pregnant women, and showed an increased number of culture failures believed related to a depression in cell-mediated immunity. Women in their second or subsequent pregnancies, even if they had never used oral contraceptives, had an increased number of chromosome breakages. The cytogenetics group also studied clinical problems; in a patient with pancytopenia and hypercellular, erythroid marrow, C-monosomy was shown in marrow cells and by banding techniques the missing chromosome was identified as number 8.

1028260

This group also reported an in vitro technique for establishing the cytotoxicity of exotoxin from *Pseudomonas* by assaying its effect on cultured mouse L-cells; this might replace more expensive in vivo testing methods.

Studies with ^{67}Ga have yielded a fascinating series of developments related to pregnancy and malignancy, including a striking reduction in uptake of gallium in the thymuses of animals either pregnant or bearing tumors. A marked increase in ^{67}Ga in uterine flushings and placenta was noted when the nuclide was given to pregnant animals. Similarly, certain interesting enzyme changes have been noted in the presence of both pregnancy and cancer.

Biological Chemistry

Introduction

Although the biological chemistry program serves primarily as a research unit, it also plays an important service function in that it furnishes specialized biochemical analyses of clinical specimens. During 1973, specific research projects have dealt with (a) effects of irradiation on biochemical properties of mammalian cells, (b) basic studies on metabolic pathways and membranes in cancer cells, (c) regulatory mechanisms that control lipid and sterol metabolism in cancer cells, (d) biosynthesis of pulmonary surfactant and related biochemical studies in normal and neoplastic lung tissue, and (e) chemical synthesis of metabolic lipid intermediates with carbon-14 (^{14}C) and tritium (^{3}H) tags for use in enzymic studies.

A brief description of the published or essentially completed work for these projects constitutes this progress report. Because of space limitations, the reader is referred to our published articles (cited as footnotes for each project) that contain explicit details about the data obtained and the pertinent sources of related literature.

Radiation-Induced Metabolic Alterations in Adipose Tissue*

Last year our group described a significant metabolic effect that X irradiation causes in adipose tissue, a major energy source in mammals. Using rats as experimental animals, we were able to demonstrate that irradiation seriously impairs the release of free fatty acids from these fat depots. The impaired availability of fat as a source of calories can be responsible for a number of the clinical symptoms associated with the radiation syndrome, and we believe it is important to determine the biochemical mechanisms responsible. For example, it is known that the levels of cyclic AMP can control the rate of release of free fatty acids from triacylglycerols in adipose tissue by influencing the conversion of inactive hormone-sensitive lipase into an active form. However, our recent results show that irradiation does not cause any striking change in the cyclic AMP levels (Table 35). In view of this, we are now investigating other factors that influence the release of free fatty acids from adipose cells. We are determining the effect of X irradiation on the lipid composition of plasma membranes and the endoplasmic reticulum of fat cells to deter-

*Lee, T-c., Stephens, N., Moehl, A., and Snyder, F., a study in progress.

mi... whether it alters hydrophobic interactions in membrane structures, which could effect the release of free fatty acids from adipose tissue. An interesting preliminary observation is that irradiation causes an increase in the choline plasmalogens present in adipose cells. We also plan to determine the level of ATP in the fat pad of control and irradiated animals, since ATP is required to convert inactive hormone-sensitive triacylglycerol lipase to active lipase.

TABLE 35

CYCLIC AMP LEVELS IN FAT PADS OF NORMAL AND IRRADIATED RATS

Animals	Control	X Irradiated	P
	Pmoles/mg tissue		
Female	0.40 ± 0.03 (18)	0.46 ± 0.03 (16)	0.2-0.3
Male	0.30 ± 0.03 (24)	0.31 ± 0.03 (26)	0.8-0.9

The number of samples analyzed are in parentheses.

Numbers preceded by the ± are standard error of means and the probability for a significant difference between the control and X-irradiated values are designated in Column P.

Biosynthesis of Pulmonary Surfactant and Related Lung Studies*

Pulmonary surfactant is the substance that lines the alveolar wall and maintains alveolar stability during compression of the lung. Alveolar collapse ensues when surfactant production is insufficient, as in hyaline membrane disease and pulmonary artery occlusion.

Although it is known that lung surfactant lipid consists mainly of a saturated phospholipid (1,-2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine), instead of the usual unsaturated types of phospholipid, its biosynthesis is still poorly understood. Our interest in the biochemistry of pulmonary surfactants is from the point of view of its role in lung neoplasia and in lung damage induced by irradiation and environmental pollutants.

Recently we found that mouse pulmonary adenomas induced by urethan contain significant quantities (≃28%) of the disaturated choline-containing phospholipids and that at least 60% of this fraction is of the dipalmitoyl (16:0) type species (Table 36). Since the urethan-induced adenoma consists exclusively of alveolar type II cells, we thought that cell-free preparations of this tumor could be an ideal system for carrying out enzymological studies to determine how the unusual structure of the saturated surfactant lipid was formed. Our results revealed that homogenates of the tumors are capable of incorporating large quantities of [1-^{14}C]palmitic acid into the surfactant type lipid, at both the 1- and 2-position of the glycerol moiety (Table 37). Normal lung and squamous-cell carcinoma also incorporated palmitic acid into phosphatidylcholine, albeit at a much slower rate, and the two tumors, like other neoplasms, also synthesized *O*-alkyl lipids from long-chain fatty alcohols. Although the adenoma is a tumor, the facts that its individual cells are similar to normal type II epithelial cells and that its incorporation of [1-^{14}C]palmitic acid into phosphatidylcholine is different from that of the squamous-cell

*Snyder, C. (ORNL), Malone B., Nettesheim, P. (ORNL), and Snyder, F. Cancer Res. 33, 2437-2443, 1973.

1028261

carcinoma indicate that it should be a useful system for elucidating the biochemical mechanisms responsible for the biosynthesis of lung surfactant. The current emphasis in these experiments is to complete kinetic studies that are expected to unravel the mechanism responsible for the biosynthesis of pulmonary surfactant in subcellular fractions of the adenoma.

TABLE 36

FATTY-ACID COMPOSITION OF MOLECULAR SPECIES OF PHOSPHATIDYLCHOLINE IN URETHAN-INDUCED PULMONARY ADENOMA

Molecular Species* (Number of Double Bonds)	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:6
Δ0 (28%)	12	80	0	2.1	4.9	0	0	0
Δ1 (24%)	2.1	42	28	2.4	24	0	0	0
Δ2 (16%)	0	31	3.2	5.8	18	40	0	0
Δ3 or more (32%)	0	35	1.6	7.5	7.8	4.0	26	7.7
Phosphatidylcholine fraction	4.1	52	6.2	4.0	7.5	7.7	12	3.8

* Based on analyses of a pooled sample using phospholipase C, preparation of the acetate derivatives of diacylglycerols, and argentation thin-layer chromatography in $CHCl_3$:hexane (90:10). The values in parentheses represent the weight percentage of the various molecular species of phosphatidylcholine.

TABLE 37

INCORPORATION OF [1-^{14}C] PALMITIC ACID INTO THE 1- AND 2-POSITIONS OF PHOSPHATIDYLCHOLINE AND PHOSPHATIDIC ACID OF THE URETHAN-INDUCED PULMONARY ADENOMA

Incubation samples used for this experiment contained adenoma homogenate, ATP, CoA, and Mg++ in Tris buffer at pH 7.1.

Fraction	Ratio of ^{14}C (2-position: 1-position)
Phosphatidylcholine	2.4,* 2.4, 1.9
Phosphatidic acid	1.2,* 1.2

* Obtained after a 20-min incubation period, whereas the other three samples were obtained after a 90-min incubation period.

Basic Studies on Novel Biosynthetic Pathways in Cancer Cells and Normal Tissues

Considerable work has been published on the glycerolipids of cancer cells. Much of it relates to composition, but in recent years, many metabolic studies have centered on glycerolipids containing ether linkages, since it is this group of lipids that is characteristically elevated in most tumors. Results from our research program have led to the discovery of a number of metabolic pathways that are responsible for the assembly and degradation of ether-linked lipids in cancer cells and in some other cell types that contain ether lipids. In general the anabolic pathways for the ether-linked aliphatic chains occur in almost all cancer cells investigated so far, whereas the catabolic pathways for the ether-linked aliphatic moieties are mainly found in normal tissue, particularly liver. Brief accounts of specific projects completed during 1973 comprise the remainder of this section.

*Pathway for the Catabolism and Redistribution of Phospholipids.** During the course of studies related to the biosynthesis of plasmalogens, we found an enzyme requiring Mg^{++} that has phospholipase D-type activity, but appears to act only on glycerophospholipids containing no ester groups at the 2-position of the glycerol (Fig. 44). Phospholipase D, which removes the base groups from phospholipids, previously had been demonstrated only in plants. The presence of this enzyme in several mammalian tissues, including tumors, provides a pathway for the redistribution of ether (and ester) linkages in different classes of glycerolipids and also provides an important catabolic reaction for phospholipids. The enzyme is also likely to act on glycerophospholipids containing ester groups, and could prove to be an essential pathway in the control of phospholipid metabolism.

Ether Lipids as Reservoirs for Essential Fatty Acids.† In many tumors, we have observed that the ether-linked phospholipids contain unusually high quantities of unsaturated fatty acids at the *sn*-2-position of the glycerol moiety. Since the ether-containing glycerolipids are more resistant to hydrolysis than those containing only ester groups, we wondered if the ether lipids might serve as a reservoir for essential fatty acids, such as arachidonic acid. If this could be established, the implication is highly significant, since essential fatty acids serve as precursors of the prostaglandin hormones and also they may be essential for membrane function.

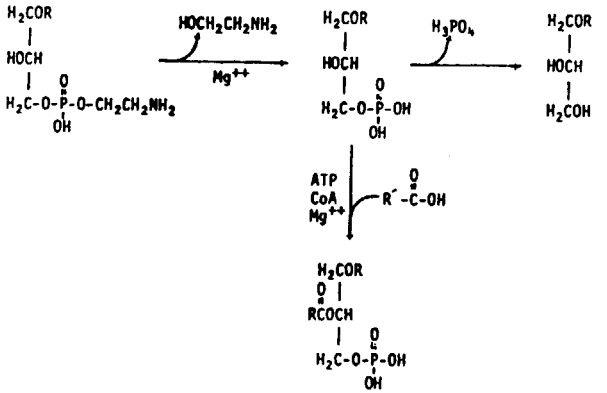


Fig. 44 A lysophospholipase-D pathway in the metabolism of lipids by brain microsomes.

We investigated the possible role of ether-linked glycerolipids as essential fatty acid reservoirs by using a well studied organ (rat testes) that is extremely responsive to essential fatty acid deficiency caused by feeding rats a fat-free diet. Using [^{3}H]arachidonic acid as a tracer, we found that this essential fatty acid was conserved in impressive quantities in the ether lipids, particularly the ethanolamine plasmalogens. Subsequent enzymic experiments revealed that the arachidonic acid was incorporated into preformed ether-containing lipids of the testes by replacement of other ester groups via a deacylation-acylation reaction as depicted in Fig. 45. These studies suggest that the ether-containing lipids may play an important role in the metabolism of essential fatty acids.

*Wykle, R. L., and Schremmer, J. M. J. Biol. Chem. 249, 1742-1746, 1974.

† Blank, M. L., Wykle, R. L., and Snyder, F. Biochim. Biophys. Acta. 316, 28-34, 1973 and Wykle, R. L., Blank, M. L., and Snyder, F. Biochim. Biophy. Acta. 326, 26-33, 1973.

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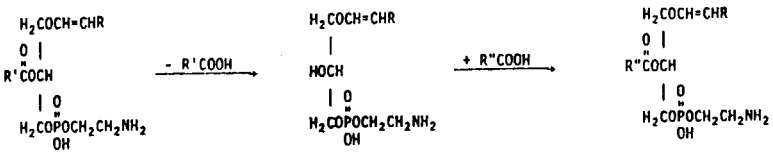


Fig. 45 A deacylation-reacylation reaction involving an ethanolamine plasmalogen as the substrate (R"COOH is arachidonic acid whereas R'COOH is some other less saturated acid).

An Alternate Enzymic Route for the Synthesis of the Alkyl Analog of Phosphatidic Acid Involving Alkylglycerol in Tumors * Phosphatidic acid (1,2-diacyl-*sn*-glycerol-3-P) and its *O*-alkyl analog are key intermediates in the biosynthesis of ester- and ether-linked phospholipids. Earlier work from our laboratory demonstrated that the alkyl analog of phosphatidic acid is formed by enzymic reduction of alkyldihydroxyacetone-P and subsequent acylation. However, during 1973, we obtained evidence for an alternate pathway for its formation from hexadecylglycerol (Fig. 46). The phosphorylation step in this pathway appears to occur via 1-alkyl-*sn*-glycerol phosphotransferase and not via 1-alkyl-2-acyl-*sn*-glycerol phosphotransferase. Futher investigation of the enzyme is being continued to unequivocally elucidate the sequence of the phosphorylation and acylation steps.

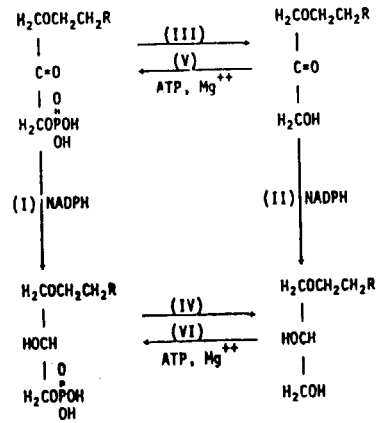


Fig. 46 Phosphotransferase, phosphohydrolase, and reductase activities involved in the metabolism of alkyldihydroxyacetone-P and alkyldihydroxyacetone. The reactions are catalyzed by (I) $NADPH_2$:alkyldihydroxyacetone-P oxidoreductase, (II) $NADPH_2$:alkyldihydroxyacetone oxidoreductase, (III) alkyldihydroxyacetone-P phosphohydrolase, (IV) 1-alkyl-*sn*-glycerol-3-P phosphohydrolase, (V) ATP:alkyldihydroxyacetone phosphotransferase, and (VI) ATP:alkylglycerol phosphotransferase.

*Chae, K. (Research Triangle Park, N.C.) Piantadosi, C. (U. of North Carolina, Chapel Hill), and Snyder, F. Biochem. Biophy. Res. Commun. 51, 119-124, 1973.

This alternate pathway has particular significance in cells that do not have the enzyme to form alkyldihydroxyacetone-P from long-chain fatty alcohols and acyldihydroxyacetone-P. Alkylglycerol phosphotransferase would permit these cells to salvage alkylglycerols generated by degradative enzymes acting on more complex ether-linked glycerolipids and to reintroduce such alkylglycerols into biosynthetic pathways.

*Reductase, Phosphatase, and Kinase Activities in the Metabolism of Alkyldihydroxyacetone Phosphate and Alkyldihydroxyacetone in Cancer Cells.** Kinetic studies with preputial gland tumor microsomes that were completed during the past year demonstrate the existence of a pathway that permits the interconversion of metabolic intermediary ether-lipid products, i.e., alkyldihydroxyacetone-P, alkylglycerol-P, alkyldihydroxyacetone, and alkylglycerol (Fig. 46). This type of investigation was made possible by synthesizing [1-^{14}C]octadecyldihydroxyacetone-P and [1^{14}C]octadecyldihydroxyacetone in the organic laboratory (See last part of this section). These experiments were carried out to establish what interrelationships existed between various metabolic intermediates that we have previously detected in a variety of tumor preparations. Such information was necessary to determine the reversibility and properties of the primary reaction steps (Fig. 46, I-IV) necessary for the biosynthesis of the alkyl phospholipids.

Reactions (I) and (II) (Fig. 46). Alkyldihydroxyacetone-P is reduced to 1-alkyl-*sn*-glycerol-3-P by a microsomal reductase that is highly specific for NADPH and optimally active at pH 7.0. However, higher levels of NADH can substitute for the NADPH. The apparent K_m for the alkyldihydroxyacetone-P reductase is 0.12 mM for NADPH and 3.2 mM for NADH. In contrast, only NADPH is utilized by the reductase that reduces alkyldihydroxyacetone to the alkylglycerol. With the alkyldihydroxyacetone reductase, the apparent K_m for NADPH is 0.38 mM.

Reactions (III) and (IV) (Fig. 46). The phosphatase present in the tumor microsomes is maximally inhibited at NaF concentrations greater than 40 mM; less than 4% of the alkyldihydroxyacetone-P was dephosphorylated during a 5-min incubation under these conditions. However, during longer incubation periods at low concentrations of NaF, significant quantities of alkyldihydroxyacetone and alkylglycerol are produced.

Reaction (V) (Fig. 46). In the presence of ATP and Mg^{++}, alkyldihydroxyacetone is phosphorylated to alkyldihydroxyacetone-P by a kinase in the tumor. The apparent K_m for ATP was 3.6 mM. The highest specific activity for the alkyldihydroxyacetone phosphotransferase was located in the microsomal membranes. These data demonstrate how alkyldihydroxyacetone, like alkylglycerol, can re-enter the pathway responsible for the biosynthesis of complex ether lipids in cancerous tissues.

Biosynthesis of Alkyl Lipids: Displacement of the Acyl Moiety of Acyldihydroxyacetone Phosphate with Fatty Alcohol Analogs.† Utilization of long-chain fatty alcohols for the biosynthesis of the *O*-alkyl moiety at the *sn*-1-position of glycerolipids is now firmly established in tumors. With few exceptions, the *O*-alkyl and *O*-alk-1-enyl moieties of glycerolipids consist of 16:0, 18:0, and 18:1 carbon chains, i.e., polyunsaturation is virtually absent. This unusual composition could be due to the specificity of the enzymes

*Chae, K., Piantadosi, C., and Snyder, F. J. Biol. Chem. 248, 6718-6723, 1973.

†Snyder, F., Clark, M. (U. of Calif., San Francisco), and Piantadosi, C. Biochem. Biophys. Res. Commun. 53, 350-356, 1973.

1028263

that are involved in the incorporation of the alcohol moiety into alkyldihydroxyacetone-P or to the reductase that converts fatty acids to fatty alcohols. In experiments completed in 1973, we examined the enzymic displacement of the acyl moiety of acyldihydroxyacetone-P with several fatty alcohol analogs to determine which portions of the alcohol chain are critical for its participation as a substrate in formation of the *O*-alkyl linkage.

Our data show that the enzyme that catalyzes the formation of alkyldihydroxyacetone-P from fatty alcohols and acyldihydroxyacetone-P is not highly selective, although it cannot utilize alcohols that have functional groups (1,2- or 1,16-diols) at either end of the aliphatic chain. Also substituting a methyl group adjacent to the terminal carbon atom (iso type) of the alcohol appears to greatly hinder its reactivity as compared to when the methyl group is substituted in an anteiso position. Thus it would appear that the type of ether-linked chains occurring in nature is determined by the specificity of the acyl-CoA reductase which forms the fatty alcohol. These experiments also indicated that the phosphatase in tumors that dephosphorylates alkyldihydroxyacetone-P is markedly influenced by the nature of the *O*-alkyl moiety.

Regulation of Lipid and Sterol Metabolism in Cancer Cells

*Dietary Control of Stearyl CoA and Alkylacylglycerophosphorylethanolamine Desaturases in Tumors.** Our recent work on the biosythesis of plasmalogens by tumor cell preparations has pointed out that the properties of alkylacylglycerophosphorylethanolamine (alkylacyl-GPE) desaturase resembles those of stearyl CoA desaturase. Both reactions require molecular oxygen and a reduced pyridine nucleotide, and are inhibited by cyanide, but not by carbon monoxide. The stearyl CoA desaturation system has been shown to consist of at least four protein components, i.e., NADH-cytochrome b_5 reductase, NADPH-specific flavoprotein, cytochrome b_5, and a cyanide-sensitive factor. In normal liver, stearyl CoA desaturase activity can be significantly induced when fasted rats are refed or when rats are maintained on a fat-free diet. This response is thought to be regulated by the amount of the terminal enzyme(s) of the desaturation system, the cyanide-sensitive factor(s).

Our experiments were carried out to determine whether the activity of alkylacyl-GPE desaturase is controlled by dietary conditions in a manner similar to that for stearyl CoA desaturase. We compared the response of stearyl CoA desaturase in tumors of rats maintained on normal and fat-free diets; the stearyl CoA desaturase activities in control and host livers were also compared. The Fischer R-3259 sarcoma was selected for these studies because it contains relatively high levels of alkylacyl-GPE desaturase.

The stearyl CoA desaturase activity significantly increased in control livers, host livers, and tumors in rats maintained on the fat-free diet over that in the same tissues from animals maintained on laboratory chow; however, no increase of alkylacyl-GPE desaturase was observed in tumors of rats maintained on the fat-free diet (Table 38). These findings suggest that the two desaturase systems are not identical and that they are probably controlled by different components of the microsomal electron transport system, i.e., the cyanide-sensitive factors. The fact that stearyl CoA desaturase activity was lower in

*Lee, T-c, Wykle, R. L., Blank, M. L., and Snyder, F. Biochem. Biophys. Res. Commun., 55, 574-579, 1973.

livers of tumor-bearing animals than in those of control animals, supports the concept of others that indicates host livers tend to acquire the enzymic composition of immature livers.

TABLE 38
EFFECT OF ESSENTIAL FATTY-ACID DEFICIENCY ON STEARYL CoA AND ALKYLACYL-GPE DESATURASE ACTIVITIES*

Tissue	Stearyl CoA desaturase		Alkylacyl-GPE desaturase	
	Chow	Fat-free	Chow	Fat-free
	nmoles/min/mg protein			
Normal livers	0.72 ± 0.07 (4)	4.80 ± 0.43 (4)	†	n.d.
Host livers	0.12 ± 0.05 (7)	2.60 ± 0.43 (6)	n.d.	n.d.
Tumors	0.06 ± 0.01‡ (5)	0.12 ± 0.02‡ (5)	0.029 ± 0.004 § (3)	0.025 ± 0.006 § (3)

* The means S.E.; the numbers in parentheses refer to the number of experiments used to obtain mean values.
† Alkylacyl-GPE desaturase was not detectable in normal liver microsomes; n.d. = not determined.
‡ $P < 0.05$.
§ $P = 0.5$ to 0.6 (no significant difference in values).

*Tetrahydropteridine-Dependent Cleavage Enzyme for O-Alkyl Lipids: Substrate Specificity.** Alkylglycerol-cleavage activity in mammals is located primarily in liver and to some extent in intestines, whereas brain and adipose tissues possess barely detectable activities. Previously, in a survey of neoplastic cells, a rich source of ether lipids, we found extremely low levels of cleavage activity except in two Morris hepatomas that closely resembled normal rat liver; the activity in the slower growing hepatoma was almost the same as found in normal liver. The cleavage enzyme appears to be most active in cells that contain the lowest levels of *O*-alkyl lipids.

Little is known about the specificity of the cleavage enzyme. It fails to distinguish optical enantiomorphs or the 1- or 2-isomers of alkylglycerols and it oxidized 14:0, 16:0, and 18:0 *O*-alkyl chains at similar rates, whereas alkylglycerols possessing shorter chains are poorly oxidized.

In view of the apparent importance of the *O*-alkyl-cleavage enzyme in controlling tissue levels of ether lipids, we felt compelled to test the specificity of this enzyme for various types of alkyl glycerolipids that are found in nature. In these experiments we determined the influence of various substituents (acyl, ketone, phosphate, phosphorylethanolamine) on alkylglycerol-cleavage activity associated with the microsomal fraction of rat liver. Our results show that the *O*-alkyl-cleavage enzyme utilizes all isomeric forms of alkylglycerols and 1-alkyl-*sn*-glycero-3-phosphorylethanolamine as substrates. In contrast, the *O*-alkyl portion of glycerolipids containing either acyl, phosphate, or ketone groupings on the glycerol moiety is not oxidized. Therefore, it appears that other enzymes, i.e., lipases, phospholipases, and phosphatases are probably important regulators of the *O*-alkyl-cleavage enzyme under physiological conditions. It is interesting that the intermediates (alkyldihydroxyacetone-P, alkyldihydroxyacetone, and alkylglycerol-P) involved in the biosynthesis of alkyl glycerolipids are not substrates for the *O*-alkyl-cleavage enzyme.

*A Tissue-Culture System for Studies of the Regulation of Ether-Linked and Ester-Linked Aliphatic Moieties in Glycerolipids.** Although the ether-linked glycerolipids are generally prominent in neoplastic cells and their essential biosynthetic routes have been established, neither their physiological function nor their role in the neoplastic process is presently known. However, accumulating evidence indicates that surface membrances of cells from certain microorganisms and mammals contain significant quantities of ether-linked lipids; even in rat liver where the quantity of ether lipids is extremely small, we have previously shown that they are concentrated in the plasma membranes.

Factors that regulate the formation and degradation of ether and ester linkages in lipid molecules have not been compared because of the lack of a suitable biological system. Results by others indicate that the formation of ether lipids is under hormonal control and crucial regulatory enzymes involved in their metabolism are glycerophosphate dehydrogenase and acyl-CoA reductase. Previous studies from our laboratory showed that suspension cultures of mouse-skin fibroblasts (L-M cells) contained significant quantities of ether lipids, whereas monolayers of the same type cells did not. Furthermore, microsomal preparations from the L-M cells grown in suspension cultures actively catalyze the biosynthesis of alkyl glycerolipids.

We have now extended these observations by carrying out metabolic experiments with L-M fibroblasts in monolayer and suspension cultures. The data demonstrate that these two conditions of growth offer a simple system for manipulating the diversion of aliphatic precursors into ester- and ether-linked lipids, so that the cellular regulatory mechanisms responsible for the accumulation of ether-linked lipids in certain tissues can be investigated in vitro.

The incorporation of [1-^{14}C]hexadecanol and [1-^{14}C]palmitic acid into ether- and ester-linked lipids was investigated using suspension and monolayer cultures of L-M fibroblasts (Table 39). Cells maintained in suspension cultures incorporated relatively high amounts of [1-^{14}C]hexadecanol into alkyldiacylglycerols, whereas the same cells grown as monolayers did not. The suspension cultures also synthesized small amounts of ethanolamine phasmalogens when the cells were incubated for 6 hr, but none of the other lipid classes contained ^{14}C in the *O*-alk-1-enyl moieties. In the monolayer cultures most of the label from the hexadecanol was incorporated into the acyl moieties, indicating that the enzymes responsible for oxidation of the alcohol is extremely active under these growth conditions.

Of the total activity incorporated into the lipids of the suspension cultures, 2 to 5% of the ^{14}C was present in the alkyldiaclglycerols when palmitic acid was the precursor and 5 to 15% when hexadecanol was the precursor. Approximately 1% of the incorporated ^{14}C was in the alkyldiacylglycerol fraction from the monolayer cultures. Analysis of the alkyldiacylglycerols isolated from the suspension cultures incubated with hexadecanol demonstrated that approximately 60% of the ^{14}C was in the alkyl moieties, whereas the alkyldiacylglycerols isolated from the suspension cultures incubated with palmitic acid contained about 35% of the ^{14}C in the alkyl moieties. The incorporation of the label from

*Snyder, F., Malone, B., and Piantadosi, C. Biochim. Biophys. Acta, 316, 259-265, 1973.

*Blank, M. L., and Snyder, F. Arch. Biochem. Biophys. 160, 100-105, 1974.

1028264

the palmitic acid into *O*-alkyl moieties indicates that acyl-CoA reductase activities are present at a much higher level in the L-M fibroblasts maintained in suspension cultures than in the monolayer cultures.

TABLE 39

INCORPORATION OF [1-^{14}C] PALMITIC ACID AND [1-^{14}C] HEXADECANOL INTO LIPIDS OF L-M FIBROBLASTS IN MONOLAYERS AND SUSPENSION CULTURES

	Incorporation of labeled precursors (% ^{14}C Incorporated)*			
	Monolayer cultures†		Suspension cultures†	
Lipid class	[1-^{14}C] Palmitic acid	[1-^{14}C] Hexadecanol	[1-^{14}C] Palmitic acid	[1-^{14}C] Hexadecanol
Alkyldiacyl-glycerols	1.6, 1.5	1.0, 1.3	5.3, 5.1	14, 13
Triacylglycerols	13, 11	5.4, 5.6	38, 38	33, 32
Ethanolamine phosphatides	14, 14	20, 20	7.3, 7.2	8.3, 8.7
Serine and inositol phosphatides	8.0, 8.2	9.4, 8.5	3.7, 3.0	3.0, 3.2
Choline phosphatides	54, 55	52, 54	44, 44	30, 33
Sphingomyelin	9.1, 9.7	12, 11	1.2, 1.3	0.5, 0.5
Total alkyl glycerolipids‡	2.0	4.0	4.0	15
Total quantity of precursor incorporated into L-M cells	31, 27	22, 24	48, 48	55, 63

* Values are expressed as percentage of total ^{14}C recovered in the products resolved on Silica Gel G using hexane-diethyl ether-acetic acid (80:20:1, v/v) or on Silica Gel HR using chloroform-methanol-acetic acid-water (50:25:8:2, v/v) for development. Each value represents the distribution of ^{14}C in lipid classes expressed as percentage of the total radioactivity incorporated into the cellular lipids. The values do not include the radioactivity present in the media or the radioactivity present in the free fatty acids or free fatty alcohols of the cellular lipids.

† Cells ($\simeq 1 \times 10^6$ cells/ml) grown 3 hr in the presence of the labeled precursors (0.33 μCi/ml media).

‡ Expressed as alkylglycerols liberated after Vitride reduction of a pooled sample of total lipids; chromatography was done on Silica Gel G using diethyl ether-water (100:0.5, v/v) for development.

In some experiments, the *O*-alkyl moieties of the cells in suspension cultures were prelabeled with [1-^{14}C]hexadecanol so that the turnover of the *O*-alkyl moieties could be traced when the cells were transferred back to monolayer cultures. After these cells grew overnight as monolayers, they contained essentially no labeled alkyldiacylglycerols, although substantial amounts of labeled acyl groups were still present. The incorporation of [1-^{14}C]hexadecanol into the alkyl moieties of the glycerolipids in the cells maintained in suspension culture could also be altered by pH of the growth media. At a pH of 6.2, the ^{14}C associated with the alkyldiacylglycerols was 40 to 50% lower than that observed at pH 8.0.

The tissue-culture systems described show that the metabolism of ether- and ester-linked lipids can be altered as a result of pH or the growth condition. Although the

1028265

mechanism for such changes needs to be clarified, the data clearly demonstrate that L-M cells can be manipulated so that the regulatory factors involved in the metabolism of glycerolipids that contain *O*-alkyl, *O*-alk-1-enyl, and acyl moieties can be investigated. An understanding of such factors could provide the necessary insights to explain why most neoplastic cells contain higher levels of ether-linked glycerolipids than normal tissues. In this connection, it is interesting to note that others have reported that L-M cells grown in suspension cultures cause essentially 100% incidence of tumors (fibrosarcomas) when they are injected into mice, whereas when the same cells are grown in monolayers, the incidence of tumors after reinjection into mice is extremely low ($\simeq$10%).

*Regulatory Studies Related to Cholesterol Metabolism in Rats Bearing 7777 Morris Hepatomas.** In a previous investigation we had observed elevated levels of cholesterol (free and esterified) in Morris hepatomas 7777 and 7794A. During the past year we completed a series of experiments that were designed to determine the metabolic basis for the increased levels of cholesterol in the blood of the tumor-bearing animals.

Analysis of plasma, tumor, and livers, 1 hr after I.V. injection of [7-^{3}H]cholesterol or *rac*-[5-^{3}H]mevalonic acid, showed that the liver from the tumor-bearing rats removed cholesterol from the blood more efficiently than the livers of the control animals; only small amounts of the injected cholesterol were incorporated into the tumors (Table 40). However, 7777 hepatomas synthesized more free cholesterol and cholesterol esters from the injected *rac*-[5-^{3}H]mevalonic acid than either the host or control livers. The plasma from both groups of rats contained essentially the same amount of phosphatidylcholine-cholesterol acyltransferase (EC 2.3.1) activity and the labeling of cholesterol esters reflected the compositional pattern of the acyl moieties for this fraction.

TABLE 40

SPECIFIC ACTIVITIES OF TUMOR AND LIVER TISSUES AFTER INJECTION OF [7-^{3}H] CHOLESTEROL AND RAC-[5-^{3}H] MEVALONIC ACID

Two 7777 tumor-bearing and two conrol rats were given injections of either 8 μCi of [7-^{3}H] cholesterol or 20 μCi rac-[5-^{3}H] mevalonic acid, each via the tail vein. Rats were sacrificed after 1 hr.

	Specific activity (cpm/g tissue x 10^{-3})	
Tissue	[7-^{3}H] cholesterol *rac*-[5-^{3}H] mevalonic acid	*rac*-[5-^{3}H] mevalonic acid*
7777 Hepatoma	1.0, 1.3	25.0, 16.0
Host livers	150, 110	19.3, 12.4
Control livers	70, 84	16.4, 14.5

* The total lipid radioactivity contained 16%, 11%, and 5% ^{3}H in the cholesterol esters for the 7777 hepatomas, host livers, and control livers, respectively.

On the basis of our results, we suggest that the presence of 7777 Morris hepatomas perturbs cholesterol metabolism in the host animal by synthesizing considerable amounts of cholesterol and releasing it into the blood. While processes for the normal clearance of

*Grigor, M. R. (MRC Biochemistry Research Group, Dunedin, New Zealand). Blank, M. L., and Snyder, F. Cancer Res., 33, 1870-1874, 1973.

the blood cholesterol are present, the increased uptake of cholesterol by the host liver is not sufficient to remove the elevated amount of cholesterol released by the tumor. These data demonstrate that tumors can markedly influence regulatory processes in host livers.

Chemical Snythesis of Metabolic Lipid Intermediates: [1-^{14}C]Octadecyldihydroxyacetone Phosphate and [1-^{14}C]Octadecyldihydroxyacetone.*

Previously we had developd a method for the total chemical synthesis of unlabled *O*-alkyldihydroxyacetone phosphate and *O*-alkyldihydroxyacetone, important intermediates in the metabolism of ether-linked glycerolipids. However, to study the metabolism of these compounds, it became necessary to synthesize them with radioactive labels.

The sequence of reactions used to prepare the ^{14}C-labeled compounds was based on the earlier procedure developed for the synthesis of unlabeled *O*-alkyldihydroxyacetone phosphate and its derivatives. The specific activities of the purified products, [1-^{14}C]alkyldihydroxyacetone-P and [1-^{14}C]alkyldihydroxyacetone, were 2.46 millicuries per millimole. Their radiopurities, as judged by thin-layer chromatographic zonal profile scans, were >99%.

Marmoset Research Project

Actively induced Thrombocytopenia in the Marmoset: A Possible Autoimmune Model

Since one of our primary interests in the marmoset has been tissue transplantation, this past year we extended our isoimmunization studies to include leukocyte antigens with the aim of obtaining immune reagents for typing purposes (D. Barnhart and N. Gengozian). Following the procedure used successfully in the development of reagents for red cell antigens (J. Med. Primatol. 1, 172, 1972), inter- and intraspecies immunization of marmosets with white blood cell (WBC) preparations suspended in Freund's adjuvant were performed. In the initial studies, two *S. oedipus* marmosets were immunized with WBC from *S. fuscicollis* marmosets, an interspecies or xenogeneic combination, and three received cells from unrelated *S. oedipus* animals, an intraspecies or allogeneic combination. The immunization protocol consisted of three weekly intramuscular injections of 9 to 30 × 10^6 WBC suspended in complete Freund's adjuvant; the dose given was dependent upon the yield obtained in the separation of WBC from 4 ml of whole blood. Unexpectedly, the two marmosets injected with xenogeneic cells became severely anemic, one within the first week after the initial injection and the other on the 33rd day; both animals subsequently died, the first on the 29th day and the second on the 65th day. No

*Piantadosi, C., Chae, K., Ishaq, K. S. (U. of North Carolina, Chapel Hill), and Snyder, F. J. Pharm. Sci. 62, 320-322, 1973.

1028266

anemia or mortality occurred among the three marmosets inoculated with allogeneic (*S. oedipus*) cells.

An examination of our immunizing preparations revealed a possible explanation for the above results. The procurement of WBC involved separation of heparinized blood on a Methocel-Isopaque gradient. While eliminating almost all red blood cells, the suspensions contained platelets in addition to the desired WBC. Subsequent platelet counts on separated fractions indicated that the animals had also been immunized with platelets ranging in number from 100 to 800 × 10^6 per inoculum. Following these observations an additional four *S. oedipus* marmosets were immunized in the same manner with *S. fuscicollis* WBC-platelet preparations. All four animals died within the time period of 20 to 32 days after the initial injection; three of the four showed a severe thrombocytopenia and variable anemia prior to death (see Table 41, Nos. 3 to 6). The platelet counts and the percent hematocrit for two animals of this group are shown in Fig. 47 (A).

TABLE 41

SUMMARY OF CLINICAL AND LABORATORY DATA ON *S. OEDIPUS* MARMOSETS IMMUNIZED WITH *S. FUSCICOLLIS* WHITE BLOOD CELLS AND PLATELETS

Animal number	Number of injections	Day of death*	Thrombocytopenia	Anemia	Antibody to *S. fuscicollis*		Antibody to *S. oedipus*	
					Platelets	White cells	Platelets	White cells
1.	3	29	?	+ (6)†	–	–	–	–
2.	3	65	?	+ (33)	2‡	–	–	–
3.	3	20	+ (17)§	+ (17)	16	32	2	–
4.	3	32	–	–	nt‖	nt	nt	nt
5.	3	24	+ (17)	+ (21)	16	32	–	–
6.	3	25	+ (20)	+ (17)	32	8	2	–
7.	1	35	+ (21)	+ (24)	16	–	–	–
8.	1	49	+ (38)	–	8	–	–	–
9.	1	Alive	+ (24)	+ (27)	32	–	–	–

* Recorded from time of initial injection.

† + indicates animal had a hematocrit of 30% or less after immunization. (Percent hematocrits in all animals ranged from 40 to 50 prior to initiation of study.) Number in parentheses indicates day after initial injection on which the anemia was first evident.

‡ Numbers represent reciprocal of highest serum dilution at which agglutination was recorded. Serum obtained at time of acute thrombocytopenia or anemia.

§ + indicates animal experienced > 50% decrease in platelets following immunization. Number in parentheses indicates day after initial injection on which thrombocytopenia was first evident.

‖ nt = not tested due to lack of serum.

To determine whether the thromobocytopenia phenomenon induced in the xenogeneic combinations was dose-dependent, an additional three *S. oedipus* marmosets were given a single injection of *S. fuscicollis* cells in adjuvant. Two died on days 35 and 49, respectively, each animal experiencing a severe thrombocytopenia with no or only a mild anemia. The third animal became thrombocytopenic on day 24 and then showed a complete absence of platelets for a period of 18 days; during the next 10 days there was a

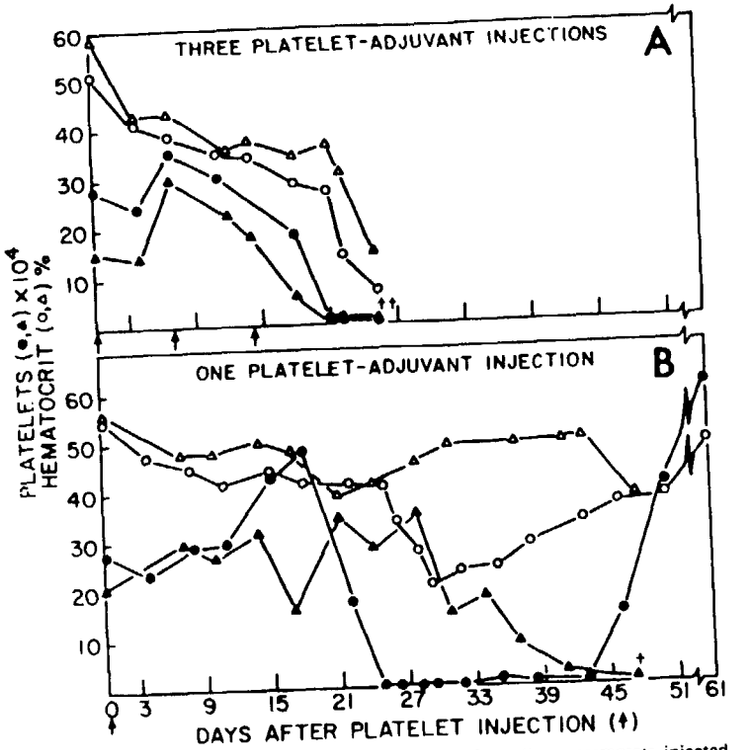


Fig. 47 Induction of thrombocytopenia and anemia in *S. oedipus* marmosets injected with platelets (suspended in Freund's adjuvant) from *S. fuscicollis* marmosets. Figures A and B show the hematologic responses in four marmosets immunized with the xenogeneic platelet-adjuvant preparations, two receiving 3 weekly inoculations and two receiving a single injection. + indicates death of animal.

sharp increase in platelets, the count rising above the preimmunization level. Figure 47 (B) depicts the hematocrit and platelet values for this animal and one of the two that died in this single-injection group.

Serum samples obtained from eight animals during the acute thrombocytopenic-anemic phase were titrated against the immunizing xenogeneic (*S. fuscicollis*) platelets. Six contained significant agglutinating activity while one showed an equivocal titer of 1 (log$_2$) (see Table 41). Antibody activity toward the xenogeneic WBC was found in only three cases. With one exception (No. 9), we could not test for antibody to autologous platelets since the animals died before any recovery of their platelets had occurred. There remained the possibility, however, that the serum of these animals would contain activity directed toward platelets of their own species, i.e., *S. oedipus*. Therefore, sera were titrated with allogeneic *S. oedipus* platelets. In only two was it possible to demonstrate agglutinating activity and this only at a 1:2 serum dilution; in the others, the results were not reproducible and were therefore recorded as negative. A similar finding was made with the single animal that had shown a spontaneous return of its platelets, i. e. equivocal

agglutination tests with both allogeneic and autologous platelets. Tests for antibodies to WBC and platelets were negative in the initial group of three *S. oedipus* marmosets that had survived without any apparent ill effects following immunization with allogeneic (*S. oedipus*) cells.

Gross morphologic observations among the animals dying in the xenogeneic groups revealed several to have focal areas of petechiae, ecchymoses, and hemorrhage on the skin and along the gastrointestinal tract. The severity of such bleeding appeared to be related to the degree of anemia experienced. Splenic enlargement was not found in any of the dying animals and their bone marrow showed either a normal or slightly increased activity of the megakaryocytic elements.

Controls for the above studies included inoculation of *S. oedipus* marmosets with Freund's adjuvant alone. No hematologic alterations or ill effects were noted; nor were any anticipated, since previous extensive studies on red cell immunizations with Freund's adjuvant did not induce the thrombocytopenic state. Injection of autologous cells and platelets in adjuvant were also without effect. It should be noted that in the experimental xenogeneic combinations described above, the white blood cell count and differential were not altered significantly from the preinjection level even though a few animals did develop leukoagglutinating antibodies to the xenogeneic cells and presumed antigenic similarities exist between platelets and white cells in this species as in others. Although red cell agglutinins were formed in response to the WBC-platelet inocula, absorption studies showed no relationship to the platelet antibodies when these were present.

The acute and severe thrombocytopenia manifested in the marmosets which subsequently led to death following immunization with marmoset platelets appears analogous to the spontaneously occurring clinical disease known as idiopathic thrombocytopenic purpura (ITP). Identification of an antiplatelet factor as a 7S antibody which could induce thrombocytopenia when transferred to normal recipients, the specificity of the factor, and finally demonstration of its production by spleen cells in culture has led to an autoimmune concept of this disease. Much of the information concerning the possible etiology and treatment of ITP has been derived from clinical case studies since a suitable experimental model is lacking. Although thromobocytopenia can be induced in dogs, rats, and rabbits by injection of heterologous antiplatelet antiserum, this passive form of the disease can contribute only indirectly and, perhaps incorrectly, to our understanding of ITP in man. The induction of thrombocytopenia and antiplatelet antibody formation in marmosets by injection of platelets from closely related animals (other species of marmosets) suggests that an animal model can be developed which will more closely approximate the active autoimmune process presumed to be involved in the pathogenesis of ITP in man.

External Uterine Palpation to Identify Stages of Pregnancy in the Marmoset

The development, maintenance, and propagation of our marmoset colony have necessitated efforts not entirely within the scope of our immunologic research goals. While many of these have involved basic animal husbandry problems common to all laboratory animals and need not be mentioned, some have yielded information that would be specifically pertinent to those anticipating working with the marmoset. The present report documents one such aspect of colony management, detection of pregnancy and

1028267

estimation of delivery times, data which we have accumulated over a period of 5 years in the establishment of our breeding nucleus of marmosets (N. Gengozian, T. Smith, and D. Gosslee*). The ability to detect pregnancy in a marmoset and to estimate the days post-conception would both facilitate colony management and permit investigators to plan experiments using fetal tissues of known ages. Unfortunately, although the twinning characteristics of the marmoset are extensively documented (Gengozian, N. In: Medical Primatology, 1970, 2nd Conf., Exp. Med. Surg. Primates, New York, 1969, pp. 926-938; Hampton, J. K., Jr. et al. Science 150, 915-917, 1965; Wolfe, L. G., et al. In: Breeding Primates, Proc. Int. Symp. on Breeding Non-human Primates for Lab. Use, Berne, 1972, pp. 145-157), substantiating the early reports of Hill (Philos. Trans. R. Soc. Lond. 221B, 45-178, 1932) and Wislocki (Amer. J. Anat. 64, 445-477, 1939), our knowledge of its reproductive physiology is limited. For example, the lack of any obvious estrous or menstrual cycle handicaps efforts at obtaining timed pregnancies. The establishment of our colony has enabled us to evaluate external uterine palpation, a simple procedure, for detecting pregnancies at various stages of gestation.

Initially, we attempted to use increase of body weight as a means of detecting and following pregnant animals. However, it soon became apparent that this was quite insensitive and too crude a method to provide a reliable measure of the postconception period; weight changes among the animals would fluctuate greatly from week to week, preventing early detection of pregnancies. Therefore, all females were routinely palpated in the region of the uterus by applying slight pressure with the thumb and forefinger on the ventral and median aspects of the abdominal wall at its most caudal point, immediately above the pubic glandular pad. The earliest palpable uterus indicative of pregnancy feels like a firm, rigid sphere, measuring approximately 0.5 cm in diameter. With time, as a sphere, it increases in size toward the midportion of the abdomen (Fig. 48). Measurements were made by placing a centimeter ruler by the thumb and forefinger, approximating the mid-point (diameter) of the uterine "sphere". We made all size estimates to the nearest one-half centimeter.

Although a few animals were palpated at least three times at monthly intervals throughout pregnancy, the majority were examined only once to estimate the uterine size. This precaution was taken to avoid undue stress to the animal. In each case the size was recorded, and then after a normal, full-term delivery of twins, the number of days to delivery after detection of pregnancy was plotted against the uterine size. The data for 92 measurements and the mean days to delivery (D) for each 0.5 cm increment in diameter (d) are shown in Table 42 and Fig. 49.

A growth curve model relating size to an exponential function of age is assumed, or

$$\log (d_i) = a' + b' (G - D_i)$$

where d_i is the diameter of the ith uterus, D_i is the corresponding days to delivery, G is the greatest gestation period and $i = 1, 2, 3 \ldots 92$. Since we wish to estimate D, given d, the model is written as

$$D_i = a + b \log (d_i + c) + e_i$$

The constant c is included to allow for bias in measuring the diameter. The constants a, b, and c were estimated by minimizing the sum of squares of deviations, e_i, from the model,

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1028268



Fig. 48 Photographs illustrating external uterine palpation of pregnant marmosets. A. Demonstrates method of holding marmoset and palpation with thumb and forefinger. Centimeter ruler has been intentionally displaced from point of measurement for illustration. Uterine diameter is approximately 2 cm on this animal. B. Close-up view of another marmoset showing actual measurement procedure. In this, the diameter is approximately 1.5 cm.

using the data for the initial group of 92 marmosets.

The resulting equation

$$\hat{D}_i = 130.6 - 162.7 \log (d_i + 0.5)$$

was then used to predict delivery times of pregnant animals over a 3-year period (Table 43). Of the 178 estimates, 12/178 or 7% of the animals delivered on the day predicted, 108 or 61% delivered within 5 days of the anticipated parturition date. The standard errors of the estimates of a and b are 2.1 and 5.5, respectively. The standard error of the deviations from the model is 9.3 days for the initial group while the standard error of the 178 deviations shown in Table 43 is 6.5. The mean days to delivery for the test group of 178 estimates are also plotted in Fig. 49.

The gestation period was estimated by using the empirical model

$$D_i = (G - ad_i)\, e^{-bd_i}$$

The least squares estimates of the parameters are G = 158.8, a = 11.3 and b = .38 for the group of 178 animals. The standard error of the estimate of G is 12.7 days. Convergence was not obtained for the group of 92 animals but a graphical estimate of G was made, yielding a value of 161 days. The growth curve model was not used, since the logarithm of a true diameter of zero, $d_i + c = 0$, is minus infinity.

TABLE 42

MEAN DAYS TO DELIVERY FOR EACH GROUP FOR EACH DIAMETER

Diameter (cm) d	Initial group D*	n*	Test group D*	n*
0.5	132.3	11	130.0	14
1.0	97.9	11	94.4	23
1.5	81.0	26	78.8	41
2.0	70.7	12	69.7	45
2.5	52.1	11	50.2	36
3.0	42.6	15	42.0	14
3.5	29.1	6	28.0	5
Total	–	92	–	178

* Means days to delivery (D) for the number of animals (n).

Our results indicate the external uterine palpation offers a simple yet accurate means of identifying pregnant marmosets at different stages of gestation. The practical and investigational applications are obvious. Thus, with injections or feeding of drugs or compounds at known gestational ages, we can more readily design and interpret experiments aimed at altering normal physiologic development. Similarly, the ability to interrupt pregnancies to procure embryonic or fetal tissues at known developmental stages would be a distinct asset. We have already obtained fetuses through spontaneous abortions of animals previously palpated, permitting us to relate the crown-rump length and other aspects of the physical and physiological growth to the approximate stage of gestation. Occasionally, we have recorded "false-positive" pregnancies, i.e., no subsequent delivery from an animal previously palpated one or more times and recorded as being pregnant. We interpret these, however, as instances of embryonic death and intrauterine resorption or spontaneous early abortions not detected because of the female's eating the aborted material. The occurrence of such unproductive pregnancies among marmosets has already been suggested by Hampton et al (Endocrinology 85, 171-174, 1969).

TABLE 43

DISTRIBUTION OF THE DEVIATIONS IN THE TEST GROUP

Diameter (cm) d	Predicted $\hat{D}$	Number in each day interval: Underestimated > 6	Underestimated 1–5	0	Overestimated 1–5	Overestimated > 6	Total number
0.5	131	3	4	1	4	2	14
1.0	102	14	5	0	4	0	23
1.5	82	13	16	6	6	0	41
2.0	66	1	7	3	16	18	45
2.5	53	10	11	1	13	1	36
3.0	42	3	2	1	6	2	14
3.5	33	3	1	0	1	0	5
Total		47	46	12	50	23	178

Although we have also used our model and data to estimate the gestation period, the estimates are subject to several sources of error. The errors due to sampling and observation are indicated by the standard error of 12.7 days of the estimate of a 158.8 day period.

1028269

The model for estimating the gestation period was chosen on empirical grounds and other models would yield different values. Heretofore, estimates have been derived from noting the interbirth intervals of paired marmosets producing live young or the time lapsing from an initial pairing to parturition. These have provided estimates of 140 to 150 days, with most investigators accepting the lower time span because interbirth intervals as short as 132 days have been reported (sp. *Leontocebus rosalia*). However, a true "normal" gestation period under laboratory conditions may be difficult to obtain in view of the tendency of marmosets in captivity to have abortions and premature deliveries. Therefore, if some qualifications are to be placed on the shorter intervals recorded, the value of 158.8 days derived from our palpation data, even with its high standard error, would suggest that the 140 to 150-day gestation period be accepted as minimal estimates for the true gestation time.

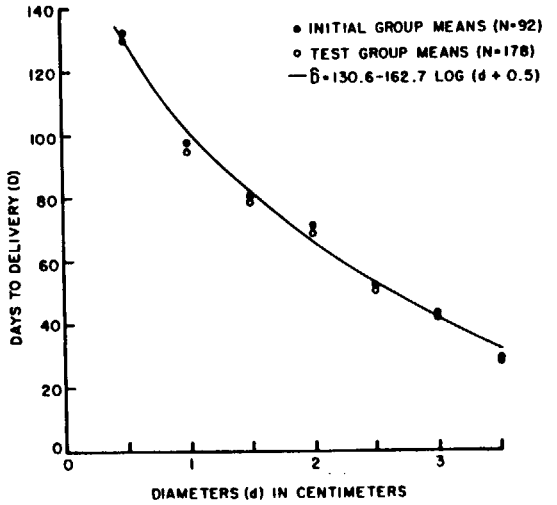


Fig. 49 Relation between days to delivery and diameter of the uterus.

Marmoset Pathology Studies

Dental Development in Laboratory-Bred Marmosets

(*S. fuscicollis sp.*)

Because we need an objective means of determining the age of wild-caught marmosets brought into our colony, we have begun a study to try and use tooth and skull development of laboratory-bred animals (whose age is known) for this purpose. A life history of dental development and attrition is needed to do this. We need to know first the temporal course of eruption of both the deciduous and permanent teeth. This timetable would then define such time periods as infancy, childhood, adolescence, and the beginning of adulthood when maturity of the alimentary system would have been attained. To determine this

time for *S. fuscicollis*, the commonest marmoset in our colony, we (Paul Crawford*, Gretchen Humason, C. C. Lushbaugh, and N. Gengozian) studied the dentition of 9 prenatal, 10 neonatal (2 to 14 days), and 40 (4 to 42 weeks of age) animals. All skulls were defleshed, macerated in 1:25 papain and in 1:1000 EDTA solution at 40°C, washed in running water, defatted in acetone and then bleached by hydrogen peroxide. The times required were varied with the age of the animal (size and calcification of the skull). The dental formula for each animal was then determined by means of a dissecting microscope and was documented by photography.

The results, summarized in Table 44, show that the marmoset has an amazingly rapid tooth development; the first incisors erupting in utero sometime between 100 to 110 days of gestational age when the animal is about 7.0 cm in crown-rump length. At birth (120 to 140 days of uterine development) the animals already have 12 teeth (dental formula = $i_1\ i_2 c$, Table 44) and are capable of biting handlers and nibbling solid food, an observation that explains our consistent finding of vegetable debris in the stomachs of dead one-day-old animals. By the end of 1 week of life, the first deciduous molars (lower m_1), have

TABLE 44

DENTAL FORMULAE (LEFT LOWER JAW) OF LABORATORY-BRED MARMOSETS

Age	Formula*	Developmental Stage
Birth	$i_1\ i_2\ c$	1
1 week	$i_1\ i_2\ c\ m_1$	2
1 month	$i_1\ i_2\ c\ m_1\ m_2\ m_3$	3
4 months	$i_1\ i_2\ c\ m_1\ m_2\ m_3\ M_1$	4
6 months	$I_1\ I_2\ c\ m_1\ m_2\ m_3\ M_1\ M_2$	5
8 months	$I_1\ I_2\ c\ P_1\ P_2\ P_3\ M_1\ M_2$	6
9 months	$I_1\ I_2\ C\ P_1\ P_2\ P_3\ M_1\ M_2$	7

* Symbols:	Deciduous	Permanent
	i = incisors	I
	c = canine	C
	m = molars	M
	premolars	P

erupted and by 30 days all 12 (three on each side, upper and lower jaw) have erupted and are fully developed when the dental formula = $i_1\ i_2 c\ m_1\ m_2\ m_3$. About 4 months of age the first permanent molar erupts and is followed at 6 months by the first two permanent incisors and the second permanent molar. Between 6 and 8 months, the first premolars erupt in sequence. At 9 months the permanent canines erupt and full dentition is established. Only 1 of the 38 animals with complete permanent dentition studied here had a third molar. Apparently this tooth is being deleted in the evolutionary process. (We are finding in an ongoing study of dental attrition in aged marmosets that a single third molar, always upper, is present in 1 of 20 animals and does not erupt consistently in early adulthood.) Since the third lower premolar looks like a tricuspid, the adult marmoset appears in some instances, like man, to have three permanent molars and only two premolars, but this is not true according to our study.

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1028270

As can be seen from the table of dental formulae and the ages when the various teeth erupt, the age of a young marmoset can be estimated with an error of less than a month by dental examination up to 9 months of age. More precision can be obtained if the eruption times of upper and lower and right and left sets are compared. We found no difference between the eruption times for the sexes (37 M, 22 F), and none between species and subspecies.* During full sexual development, the upper canines of the male become longer and sturdier than those of the female.

An occasional animal suffering nutritional deficiency secondary to chronic enteritis did show some retardation in the development scheme but this was inconsistent. Our study of wear, decay, and loss of teeth with age is far enough along to encourage its continuance. We are finding that the aged marmoset (7 to 8 years old) has lost one or more molars and worn others to the gum line, a finding that suggests, among other things, that commercial diets are abrasive. This part of the study will obviously be required to determine the actual age of wild-caught marmosets that develop colon cancer at a later time in our colony, but its use rests on the questionably valid assumption that the rate of dental damage in the wild is the same as that in the laboratory.

In Table 45 we have extended Shultz's comparison of the ages of eruption of the teeth of macaque, chimpanzee, and man to include those of the marmoset (Shultz, A. H., Am. J. Phy. Anthropol. 29, 489-581, 1935). This comparison emphasizes the remarkably rapid dental development of the marmoset. If the slow-to-erupt third molar of man is ignored and only the time that is required for eruption of the canines is used for comparing total dental development time of the four species, that of the marmoset is >12 times faster than that of man, >8 times that of the chimpanzee, and >5 times that of the macaque. If this relative index of maturation could be extrapolated to other metabolic systems, it would suggest that one "marmoset month" might be roughly equal metabolically to one "human year". If true, this disparately rapid metabolic rate would enforce our belief that the marmoset offers an excellent primate model for experimental study of many human diseases including alimentary tract neoplasia. Here this rapid metabolic rate would explain in part what appears to be a remarkably short induction period for colon cancer, which we observed in a colony-bred marmoset only 45 months of age and in a wild-caught

*List of Species Used

Purebred	Code	No. Animals
S.f. illigeri	i	30
S.f. leucogenys	lc	2
S. f. lagonotus	la	3
S.f. nigrifrons	nif	2
S.o. oedipus	oed	2
S. nigricollis	nig	2
Hybrid		
S.l., la × i		9
S.l., lc × i		9
	Total	59

TABLE 45

COMPARISON OF THE ABSOLUTE AGES OF DENTAL DEVELOPMENT IN MAN, CHIMPANZEE, MACAQUE, AND MARMOSET

Dental Stage	Man	Chimp	"Rhesus"	Marmoset
		(Years)		(Months)
Deciduous				
First tooth	0.5	0.3	0.1	-1
All milk teeth	2.5	1.5	0.5	1
Permanent				
First molar	6	3	1.5	4
First incisors and second molar	7-9 12-14	5-7	3-4	4-6
Premolars	10-12	6-7	4	6-8
Canines	11-12	7-8	4-5	8-9
Third molar	18-30	9-10	6-8	–

male marmoset that died from this cancer after only 13 months in our colony. The criticism that all primates are too long-lived and too slow to mature and age for use in carcinogenesis studies would not seem to apply to marmosets in light of their rapid (less than 5 months) intrauterine development, short adolescence (this dental study), and Gengozian's clinical observation that sexual maturity, as evidenced by second-generation laboratory breeding, is attained by this species within 20 to 24 months.

Gonadal Maturation in the Marmoset

The fact that, in man, dental and organic sexual maturity are reached at about the same time, prompted us to try to determine in the marmoset what stage of gonadal maturity conforms with dental stages of development so it could be used as another yardstick for estimating the age of marmosets caught in the wild (C. C. Lushbaugh, Gretchen Humason, and N. Gengozian). For this histologic study we had available 33 males and 20 females from colony-bred animals 6 to 20 months of age. These animals had all died from acute enteritis at various times during the last 6 years; tissue preservation was good and no chronic diseases were present that could have altered gonadal development. Briefly this study showed, as might be expected, that gonadal maturity is achieved several months before successful breeding is obtained. The histologic appearance of adult testes with abundant sperm production was found in 1 of 4 animals 13 months old, in 4 of 7 that were 15 months old, and uniformly after 18 months. In females, corpora lutea indicating that ovulation had occurred were not found until the animals were 12 or more months of age. For 6 months before that event, follicular atresia is extensive, and many ova are seen in process of being shed in multiple ovular follicles undergoing atresic degeneration. These signs of sexual immaturity subside gradually so that follicular destruction by atresia becomes minimal, although constantly present after 15 to 16 months of age. During this period, PAS-positive ringlets of former zona pellucida, left from ova destroyed during follicular atresia, accumulate in the ovarian hilum and are then gradually absorbed by 18 to 20 months of age. The occurrence of this highly antigenic glycoprotein in the ovarian hilum diminishes during the bloom of a female's sexual life and then recurs in her old age.

1028271

The prepubertal marmoset ovary is easily identified by these histologic criteria during the extensive follicular atresia of this period. This process has largely abated in the pubertal marmoset ovary which can be identified by the presence of corpora lutea that become multiple and much greater in relative size than even those of human pregnancy. A hasty scan of adult ovaries of various ages indicates that the number of primary and secondary ovarian follicles can be used as a rough indicator of age, since these numbers decrease progressively until a fibrous, anovular ovary finally results. Until now the oldest laboratory-bred female to die has been only 9 years old. She still had what appeared to be a large number of primary and secondary follicles, suggesting to us that the several wild-caught females we have found to have fibrotic ovaries are over 10 years of age at least.

Eight Case of *S. oedipus* Primary Adenocarcinoma of the Cecocolon with submucosal Lymphatic Invasion of Ileum and Numerous Lymph Node Metastases

Several months after receiving support from the Animal Resources Section of the Epidemiological Division of NCI to help maintain our marmoset colony for another 6 months, we (C. C. Lushbaugh, Gretchen Humason, and N. Gengozian) observed an additional case (No. 8) of colonic cancer. This male *S. oedipus* had been imported 4 years and 2 months previously. Although he was known to be in poor condition, he died unexpectedly. His weight loss had been gradual over about 1 year, and since he was not handled daily in an active experiment, the abdominal distention that he had terminally had gone undetected. At necropsy, the abdominal contents were displaced by a massively distended ileum (Fig. 50). The terminal 2 cm of the ileum had thickened walls that became progressively thinner with distance from the ileocecal value ring which was less than 1 mm in diameter. Above this obstruction the ileum was filled with 15 grams of normal-appearing solid feces and fluid. The walls of the cecum just below the ileocecal vaive ring were about twice as thick as the walls of the ileum and the opposite wall of the cecum. The walls of the colon within 5 mm above the valve ring were of normal size. The two lymph nodes in the mesentery adjacent to the ileocecal valve were enlarged and semitranslucent in appearance. The lymph nodes of the mesentery and around the pancreas were also enlarged but most of these nodes were opaque looking.

Histologically, the gross impression of primary mucinous adenocarcinoma of the cecum and secondary involvement of the terminal ileum was easily confirmed in spite of poor postmortem preservation. There was extensive submucosal invasion of lymphatics by mucin-producing carcinoma cells that were arranged in glandular spaces, and massive hypertrophy and hyperplasia of intestinal muscle coats and connective tissues as described in rodent colon cancer by Harold Stewart years ago. There were multiple discrete and confluent foci of adenocarcinoma in the mucosal remnants that could not be differentiated as to whether they were primary foci or secondary sites of mucosal invasion. The two large ileocecal nodes contained large masses of adenocarcinoma cells in areas of fibrosis. These masses contained foamy mucin and were easily differentiated from PAS-positive histiocytic macrophages that typically are fusiform cells containing fine granular PAS+ material. These benign histiocytes are reactive to neoplasia, and, while found in masses in lymphatic channels, do not stick to one another as metastatic carcinoma cells do. A total of 5 to 15 lymph nodes examined contained metastatic cancer cells. Because of this animal's having died over a weekend, we again missed an opportunity to try to transplant or culture these rare and unusual neoplastic cells. In this case, again, we



Fig. 50 Photograph of colon (C), cecum (Ce) and ileum (il) of marmoset, necropsy No. 1054, showing sclerotic primary adenocarcinoma (Case 8) (marked by arrowheads) arising in cecal mucosa, producing obstruction of the ileocecal valve by invasion of the terminal ileum. The ileum is greatly distended and its walls thickened by muscular hypertrophy and fibrosis showing that the obstruction was partial and long-standing.

found protein deposits suggestive of amyloid in many of the lymph nodes that did not contain cancer cells. The vital statistics of this animal are listed in the table. These should be compared with those of the other 7 cancerous animals described in the 1972 Research Report (ORAU-122).

An. No.	Imported	Died	Colony Age	Wt. Loss	Sex	+LN	Amyloid Nodes
1054	5/7/69	7/6/73	4 yr 2 mo	230 g	M	5/15	8/15

This will show the remarkable consistency with which this spontaneous carcinoma of the large intestine metastasizes to regional lymph nodes, a rarity in most animal models of various types of human cancer.

Several other diseases of potential pathogenic importance to human disease are now gradually becoming defined as marmosets of greater than 8 years of age are commencing to die. One such disease involves the biliary system. We consider it a cholecystodochopathy secondary to chronic inflammation from some unknown bile constituent. Histologically, it is recognized as increasingly complex invaginations of the epithelial linings and glands. These penetrate the muscular coats as Rokitansky-Ashoff sinuses and become sufficiently hyperplastic and crowded to suggest adenocarcinoma. No case has as yet, however, fulfilled all the criteria for this diagnosis. On occasion, small gallstones are present in the bile, which is quite voluminous, and acute cholecystitis has occurred and caused death. A disease similar to this has been described in rodents fed dietary carcinogens and seems to be a common accompaniment along with pancreatic fibrosis of experimental carcinogenesis of the liver and intestinal tract in those animals.

1028272

Ultrastructural Anatomy

In Vivo Evaluation of Tumor Cell Metastasis and Death

A collaborative study (20) with Kurt G. Hofer (Florida State University) was undertaken to determine the stability of gallium-67 (^{67}Ga) localization after incorporation into neoplastic cells, and the rate of reutilization of Ga-labeled breakdown products following death and disintegration of labeled cells. Tumor cells (Ehrlich ascites, L5178Y, L1210) were double-labeled with ^{125}I-iododeoxyuridine (^{125}IUDR) and ^{67}Ga-citrate before transplantation into normal, nonradioactive hosts. The fate of the labeled cells and their progeny were monitored at daily intervals in the live mice by measuring the retention of radioactive iodine (^{125}I) and ^{67}Ga in a well-type, crystal scintillation counter. The excretion of ^{125}I from the test animals was taken as an index of tumor cell death in vivo and the anatomic distribution of IUDR was linked to the organ distribution of the labeled cells. While ^{125}I from dying cells was rapidly excreted from the test animals, a large fraction of the ^{67}Ga activity released by cell death (and, to a slight extent, by leakage from living cells) was reutilized in various tissues of the hosts. Attempts to accelerate ^{67}Ga clearance from animals by administration of stable gallium or scandium were only marginally successful. Consequently, the rate of ^{67}Ga excretion from tumor-bearing mice did not constitute a measure of the rate of tumor-cell death in vivo, and the organ distribution of ^{67}Ga activity did not provide an accurate index of tumor-cell metastasis. The shortcomings of ^{67}Ga, although not precluding its use as a medical tumor-scanning agent, suggest that ^{67}Ga is a poor tracer for quantitating the precise magnitude of neoplastic involvement and metastatic spread.

Localization of ^{67}Ga in Peritoneal Cells

The fact that ^{67}Ga does not localize specifically in tumors requires for its eventual clinical usefulness a fuller understanding of the mechanisms that control its deposition in both malignant and normal cells. We have previously reported that ^{67}Ga localizes in lysosomal-like bodies, notably, although not exclusively, in macrophages of the spontaneous AKR thymoma. Further studies on the uptake of ^{67}Ga by macrophages are needed to determine whether there are factors related to malignancy that might alter the localization of ^{67}Ga in these cells and thus provide clues to discovering the mechanism of ^{67}Ga localization in tumor tissue. We [94 (D. C. Swartzendruber and Norma L. Idoyaga-Vargas] now report electron microscopic autoradiographic studies on ^{67}Ga localization in peritoneal cells incubated with polystyrene latex spheres as a functional test of phagocytic activity.

Eight-to-nine-month-old nonleukemic AKR mice were injected intraperitoneally with 2 mCi ^{67}Ga given as a citrate. Peritoneal cells were washed three times in siliconized



Fig. 51 Electron microscopic autoradiogram of a ^{67}Ga-labeled mononuclear macrophage from the peritoneal cavity. Note the phagocytosed latex particles, LP, and silver grains associated predominantly with dense bodies.

glass centrifuge tubes and incubated 1 hr at 37°C in medium-199 containing Hanks' Balanced Salt Solution to which 0.1 ml of 3% acryl latex particles were added. The cells were subsequently pelleted and fixed in 3% glutaraldehyde, postfixed in $0s\,0_4$, embedded in Epon 812 and sectioned for electron microscopic studies. Thin sections mounted on bare copper grids were coated with Ilford L-4 nuclear emulsion by a wire-loop technique, exposed 10 to 14 days, developed in Kodak D-19, fixed in Kodak acid fixer, washed, and double-stained with uranyl acetate and lead citrate. Figure 51 shows an electron microscopic autoradiogram of a large mononuclear cell that contains phagocytosed latex particles (LP). The majority of silver grains over these ^{67}Ga-labeled mononuclear macrophages are closely associated with dense bodies. Perhaps of equal significance is the observation that both neutrophilic and eosinophilic granulocytes engulfed latex particles

1028273

but were not labeled with ^{67}Ga. Our results indicate that cellular ^{67}Ga localization is not dependent on phagocytosis and that certain types of cells exhibit degrees of selectivity for ^{67}Ga. This fact might be explained by differences in the lysosomal hydrolases contained by different types of cells. We have found that ^{67}Ga is associated with lysosomes in mononuclear macrophages. Other factors, such as cell surface phenomena, might also be involved in the affinity of certain cell types for ^{67}Ga.

Effect of Irradiation on ^{67}Ga Retention in Mouse Tissues

Another study in progress has as its goal the elucidation of the effect of X irradiation on ^{67}Ga retention in mice. We (D. C. Swartzendruber and K. F. Hübner) reported (37; see also ORAU-122) the effect of external whole-body irradiation on ^{67}Ga retention as a function of the administration of ^{67}Ga at different time intervals after irradiation. A summary of these data follows: Whole-body and tissue retention of the radionuclide ^{67}Ga was determined in adult $C3BF_1$ mice after acute whole-body X irradiation. A significant decrease in whole-body retention occurred after an exposure to 75 R. Decreased retention in soft tissues was a transitory effect and recovery of the ability to concentrate ^{67}Ga was dose dependent within the range of 100 to 950 R exposures. "Recovery" occurred at day 3 after 100 R, and between days 6 and 8 after 450 and 950 R. The radiation effect of decreased retention was most pronounced when the radionuclide was administered 24 hr after irradiation. In contrast to decreased uptake in soft tissues, ^{67}Ga concentration in the femur increased after irradiation. Blood levels decreased rapidly in irradiated mice (^{67}Ga injected 24 hr after 450 R) between 1 and 2 hr after administration of the radionuclide. Two hours after injection, its concentration was approximately 15% of that in nonirradiated, control animals. We conclude that decreased ^{67}Ga retention in mice after X irradiation is in part due to an effect on the synthesis of a relatively radiosensitive, as yet unidentified, blood or serum component that normally binds the radioisotope. Other as yet unidentified factors may also be operative, however.

Further evidence has been obtained to support our hypothesis that a serum factor is involved either directly or indirectly in the decreased ^{67}Ga retention observed in irradiated animals. The injection of 0.5 ml normal mouse serum into irradiated mice reduces the effect of irradiation on increased ^{67}Ga excretion but does not abolish it. Mouse serum collected from mice 24 hr after they received 450 R was injected into irradiated mice and it had no effect; i.e., whole-body retention was identical to that observed in irradiated animals receiving either saline injections or no injections. Horse serum was about as effective as normal mouse serum, indicating that the factor or component is not species specific.

In another experiment (Henry Mainwaring*, Norma L. Idoyaga-Vargas, and D. C. Swartzendruber) whole-body ^{67}Ga retention was determined at 1, 2, 3, 7, 14, and 20 days after its injection in irradiated mice instead of assaying ^{67}Ga retention 24 hr after its administration at different intervals after irradiation as in the previous report. As we found before, ^{67}Ga administered 24 hr after whole-body irradiation was excreted more rapidly (15 to 20% more) than in nonirradiated mice or in mice injected before or immediately after irradiation. Interestingly, the 15 to 20% differences were only noted at 1,

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2, and 3 days after ^{67}Ga injections; at 7, 14, and 20 days, the whole-body retention of ^{67}Ga was nearly the same in all groups of mice, irrespective of treatment.

The above studies form a baseline for subsequent determinations on the effect of irradiation on ^{67}Ga retention in animals bearing tumors. Studies in progress yield preliminary results that indicate ^{67}Ga retention and its tissue distribution are less severely affected by irradiation in C57BL/6 mice bearing transplanted preputial gland tumors than we observed in irradiated mice without tumors. We hope to gain clues from these experiments to further our understanding of the mechanisms of ^{67}Ga localization in both normal and malignant tissues. Further, these studies may shed light on as yet undiscovered irradiation effects at relatively low whole-body exposures, i.e., 75 R, that can be elucidated by determining the mechanism(s) that are operative or inoperative in this model system.

Induced Cytogenetic Abnormalities

Chromosome Breakage Frequencies in 977 Lymphocyte Cultures From Control Men and Woman, Pregnant Women, and Women Taking Oral Contraceptives: Results of Detailed Statistical Analyses of Data

During the last 4$^{1}/_{2}$ years our laboratory has been conducting an extensive longitudinal study of chromosome breakages in lymphocytes from control men and women, pregnant women, and women taking oral contraceptives (OC) (L. Gayle Littlefield, Eugene Joiner, Mary Beth Klepper, and Lydia Corrill). We have previously reported our findings of variability in aberration frequencies among consecutive cultures from the same individual and among cultures from different persons (ORAU-113, p. 168, ORAU-116, p. 159, and (26). This year, at the completion of data collection, we undertook a detailed statistical analysis (L. Gayle Littlefield, W. E. Lever*, F. L. Miller*) designed to determine whether synthetic estrogens and progestogens caused an increase in chromosome breakages in lymphocytes from OC users, and if so, whether the effect could be correlated with dose or length of time of exposure, and whether increased breakages persisted after cessation of the medication. In this communication we report the results of these analyses.

Description of Study. In our original protocol we planned to evaluate chromosomal breakage frequencies in a normal population of 10 men, 20 women who were currently taking OC, 20 women who had never taken OC, and 10 women before, during, and after pregnancy. Blood samples for lymphocyte culture were to be obtained from each person at intervals of 4 to 7 weeks for a total period of 2 years. As the study progressed it became evident that we would not be able to maintain the planned numbers of people in the various treatment groups because many women who enlisted in the study in one group subsequently switched to another. We continued to collect samples from each person, and at the completion of the study had accumulated 977 lymphocyte cultures from 10 men and 54 women.

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1028274

All participants were between the ages of 20 and 40, had no known phenotypic defects or chronic illnesses, and had no familial history of congenital abnormalities. At the time of each blood collection each person completed health histories describing recent incidental illnesses and medications and each woman recorded the dates of her last menstrual cycle.

We initiated 72-hr lymphocyte cultures from each blood sample, assigned code numbers to each preparation, and attempted to analyze for chromosome aberrations 100 consecutive well-stained, well-spread metaphases from each culture. Each metaphase was counted and the chromosomes analyzed by microscopically pairing chromosomes 1, 2, 3, and 16 and counting each member of groups B, E, F, and G chromosomes. All visible structural lesions were noted, and any metaphases with isolocus breaks (all classes), deletions, dicentrics, translocations, or inversions were considered to be abnormal. Five persons who were unaware of the identity of the blood donors were responsible for scoring the various preparations.

TABLE 46

TOTAL CULTURES OBTAINED FROM 10 MEN AND 54 WOMEN FROM OCTOBER 1968 TO MAY 1972

	Successful cultures	Culture failures	Total cultures initiated
Men (10)	148	11	159
Women (54)	701	117	818
	Σ 849	128	977

Total metaphases analyzed in successful preparations, 80,342.

In preparation for the statistical analyses, we encoded data on the following independent variables for each culture: culture number, donor, date of culture (month and year), age of donor at first culture, hormonal condition of each woman at time of culture, number of pregnancies, months since last pregnancy, state of current pregnancy, type of pill, months on pill, months since last pill, stage of OC or menstrual cycle (estrogenic, ovulatory, progestogenic, or unknown), sex of offspring, infection during preceding culture period (cold, viral, bacterial, and unspecified), incidental medication during preceding culture period (aspirin, antibiotics, tranquilizers, narcotics, other), and person scoring slides. Data also were encoded for the following dependent variables: success of culture, number of metaphases scored, metaphases with chromosomal breaks, number of isobreaks, deletions, dicentrics, translocations, and inversions, and when possible, the specific chromosome or chromosome group in which the lesion was observed.

Description of Sample Population. A summation of all cultures obtained from 54 women and 10 men between October 1968 and May 1972 is shown in Table 46. Of the 849 successful cultures, we were able to score 100 metaphases from 758 cultures and at least 20 metaphases from the remaining 91 cultures, for a total of 80,342 metaphases scored. To test for possible effects of current or previous hormonal treatments on culture growth and chromosomal breakage frequencies, we classified the 701 successful cultures from the 54 women according to the hormonal status of each woman at the time each culture was initiated. The various hormonal conditions and the number of cultures available for comparison in these groups are shown in Table 47.

TABLE 47

CLASSIFICATION OF SUCCESSFUL CULTURES ACCORDING TO HORMONAL STATUS OF WOMEN AT TIME EACH SPECIFIC CULTURE WAS INITIATED

	Number of cultures	
Men	148	
Women	701	
	No prior pregnancy	Prior pregnancy
No prior pill use	84	86
Prior pill use	73	86
Current pill use	120	153
Current pregnancy	52	47

Statistical Comparisons of Chromosome Breakages Among Groups Of Cultures From Women Having Different Hormonal Histories

Rates of Culture Failure. To determine where there were differences in the rate of failure in cultures obtained from the men and the various groups of women, we tabulated the percentage of cultures which did not yield analyzable metaphases in each group. These data are presented in Table 48. The average rate of culture failure in preparations from men and all nonpregnant women is (1) 11.1% compared to a rate of failure of 21.2% for

TABLE 48

RATE OF FAILURE IN CULTURES FROM MEN AND WOMEN WITH DIFFERENT HORMONAL CONDITIONS

	Successful cultures	Culture failures	Failure rate	Successful cultures	Culture failures	Failure rate
Men	148	11	6.9			
Women		No Prior Pregnancy			Prior Pregnancy	
No prior pill use	84	7	7.7	86	10	10.4
Prior pill use	73	13	15.1	86	17	16.5
Current pill use	120	12	9.1	153	24	13.6
Current pregnancy	52	14	21.2	47	20	29.9

pregnant women without prior pregnancy (2) and 29.9% for pregnant women with prior pregnancies (3). The differences between 1 and 2, and 1 and 3 are statistically significant (P less than or equal to 0.001). Our findings of an increased rate of failure in phytohemagglutinin-stimulated lymphocyte cultures from pregnant women are in agreement with several recent reports of decreased lymphoblast transformation in primiparas and multiparas. These data have been interpreted as supporting the theory that there is a depression of cell-mediated immunity during pregnancy.

1028275

Chromosome Breakage Frequencies: Unadjusted Data. As an initial comparison of chromosome breakages in the various groups of cultures, we calculated the frequency of abnormal metaphases, the number of chromosomes with breaks, and the number of isolocus breaks, deletions, dicentrics, translocations, and inversions observed in preparations from men and the various groups of women (Table 49). This tabulation of data suggested that there were differences in the number of metaphases with aberrations and the number of chromosomes with breaks among the various groups of cultures. Cultures from the men had the lowest frequency of abnormalities; whereas the highest frequency of aberrations were noted in cultures from OC users. In each group of cultures, higher breakages were noted in preparations from the women who had had previous pregnancies. In all groups, the majority of the lesions involved single chromosomes - i. e., isolocus breaks or deletions. Since the relative frequency of the various types of lesions (specific lesion: total lesions) appeared to be similar in all groups, we chose to use the number of abnormal cells per 100 cells as the criterion for statistical comparisons.

TABLE 49

CHROMOSOME BREAKAGES IN 80,342 METAPHASES FROM MEN AND WOMEN

	Abnormalities/100 metaphases*								
	Abn Cells	Abn Chrom	Isobr	Delet	Dicent	Trans	Inv	One-break Lesions†	Two-break Lesions‡
Men	5.36	5.77	3.59	1.64	0.20	0.24	0.10	5.23	0.55
Women									
No prior pill use									
No prior pregnancy	5.50	5.87	3.77	1.74	0.04	0.21	0.11	5.51	0.36
Prior pregnancy	6.57	7.30	5.05	1.90	0.04	0.24	0.07	6.95	0.35
Prior pill use									
No prior pregnancy	5.54	5.86	3.45	1.93	0.14	0.27	0.07	5.38	0.48
Prior pregnancy	6.15	6.30	4.29	1.70	0.06	0.16	0.09	5.99	0.31
Current pill use §									
No prior pregnancy	7.81	8.81	6.19	2.19	0.11	0.27	0.05	8.38	0.43
Prior pregnancy	8.06	8.75	6.11	2.10	0.16	0.33	0.05	8.21	0.54
Current pregnancy									
No prior pregnancy	4.63	5.08	3.01	1.52	0.12	0.24	0.18	4.53	0.55
Prior pregnancy	7.26	8.31	5.33	2.27	0.24	0.18	0.29	7.60	0.71

* Symbol abbreviations:
Abn = abnormal, Chrom = chromosomes, Isobr = isolocus breaks, Delet = deletions, Dicent = dicentrics, Trans = translocations, Inv = inversions.
† Isolocus breaks plus deletions.
‡ Dicentrics plus translocations plus inversions.
§ Includes data from four women taking sequential medications.

Comparison of Breakages in Cultures From Women Taking Different Types (Sequential-*vs*-*Combination-type) OC.* We obtained cultures from 31 women during times when they were taking OC. Four of the women were taking sequential-type medications (estrogen only, first 15 to 16 days of the cycle followed by 5 days estrogen plus progestogen), whereas the other 27 women were using combination-type medications (estrogen plus progestogen regimen for 20 or 21 days). In earlier tabulations of average breakage frequencies in cultures from individual women, we had been impressed with an unusually high frequency of breakage in cultures from three of the four sequential OC users. We calculated the average breakage frequencies in 35 cultures obtained from these

four women while they were taking sequential OC and obtained a mean of 13.65 abnormal cells per 100 cells compared to a mean of 7.2 for the combination pill users. Because of the discrepancies in breakages between the combination and sequential users and since at least two sequential-type medications (C-Quens and Provest) have been taken off the market and other sequentials are being prescribed infrequently, we excluded *all* cultures from the sequential users from the subsequent comparisons of breakage frequencies among the groups of women.

Comparison of Breakages in Cultures From Women Taking Combination-Type OC Compared to Women With Other Hormonal Histories. After excluding from the various groups of cultures all cultures from women who had taken sequential medications, we recalculated the mean number of abnormal metaphases/100 cells and the standard deviations of the means for the various groups of cultures obtained from women with different hormonal histories (Table 50). The variances of the means are weighted sums of variances of the individual subjects where only the cultures in a given group contribute to the variance for that group. That is if $\bar{\bar{P}}$ is the group mean and $\bar{P}_1 \ldots, \bar{P}_k$ are the subject means in the group based on $n_1 \ldots, n_k$ cultures, the estimate of the variance of $\bar{\bar{P}}$ is

$$S^2_{\bar{\bar{P}}} = \sum_{i=1}^{k} \left(\frac{n_i}{N}\right)^2 S^2_{\bar{P}_i}$$

when

$$N = \sum_{i=1}^{k} n_i \quad \text{and} \quad \bar{\bar{P}} = \sum_{i=1}^{k} \frac{n_i}{N} \bar{P}_i .$$

To determine whether the apparent differences in breakages among these groups of cultures were significant (P less than or equal to .05) we performed six nonindependent tests of significance on these means. It should be noted that in these statistical comparisons we dealt with the data on a per culture basis, and the significance tests we performed assumed that the cultures were independent of one another. This is clearly not true since multiple cultures were taken from each subject and many women changed hormonal status during the study. The effect of this nonindependence is difficult to determine, but given the relatively large number of subjects contributing to each group, we hope that the differences observed are at least a useful indication of the effects of hormonal condition on chromosome aberration frequencies. The results of the statistical comparisons of the group means showed (1) Among women who had never been pregnant, breakages in cultures from current pill users were significantly higher than breakages in women who had never taken OC, whereas breakages in these two groups were not significantly different when the women had had previous pregnancies. (2) Regardless of pregnancy history, current pill users had significantly higher chromosome breakages than women not on OC but who were formerly pill users. (3) Given no pill use, the women who had had previous pregnancies did not differ significantly from those who had never had pregnancies. (4) Breakage in cultures obtained from women during their second or subsequent pregnancy were significantly higher than breakages in women who had never been pregnant nor used OC.

1028276

TABLE 50

COMPARISON OF CHROMOSOME-BREAKAGE FREQUENCIES IN CULTURES FROM MEN AND WOMEN

	N†	$\bar{\bar{P}}$	$S_{\bar{\bar{P}}}$	N	$\bar{\bar{P}}$	$S_{\bar{\bar{P}}}$
Men	148	5.4	.34			
Women*	No prior pregnancy			Prior pregnancy		
No prior pill use	84	5.5	.40	86	6.6	.59
Prior pill use	68	5.4	.48	82	5.9	.46
Current pill use	112	7.2	.43	124	7.2	.33
Current pregnancy	39	4.7	.41	43	7.9	.66

* Excluding all cultures from four women who had taken sequential-type medications during the course of the study.

† N = number of cultures, $\bar{\bar{P}}$ = mean (number of abnormal cells per 100 cells), $S_{\bar{\bar{P}}}$ = standard error of the mean.

Tests for Variations in Aberration Frequencies During Different Stages of Menstrual Cycle. The observation of a significant increase in the frequency of chromosome breakages in cultures from nulligravidas using OC compared to nulligravidas who had never taken these hormones suggested a possible correlation between exogenous estrogens and/or progestogens and cytogenetic aberrations in lymphocytes. Since circulating levels of endogenous estrogens and progestogens undergo cyclic fluctuations, we next attempted to determine whether breakages varied consistently during different stages of the menstrual cycle in cultures from control women and OC users. Assuming that ovulation usually occurs 14 to 16 days before the onset of menses in women not taking OC, and considering the first day of menses to be day one of the cycle, we arbitrarily divided the cycle into three stages; we considered cultures obtained between days minus one and minus 12 to be "progestogenic;" between days minus 13 and minus 16 to be "ovulatory," and between day minus 17 and the last day of the preceding menses to be "estrogenic." The frequency of chromosomal breakages observed in cultures obtained from women during these stages of the menstrual cycle are shown in Table 51. Although there appears to be some differences in breakages in cultures obtained during different stages of the menstrual cycle from women with specific hormonal statuses (i.e., high breakages during midcycle in pill users with prior pregnancies), no specific trend in breakages could be detected among cultures from the six groups of women.

Corrections for Possible Effects of Nonhormonal Exposures On Aberration Frequencies. The lack of correlation between stage of the menstrual cycle and breakage frequencies indicated that the overall increases in breakages in pill users could not be directly correlated with "dose" of hormones (i.e., circulating concentrations of endogenous and exogenous hormones). We next attempted to determine whether the differences in breakages among the various groups of women could be attributed to any known external factors to which the women had been exposed. We considered as possible covariates the incidental infections and medications reported on health records of each subject. We first determined the mean breakage frequency for all cultures obtained from persons who reported having a specific infection or having taken specific medications during the preceding month, and compared these means to the breakages noted in persons who had not reported exposures to illnesses or medications (Table 52). An increase in average chromo-

TABLE 51

THE PERCENTAGE OF ABNORMAL METAPHASES IN RELATION TO STAGES OF THE MENSTRUAL CYCLE

	Stage of Cycle*									
	1		2		3		Unknown		All	
Pill use	Never pregnant									
Never	37†	*6.5‡*	15	*4.1*	17	*4.2*	15	*6.0*	84	*5.5*
Prior	22	*4.9*	8	*5.6*	27	*5.7*	11	*5.8*	68	*5.4*
Current	51	*6.8*	13	*7.5*	40	*7.2*	8	*9.2*	112	*7.2*
Pill use	Prior pregnancy									
Never	32	*6.7*	11	*4.8*	35	*7.2*	8	*5.6*	86	*6.6*
Prior	42	*6.4*	9	*5.9*	16	*6.4*	15	*3.9*	82	*5.9*
Current	47	*7.0*	15	*10.2*	52	*6.3*	10	*8.2*	124	*7.2*

* 1 = "estrogenic"; 2 = "ovulatory"; 3 = "progestogenic"; unknown = stage of cycle could not be determined from health sheets.

† Number of cultures

‡ Italicized numbers denote abnormal cells/100 cells.

some breakages was noted in cultures from persons having both bacterial and viral infections, and from those who had taken antibiotics and narcotics. To correct for the influence of these factors on chromosome breakages, we selected the covariate showing the largest positive effect, bacterial infection, and decreased the percentage of abnormal metaphases in each culture obtained under this condition by 2.12% (the difference between the culture means for subjects having bacterial infections and subjects not having bacterial infections in the month preceding the culture). The correction for bacterial

TABLE 52

CORRECTIONS FOR POSITIVE EFFECTS OF COVARIATES ON CHROMOSOME BREAKAGE FREQUENCIES

Cultures (786)*	Raw data			Adjusted for bacterial infection			Then adjusted for viral infection			Then adjusted for narcotics medication		
	No	Yes	Dt	No	Yes	D	No	Yes	D	No	Yes	D
Infections	6.2	6.3	.075	6.2	5.9	–	6.2	5.9	–	6.2	5.8	–
Cold	6.3	5.9	—	6.2	5.7	–	6.2	5.6	–	6.2	5.6	–
Viral	6.2	6.7	.486	6.1	6.5	.401	6.1	6.1	–	6.1	6.1	–
Bacterial	6.1	8.3	2.120	6.1	6.1	–	6.1	6.1	–	6.1	6.1	–
Unspecified	6.3	5.8	–	6.2	5.8	–	6.1	5.7	–	6.1	5.7	–
Medications	6.4	6.2	–	6.4	6.1	–	6.4	6.0	–	6.4	6.0	–
Aspirin	6.3	6.2	—	6.2	6.1	–	6.2	6.0	–	6.2	6.0	–
Antibiotics	6.2	6.9	.702	6.1	6.1	–	6.1	6.0	–	6.1	6.0	–
Tranquilizers	6.3	5.6	—	6.2	5.4	–	6.2	5.4	–	6.1	5.4	–
Narcotics	6.2	6.7	.516	6.1	6.5	.388	6.1	6.4	.321	6.1	6.1	–
Others	6.3	6.1	–	6.2	6.0	–	6.2	6.0	–	6.2	5.9	–

* Cultures from women using sequential-type OC not included.

† Relative increase in breakage frequencies in cultures obtained from persons exposed to infections or medications during the preceding month compared to cultures from persons reporting no infection or medication.

1028277

infections also acted as a correction for the covariate antibiotic medication (... might have been expected since most bacterial infections are treated with antibiotics).

With the data adjusted for bacterial infection and antibiotics, two other covariates, viral infection and narcotics medication, still showed a positive effect on the percentage of abnormal metaphases. The data were next adjusted for effects of viral infection, and lastly for effects of narcotics. This eliminated any positive effect "due to" a covariate.

TABLE 53

STATISTICAL COMPARISON OF CHROMOSOME-BREAKAGE FREQUENCIES (ADJUSTED MEANS)* IN CULTURES FROM MEN AND WOMEN

	N†	$\bar{p}$	$S\bar{p}$	N	$\bar{p}$	$S\bar{p}$
Men	148	5.3	.34			
Women	No prior pregnancy			Prior pregnancy		
No prior pill use	84	5.3	.40	86	6.5	.59
Prior pill use	68	5.2	.48	82	5.6	.46
Current pill use	112	7.1	.43	124	7.2	.33
Pregnant	39	4.5	.41	43	7.6	.65

* Cultures adjusted for effects due to bacterial and viral infections and antibiotic and narcotic medications.

† N = number of cultures; $\bar{p}$ = mean (number of abnormal cells/100 cells); $S\bar{p}$ = standard error of the mean.

Following this series of adjustments for covariates, the selected comparisons of group means (Table 50) were repeated for the adjusted means (Table 53) with identical results. The lack of change in the results of any of the significance tests on the group means was probably due to the approximately equal distribution of covariates among the various groups.

Evaluation of Breakage Frequencies as a Function of Time Off Pill. Although breakages in cultures from nulligravidas who were currently taking OC were significantly higher than cultures from nulligravidas who had never taken synthetic hormones, it was interesting to note that breakages in cultures from women who had previously taken OC were not different from control values. As a final test of the data, we attempted to determine whether the decrease in aberration frequencies in cultures from women who stopped taking OC could be correlated with time off pill. Calculating the percent abnormal cells in individual cultures from women in this category in relation to the number of months between administration of the last pill and the date the cultures were obtained, we could not detect a decrease in aberration frequency as a function of time off pill.

Discussion. Although our primary objective was to determine whether synthetic estrogens and progestogens caused chromosome damage in lymphocytes from OC users, several other significant observations were made in our study. We observed that chromosome breakages (particularly isolocus lesions) are a common occurrence in lymphocyte cultures from "control" men and women, and that the frequency of breakages varies considerably, both among consecutive cultures from the same person, and among cultures from different people. We also observed that average breakages in series of cultures from some persons are higher than in preparations from others, suggesting that there may be differences among individuals in "susceptibility" to lymphcyte chromosome damage.

ıe present statistical comparisons showed significantly higher *average* breakages in lymphocyte cultures from nulligravidas who were taking OC and in pregnant women who had had previous pregnancies than in nulligravidas who had never taken the pill; however, higher breakages were not observed in all cultures from women in these two groups. The variability in aberration frequencies in cultures from these women is not surprising, since any damage incurred as a result of hormonal exposure would be superimposed on an unknown and inconstant frequency of "background" breakages. In an effort to reduce this background, and to define the effects of female hormones more precisely, we applied statistical corrections for increased cytogenetic damage that may have been induced by other agents that have been reported to cause chromosome breakages (i.e., viruses, narcotics, antibiotics, etc.). That these data adjustments did not greatly reduce the variability in the various groups of cultures suggest that many unidentified factors may affect the frequency of chromosome breakages in lymphocytes.

Since we did not observe an increase in breakages as a function of time on pill, consistent differences in breakages during the first, middle and last third of the pill cycle, or consistently elevated breakages in cultures from all women taking OC, we must surmise that the effect of synthetic hormones on chromatin is not directly related to concentration of circulating hormones or length of time of exposure.

If synthetic hormones do not actively induce chromosome breakages what then might account for the inconsistent increases in breakages that we have observed in lymphocyte cultures from OC users? Perhaps the most plausible explanation is that female sex hormones affect other metabolic or biochemical systems with a resultant expression of increased chromosomal damage in lymphocytes. Over 80 physical, chemical, and biological agents have been shown to cause chromosome breakages in mammalian cells. Were synthetic hormones to enhance the chromosome-breaking properties of any of these or other agents, or to interfere with mechanisms of chromosome repair or the removal of cells damaged by other agents, overall increases in breakages could be observed. There is no doubt that female sex hormones exert an effect on a diversity of metabolic and biochemical parameters, and numerous subtle changes have been noted in OC users and pregnant women. Oral contraceptives have been implicated in the induction or exacerbation of several diseases, and studies in both humans and animals have suggested that estrogens and/or progestogens may affect a number of immunologic phenomena. Considering the multiplicity of physiological alterations that have been associated with synthetic and natural female hormones, it does not seem unreasonable to suspect that OC usage might secondarily create a subtlely altered in vivo milieu in which slightly increased frequencies of chromosomal breakages might occur.

Regardless of the etiology of the increased breakages that we have observed in OC users, the nebulous nature of our findings considerably complicates interpretations of our data. If agents such as OC do not directly damage lymphocyte chromosomes but in some manner induce an in vivo condition in which increased chromosome damage may occur, how does one assess the significance of the increased breakages? Apparent correlations between increased frequencies of chromosome breakages and increased risk of neoplastic disease have been noted in patients with several inherited diseases including Bloom's syndrome, ataxia telangiectasia, and Fanconi's anemia, and in populations of persons exposed to well-defined chromosome-breaking agents such as ionizing radiations. Likewise, aberrations of chromosome number or structure are typical findings in solid tumors and leukemia. The nature of the association between cytogenetic aberrations and malignant cell transformation is now known, but the fact that an association exists has

1028278

generated concern over the possible hazards of exposure to agents that alter somatic chromosomes. Our data on chromosomal aberrations in lymphocyte cultures from normal men and women indicate that chromosomal breakages are a common occurrence in this cell type, and that the frequency of chromosome breakages varies over time, both in cultures from the same person and among cultures from different people. Thus, we cannot assume that agents such as OC, which apparently result in overall increases in breakages, alter a homeostatic condition that was free from defect before exposure. If chromosome damage in lymphocytes cultured in vitro do reflect similar breakages in this cell-type in vivo, one must surmise that either these breakages are not consequential, or that under normal circumstances, mechanisms for the removal of cells bearing nonlethal type aberrations are operative. Thus if increased somatic-cell risk does exist, it would be restricted to that population of individuals who cannot eliminate somatic cells bearing aberrant chromosomes.

In summary, we have observed a significantly higher frequency of chromosomal aberrations in lymphocytes from nulligravidas taking OC and in multiparous pregnant women than in cultures from nulligravidas who have never taken OC. The effect of the synthetic hormones on aberration frequencies could not be correlated with dose, length of time on pill, or stage of pill cycle, suggesting that these agents do not directly damage lymphocyte chromosomes. Since estrogens and progestogens affect many metabolic and biochemical systems, it is possible that these substances may induce a subtlely altered in vivo condition in which increased chromosome breakages may be expressed.

Comparison of Chromosome Breakages in Lymphocytes and Fibroblasts From Control Women and Women Taking Oral Contraceptives

As previously discussed, variability in aberration frequencies in the control cultures, combined with the observation of very high breakages (>20%) in some preparations suggested that the lymphocyte may be extremely susceptible to chromosome damage induced by a variety of extraneous factors, and raised the question, does exposure to agents such as OC, which apparently results in a slight increase in average breakages in lymphocytes, also result in chromosome damage in other somatic cells? In an attempt to answer this question, we initiated a series of supplementary studies designed to determine the in vivo and in vitro effects of synthetic hormones on a second type of human cell, the skin fibroblast (L. Gayle Littlefield, J. Mailhes, and B. Lazar). By culturing peripheral blood lymphocytes and skin fibroblasts from groups of control women and pill users, we hoped to determine (1) whether there were differences in breakage frequencies between the two types of cells, (2) whether the frequency of chromosome breakages or of cytogenetically aberrant clones of cells was higher in fibroblasts from women taking OC, and (3) whether chromosome breakages were induced in fibroblasts by serum from OC users.

Description of Study. During an 18-month period, we obtained a minimum of four sets of blood samples and skin biopsies from each of five women who were taking combination type OC and from five women who had never taken synthetic hormones. All women were between the ages of 20 and 40, in good health, and had no history of congenital abnormalities or medical defects. At the time each blood sample and skin biopsy

were obtained, each woman filled out a health sheet describing recent illnesses and incidental medications taken, and recorded the dates of her last menstrual cycle. We set up the following series of cultures.

Lymphocyte preparations: We initiated two lymphocyte cultures from each blood sample: one culture was supplemented with autologous serum and the other with commercially processed fetal calf serum (FCS) (final concentrations of serum 20%). The cells were harvested after three days incubation at 37°C, and slides made for cytogenetic evaluation. The culture methods, fixation and staining procedures have been described in detail elsewhere (ORAU-116 and -122).

Fibroblast preparations: On the same day that venous blood samples were drawn for lymphocyte cultures we obtained a full-thickness skin biopsy from each woman. Primary fibroblast cultures were initiated according to previously described methods (ORAU-122). The primary cell-lines derived from the biopsies were maintained using standard tissue-culture procedures. When sufficient fibroblasts had been accumulated for the cytogenetic studies, we exposed replicate subcultures of dividing cells to fresh medium containing the following types of serum: 1) 20% FCS, 2) 20% autologous serum, 3) 20% homologous serum (from a control woman), and 4) 20% homologous serum (from an OC user). After approximately 24 hr incubation, the monolayers were trypsinized to suspend the cells and the cultures were harvested for cytogenetic evaluation. The lymphocyte and fibroblast cultures were given code numbers so that the five persons scoring the slides would not be aware of hormonal status of the tissue donor.

Scoring procedures: In evaluating these cultures for chromosome aberrations we had to take into account that, in contrast to lymphocytes which normally progress through only 1 to 3 divisions in 72-hr cultures, fibroblasts derived from primary tissue must proceed through numerous in vitro divisions before a sufficient number of cells are available for cytogenetic study. Those fibroblasts which suffered lethal-type cytogenetic injuries in vivo would be eliminated during the culture period, whereas cells bearing nonlethal-type chromosomal rearrangements might become established in culture. We evaluated all fibroblast cultures for both primary breakages that may have been induced by exposure to various sera and for stem-lines of abnormal cells that may have evolved as a consequence of in vivo and in vitro injury to chromosomes.

We attempted to score for chromosomal abnormalities 100 well-stained, well-spread metaphases from each lymphocyte and fibroblast culture. Metaphases with good morphology were counted, and those having 45 or 46 centromeres were analyzed for chromosomal breakages and rearrangements. The frequency of all types of identifiable lesions (single chromatid and isochromatid gaps and breaks, deletions, translocations, inversions, dicentrics) was determined for each culture. If a specific rearrangement was noted in three or more cells in the same replicate series of fibroblast cultures, we scored the aberrant metaphases as a stemline and did not include the lesion in breakage tabulations.

Results.

Rates of culture failure: We obtained analyzable metaphases from 20 sets of blood and fibroblast cultures from the five control women, and from 17 sets of cultures from the five pill users. However, there were differences in growth in replicate fibroblast cultures exposed for the last 24 hr of culture to the various types of sera; thus, it was frequently necessary to repeat individual series of serum experiments before we obtained analyzable metaphases in all cultures. For this reason we had to maintain several of the primary cell-lines for long periods (average time in culture for fibroblasts from control women 75 days,

1028279

TABLE 54

CHROMOSOME ABERRATIONS IN LYMPHOCYTES AND FIBROBLASTS FROM CONTROL WOMEN AND WOMEN TAKING ORAL CONTRACEPTIVES

Control women Cell type	Serum	No. Cult*	Percent Fail	No. cells scored	Abnormal Cells/100 cells: including chromatid lesions	Abnormal Cells/100 cells: excluding chromatid lesions
Lymphocytes	Autol	19 (1)	5	1713	*81*† 5.62 ± 1.17	*39* 3.04 ± 1.03
Lymphocytes	FCS	18 (2)	10	1391	*81* 5.84 ± 3.06	*35* 3.06 ± 1.00
Fibroblasts	Autol	19 (1)	5	1528	*85* 6.51 ± 1.61	*41* 3.90 ± 1.39
Fibroblasts	FCS	14 (6)	30	1002	*52* 5.57 ± 0.89	*22* 2.96 ± 0.92
Fibroblasts	Homol	17 (3)	15	1394	*74* 5.82 ± 0.89	*44* 3.63 ± 0.83
Fibroblasts	Homol OC	15 (5)	25	1113	*63* 5.84 ± 1.46	*28* 2.79 ± 0.99
OC Users						
Lymphocytes	Autol	17 (0)	0	1510	*66* 4.99 ± 1.00	*28* 2.43 ± 0.81
Lymphocytes	FCS	14 (3)	17.6	770	*48* 7.10 ± 1.39	*26* 3.83 ± 1.38
Fibroblasts	Autol	15 (2)	11.7	1447	*68* 4.60 ± 0.89	*37* 1.86 ± 0.44
Fibroblasts	FCS	13 (4)	23.5	947	*62* 6.23 ± 1.27	*26* 2.52 ± 1.04
Fibroblasts	Homol	15 (2)	11.7	1403	*70* 5.09 ± 0.70	*30* 2.15 ± 0.28
Fibroblasts	Homol OC	15 (2)	11.7	1368	*65* 4.88 ± 0.69	*33* 2.48 ± 0.54

* Success (failure) cultures with less than 20 metaphases analyzed; data from these not included in breakage tabulations.

† *81 (number of lesions observed)*; 5.62 ± 1.17 (mean ± standard error).

for cultures from OC users 70 days). As is shown in Table 54, we obtained best results in fibroblast cultures exposed to autologous serum, and poorest results in cells exposed to FCS. Because of the detrimental effects of FCS exposure on general culture growth, we frequently could not analyze the proposed 100 metaphases in these cultures.

Chromosome breakage frequencies: To determine whether there were differences in the frequencies of chromosome aberrations in the various lymphocyte and fibroblast cultures, we calculated the average number (± S.E.) of cells having chromosome breakages (both including and excluding single chromatid breaks) in the various sets of cultures (Table 54). The most frequently observed lesions in all groups of cultures were simple chromatid and chromosome breaks. Complex lesions requiring two or more breaks for their formation (i.e., chromatid exchanges, dicentrics, translocations, etc.) were seen infrequently.

The frequency of cells having damaged chromosomes appeared to be similar in all groups of cultures, and statistical comparisons of the means verified that there were no differences in breakages within series of lymphocyte and fibroblast cultures treated with the various types of sera, or among lymphocyte and fibroblast cultures from the control women compared with cultures from women taking OC. Thus in these experiments, fibroblasts exposed to autologous or FCS serum had the same frequency of breakages as lymphocytes cultured in media containing the same serum, and no increase in breakages could be detected in fibroblasts exposed to serum from OC users. Likewise, no increase in breakages was observed in lymphocytes and fibroblasts from OC users.

Evaluation of stem-lines: In addition to scoring metaphases for primary breakages, we were interested in determining whether stem-lines of aberrant cells (derived from either in vivo or in vitro injury) were more likely to evolve in fibroblasts obtained from OC users than in cultures derived from women who had not been exposed to synthetic hormones. In seven sets of cultures we were able to identify subpopulations of cells having consistent cytogenetic abnormalities. Five of the stem-lines were observed in fibroblasts from the control women and two clones were observed in fibroblasts from OC users (Table 55). As is shown in the table, we observed clones of cells having consistent structural abnormalities (i.e., translocations, inversions, dicentrics) as well as a stem-line monosomic for a D group chromosome and a pseudodiploid stem-line monosomic for a no. 3 chromosome and trisomic for a C-group chromosome. The various stem-lines were observed in cultures of ages ranging from 41 to 144 days and comprised varying percentages of the cell populations ranging from less than 1% to virtually 100%.

TABLE 55
CHROMOSOMALLY ABERRANT STEM-LINES OBSERVED IN PRIMARY FIBROBLAST CULTURES FROM CONTROL WOMEN AND OC USERS

Donor code	Fibroblast series*	Days in culture	Total number cells scored	Karyotypic description of stem-lines	Number of cells having stem-line rearrangements (percent of total cells scored)	
Control Women						
Ia	160-163	75	52	Modal chromosome number 45 with a missing group "D" chromosome (45, XX, D-)	38	(73%)
Ib	400-403	124	150	Modal chromosome number 46 with a missing 3 and an extra group "C" chromosome (46, XX, 3-, C+)	~ 150†	(100%)
2	304-307	69	400	Modal chromosome number 46 with a translocation between a "C" and a "D" group chromosome (46, XX t(C_p + D_q-))	90	(22.5%)
3	220-223	52	219	Modal chromosome number 46 with a pericentric inversion of a "D" chromosome (46, XX, inv (D_p + q-))	6	(2.7%)
4	176-179	144	90	Modal chromosome number 46 with a dicentric involving a 3 and a "C" group chromosome (45, XX, dic+)	3	(3.3%)
OC Women						
1	125-128	41	269	46 modal count with a deletion of the short arms of a "B" chromosome (46, XX, B_p-)	6	(2.2%)
2	348-351	69	400	Modal chromosome number 46 with a dicentric involving 2 "C" chromosomes (45, XX, dic+)	3	(0.8%)

* Pooled data derived from 4 replicates of the same primary fibroblast culture, exposed to either autologous, FCS, homologous or OC serum during the 24 hr period preceding harvest. No differences were observed in the percentages of the various stem-lines in cultures exposed to different sera.

† In a few metaphases the chromosomes were too contracted to positively identify the No. 3 chromosomes, making it impossible to determine if the cell had the stem-line rearrangement.

Discussion. In the present series of experiments we did not observe an increase in chromosome breakages in lymphocyte cultures from five women taking OC compared to breakages in cultures from five women who had never taken synthetic hormones. This finding is not unexpected since our larger survey study of aberrations in lymphocytes from pill users (see p. 120, this report) had demonstrated considerable variability in breakages in consecutive cultures from the same individual, and among cultures from different persons. Likewise, we had previously found that average breakages in series of cultures from some OC users were not different from breakages in cultures from some control women. Because of the variability in breakages among cultures from the same individual, we obtained four separate sets of tissue samples from each woman for the evaluation of chromosome lesions. However, since we obtained blood from only five women in each group, it is possible that our sample size was not large enough to detect the minor increases in breakages that we had observed in the larger group of OC users.

The primary objective of this study was to determine whether OC usage resulted in increased chromosome damage in cells other than lymphocytes. We chose to study fibroblasts because these cells are relatively easy to culture and are the only somatic cells other than lymphocytes that can be obtained from human volunteers with a minimum of trauma. We realized at the outset that there were some problems in using this cell-type for estimates of aberrations induced in vivo. Because of the loss of cells bearing some types of aberrations and the possible evolution of stem-lines of other abnormal cells, it follows that the aberrations observed in fibroblasts cultured in vitro for a period of several weeks would not necessarily reflect the in vivo condition of this population of cells. Thus, in an effort to determine the effect of OC on chromosomes, we not only analyzed breakages in standard cultures exposed to FCS but also exposed replicate fibroblast cultures to autologus, homologous, and homologous serum from OC users. From the series of replicate cultures we hoped to learn whether the frequency of breakages or stable rearrangements was higher in the standard cultures from the OC users, whether fibroblasts from women who had taken OC would show increased breakages or abnormal growth when re-exposed to serum containing synthetic hormonal constituents, and whether serum from OC users contained substances that could induce breakages in fibroblasts from women who had not taken oral contraceptives.

Results of cytogenetic analyses of these fibroblast cultures showed no difference in chromosome breakages in cells from OC users cultured in FCS compared to control cells cultured in the same serum, and we could not detect any evidence of breakage induction by OC serum in either the control fibroblasts or fibroblasts from OC users. Since in the OC users lymphocytes cultured in autologous plasma did not have an increased frequency of breakages, it is not surprising that serum from these women had no effect on breakages in the various fibroblast cultures. Likewise, we found no evidence for increased frequency of cytogenetically abnormal stem-lines in fibroblasts from the OC users. In fact, we observed five aberrant clones of cells in 20 primary cultures derived from skin from the control women, and only two aberrant clones in 17 primary cultures from the OC users. We do not know whether the seven abnormal cell-lines observed in these cultures arose from cells that suffered injuries in vivo, or whether the clones evolved as a result of an injury incurred

1028280

during the period of culture. But the lack of observation of a substantial number of chromosomally aberrant stem-lines in that 17 sets of cultures from the OC users certainly suggests that fibroblasts from these women are no more predisposed to the evolution of aberrant clones than are fibroblasts from women who have not taken synthetic hormones.

In addition to giving information on the lack of demonstrable effect of OC on chromosome aberrations in fibroblasts, several general observations can be made from our study. First, we found that primary chromosome breakages (chromatid and chromosome aberrations) occur with approximately equal frequencies in both lymphocytes and fibroblasts cultured in vitro. Although we cannot assume from this observation that these two cell-types have equal in vivo sensitivities to various chromosome breaking agents, we can conclude that "background" breakages are as prevalent in cultured fibroblasts as in cultured lymphocytes. We also observed variability in "spontaneous" breakages among the various fibroblast cultures. Thus, as far as background breakages are concerned, it appears that there is little advantage in using fibroblasts as opposed to lymphocytes for in vitro test procedures.

Secondly, although it is well-known that primary fibroblast cultures have a finite life span, and that changes in ploidy may occur as these cell-lines approach senescence, there have been few studies to determine how soon after culture initiation stem-lines of chromosomally aberrant cells may be observed. Consideration of the evolution of abnormal clones has considerable practical importance since the chromosomally aberrant stem-lines derived in culture do not represent the true in vivo karyotype of the individual being studied. Fibroblast cultures are routinely used in clinical studies for evaluation of tissue mosaicism in children with phenotypic defects and in prenatal diagnosis (via amniocentesis) of high-risk fetuses. It is frequently necessary to maintain clinical cultures for periods of 6 to 12 weeks before a sufficient number of cells are accumulated for cytogenetic analyses. We observed five different chromosomally aberrant cell-lines in primary cultures maintained in vitro for periods of less than 70 days. In two sets of cultures (1a and 2) the stem-lines were major components of the fibroblast population. Obviously, had these biopsies been obtained from an individual or fetus suspected of having a congenital chromosomal abnormality, it would have been impossible to know whether the population of abnormal cells was karyotypically representative of the cell donor or whether the stem-line arose de novo in culture. Our data certainly suggest that caution should be used in the interpretation of stem-line data obtained from primary fibroblast cultures from abnormal patients, since these stem-lines may not be representative of the true karyotype of the tissue donor.

C-Monosomy in Marrow Cells From a Patient With Peripheral Pancytopenia and Marrow Hyperplasia. Identification of the Missing Chromosome by Giemsa-Banding Techniques.

Although many patients with "preleukemic" conditions have been found to have normal karyotypes, a missing C-group chromosome has been reported in marrow cells from at least 10 persons with various "myeloproliferative" disorders. In six of these patients, the disease eventually terminated in either acute myelocytic or erythroleukemia. The apparent correlation between C-monosomy and abnormal marrow proliferation has prompted several investigators to speculate that the genes responsible for control of

1028281

hematopoiesis may be located on one or more C-group chromosomes, and that any excess of deficiency of C-chromatin might result in a condition conducive to abnormal marrow proliferation. Unfortunately, prior to 1970, when chromosome-banding methods were first introduced, cytogeneticists could not specifically identify the seven pairs of chromosomes comprising the C-group (i.e., autosome pairs 6, 7, 8, 9, 10, 11, 12, and the X chromosome(s) and the identity of the missing C chromosome has not been determined in any of the reported ones. Thus, it has not been possible to determine whether the missing C-chromosome in marrow cells from the 10 patients with preleukemic disorders was a member of the same chromosome pair.

We have recently observed a major stem-line of cells with a 45,-C karyotype in marrow preparations from a man with peripheral pancytopenia and hyperplasia of the marrow (L. Gayle Littlefield and Eugene E. Joiner). The clinical findings in our patient are compared to those reported in other patients with myeloproliferative disorders in Table 56. In addition to documenting another case of "C-monosomic myeloproliferative disease," we would like to report the results of our preliminary attempts (using Giemsa-banding techniques) to identify the specific missing C chromosome in our patient.

This 52-year-old man was untreated at the time of admission to ORAU Medical Division. We obtained and processed marrow samples for cytogenetic evaluation according to methods routinely used in our laboratory. Two to five drops first aspirate marrow were collected in 8 cc tissue-culture medium containing 10 units heparin and 20 μgm colchimide/cc. After incubation for 1 hr at 37°C, we prepared slides for cytogenetic analyses. We analyzed 50 metaphases from this preparation for aberrations of chromosome number or morphology. The following chromosome counts were obtained:

number of chromosomes	44	45	46
number of metaphases	1	45	4

Four metaphases were normal diploid cells with a chromosome complement of 46,XY; whereas 45 metaphases (90%) had a hypodiploid count of 45 chromosomes. Microscopic and karyotypic studies showed that the missing chromosome was consistently a member of the C group (Fig. 52). Our inability to detect any evidence of chromosomal rearrangement in the aberrant metaphases suggested that the stem-line arose as a result of a simple non-disjunction. Because of the patient's pancytopenia, lymphocyte cultures were not attempted. However, since inherited C monosomy is not compatible with life, we assume that the hypodiploid stem-line was not congenital, but karyotypically represented the abnormally proliferative marrow elements.

Having established the presence of a major clone of hypodiploid cells in this patient, we next attempted to identify the specific missing C chromosome using Giemsa-banding techniques. For reasons that are not clear, marrow chromosomes tend to be fuzzy and overly contracted. Thus banding methods which reproducibly produce good results in elongated lymphocyte or fibroblast chromosomes do not yield good resolution in marrow preparations. We attempted several banding procedures, and obtained best results using a modification of the technique of Chu (personal communication). Briefly, slides to be banded were treated for 9 to 17 min in hot buffer (44 to 56°), and stained for 5 to 7 min in a trypsin-Giemsa staining solution consisting of 26 cc 0.025 m phosphate buffer, 10 cc methanol, 4 cc 0.1% trypsin, and 1 cc Giemsa stock stain. We located metaphases having 45 chromosomes, and attempted to identify specific pairs of C chromosomes according to banding criteria proposed by the Paris Conference. In most hypodiploid metaphases only a few C-group chromosomes showed optimum banding, thus we were not able to identify

Fig. 52 Regularly stained metaphase and karyotype from a marrow cell from our patient. Note the hypodiploid count with a missing chromosome in the C group. The size and short-arm to long-arm ratio of the monosomic "C" is typical of chromosome 8.

each pair of autosomes in each cell scored. We recorded the various pairs of homologues which could be positively identified in the various metaphases, and obtained the following profile of C chromosomes in this hypodiploid cell line:

C-group chromosome Pairs	X	6	7	8	9	10	11	12
Number of metaphases scored in which specific pairs could be identified	10	18	10	0	3	5	18	15

In metaphases with reasonably good banding we could invariably identify both homologues of autosome pairs 6, 11, and 12. The single X chromosome was also prominent in several metaphases. We encountered some difficulty in distinguishing between chromosome pairs 7 and 9 since both of these chromosomes may have two prominent bands in the long arm, and in contracted metaphases appear quite similar. In spite of the problems in distinguishing between these two pairs of chromosomes, since we saw 4 chromosomes with distinct bands in the long arms in several metaphases, we believe the missing "C" was not a member of autosome pairs 7 or 9. The number 10 chromosomes in lymphocytes have three distinct bands in the long arm. Again, owing to contraction of the chromosomes, it was impossible to resolve all three bands in the long arm of chromosome number 10 in most of the patient's cells, but we were able to identify both 10 homologues in five metaphases. Assuming that all affected hypodiploid metaphases in marrow cells from this patient were missing the same C-group chromosome, we arrive at the conclusion that our patient was monosomic for chromosome number 8.

A composite of C-group chromosomes from metaphases from this patient is shown in Fig. 53. As reference we have included the C-group chromosomes from a similarly con-

TABLE 56

CLINICAL AND CYTOGENETIC FINDINGS IN 11 PATIENTS WITH C-MONOSOMIC MYELOPROLIFERATIVE DISEASE

Patient	Sex	Age (yr)	Anemia	Thrombocytopenia	Initial WBC	Marrow (initial)	Myelometaplasia	Hepatosplenomegaly	Bone-marrow chromosomes
Freireich (1)	F		+	+	"normal"	? normal	Not specified	Not specified	45, C-
(2)	F*		+	+	"normal"	? normal	Not specified	Not specified	45, C-
(3)	M*		+	+	"normal"	? normal	Not specified	Not specified	45, C-
McClure (1)	M (5)†		+	+	8,000	megaloblastic changes—late erythroid prol.	Spleen	+	45, C- (71%)
Teasdale (1)	M (8/12)*		+	developed	25,900	6% blasts	0	+	45, C- (99%)
(2)	F (13)*		+	+	5,200	Normal	Spleen, nodes	+	45, C- (100%)
Humbert (1)	F (6/12)7*		+	+	25,000	Normal	Spleen, liver	+	45, C- (11-100%)
Jackson (1)	M (54)‡		+	+	72,000	Fibrosis	Spleen, liver	+	45, C- (83%)
Rowley (1)	M (23)		+	+ (pancytopenia)	3,000	Hypoplasia	0	0	45, C- (61%)
Holden (1)	M (4)§		+	+	3,000	Fibrosis	Nodes, spleen, liver	+	45, C- (90%)
Littlefield (1)	M (52)		+	+ (pancytopenia)	3,200	Hyperplastic erythroid hyperplasia (megaloblastic changes)	No biopsy	0	45, C- (90%)

* Developed AML
† Developed EL
‡ Died, septis
§ Died, pneumonia

References:
Freireich, E. J., et al. Clin. Res. 12, 284, 1964, Abst.
McClure, P. D., et al. Arch. Intern. Med. 115, 697, 1965.
Teasdale, J. M., et al. Cancer 25, 1468, 1970.
Humbert, J. R., et al. Br. J. Haematol. 21, 705, 1971.
Jackson, J. R., et al. Arch. Intern. Med. 119, 403, 1967.
Rowley, J. D., et al. Blood 27, 782, 1966.
Holden, J. D., et al. Am J. Clin. Pathol. 55, 573, 1970.

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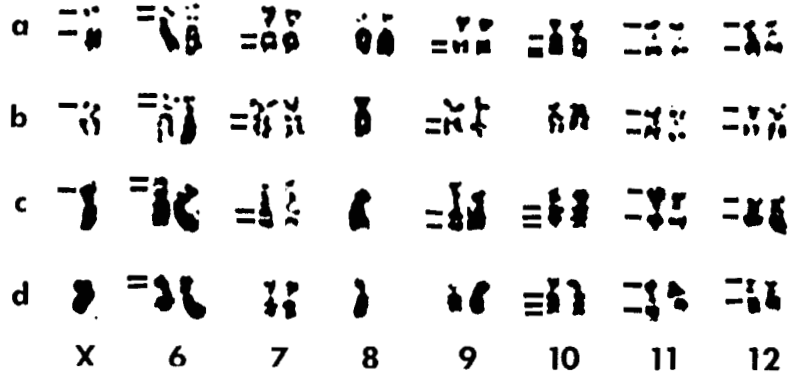


Fig. 53 a. C-banding in normal lymphocyte metaphase; b., c., d., C-group chromosomes from our patient. Note the consistent light band in the short arms of chromosome 6 and the distinctive 11 and 12 chromosomes in all metaphases from the patient. Chromosome pairs 7 and 9 have characteristic banding in metaphases b. and c., and a dark band in the short arms of the X identifies this chromosome in these two cells. Three bands in the long arms of chromosome 10 can be distinguished in metaphases c. and d.

tracted lymphocyte metaphase from a normal man.

Although Giemsa banding procedures do not yield as much detail in myeloid cells as in lymphocytes, we believe that major bands can be distinguished in the C-group chromosomes in marrow preparations, and that these bands can be used to identify specific pairs of chromosomes. If we are to determine the relationship between cytogenetic changes and malignant cell processes, all attempts should be made to specifically identify chromosomes that are monosomic or trisomic in abnormally proliferative marrow cells. In this regard, it is interesting to note that five cases of an *extra* No. 8 chromosome in patients with blastic crisis of CML or PCV have been reported during the last year. These findings in conjunction with our observation of a monosomic No. 8 chromosome in a patient with another myeloproliferative disorder may suggest a relationship between aneuploidy of this C-group chromosome and abnormal marrow proliferation.

Preliminary Studies: A Simple In Vitro Method For Estimating Toxicity of Exotoxin Produced by *Pseudomonas Aeruginosa*

Most laboratories involved in studies of the exotoxin produced by *Pseudomonas aeruginosa* use in vivo assays for estimating the relative toxicity of different preparations of exotoxin. These assays, which require determination of LD_{50} in mice, are not only costly, but do not yield quantitative data describing the effects of sublethal doses of toxin. Less expensive in vitro test systems have been used to determine the toxicity of many other microbial toxins including the exotoxin of *Corynebacterium diptheriae* and the enterotoxin of *Shigella dysenteriae*; however, in vitro methods for obtaining quantitative data on the lethality of *Pseudomonas* exotoxin have not been developed. We have recently conducted pilot studies to determine the applicability of using in vitro tests for determining the toxicity of this exotoxin. Our primary objective was to determine whether this exotoxin was lethal to various lines of mammalian cells, and if so, whether the relative number of cells killed could be correlated with concentration of the toxin to which the cells were exposed (L. Gayle Littlefield, and Mary Ann Barnhill*).

Materials and Methods. The preparations of exotoxin used in these experiments were produced from *P. aeruginosa PA*-103 by the following methods. The organisms were grown on plates containing soft agar, 1% $(NH_4)_2SO_4$, and dialyzed trypticase-soy broth. After incubation for 18 hr at 32°C the plates were frozen for 24 hr, then thawed. The liquid collected was centrifuged and passed through an ultrafiltration cell. The retained fraction was precipitated with $(NH_4)_2SO_4$, and dialyzed against a phosphate buffer for 48 hr. The crude exotoxin preparation was then filter-sterilized through a 0.45 μ membrane.

Varying concentrations of the toxin were tested for lethality in four different mammalian cell lines: primary fibroblasts derived from marmoset skin, HeLa cells, mouse L cells (NCTC clone 929) and SV-40 virus transformed hamster cells. All cell cultures were maintained at 37°C in a 5% CO_2 atmosphere in TC-199 culture medium supplemented with 5-20% fetal calf serum and 50 μg streptomycin and 50 units penicillin G/ml. The cells were initially cultured in 75 cm^2 Falcon plastic flasks, and when specific experiments were to be run, confluent monolayers were trypsinized and the number of cells counted in a Neubauer chamber. The cells were then suspended in complete medium to the desired final concentration. Since relatively small amounts of exotoxin are produced by *Pseudomonas*, it was necessary to use an assay system requiring small volumes of crude toxin preparation. For this reason we devised the following micromethod for evaluation of toxicity. Medium containing suspended cells was diluted to give a final concentration of 500 cells/ml. We then added 0.2 ml of this suspension to individual wells in a Falcon Micro-Test II plate (96 wells/plate, fluid capacity 0.396 ml). Dilutions of toxin were prepared in Hanks' Balanced Salt Solution, and 0.05 ml of various concentrations of toxin were added to specific wells. The final concentrations of the toxin tested ranged from 1 μg to 800 μg protein/ml culture medium. Generally 6 to 10 replicate wells were plated for each concentration of toxin to be tested. The cells were then incubated for varying periods of time ranging from 4 to 48 hr. At the end of the incubation period, the medium was poured from the wells, and the cells attached to the bottom of the culture vessel were fixed with 95% ethanol, and stained with a 10% solution of Giemsa stain. The number of cells plated in each well were then counted under a light microscope using a 16X objective.

Results. We conducted nine separate experiments to determine the toxicity of the crude preparations of exotoxin in the four cell lines studied. The average number of cells that plated in the control wells varied in the different experiments from 51 ± 1.3 to 139.0 ± 2.9 cells/well. The mean number of cells plated in wells containing specific concentrations of the exotoxin were calculated, and the percentage of surviving cells (compared to the number of control cells plated in that specific experiment) was determined for each toxin concentration. The percentages of surviving cells, after exposure to various concentrations of toxin, are plotted in Fig. 54. As is evident from the graph, the different cell lines exhibited different sensitivities to the toxin, and the length of time of incubation dramatically affected the percentage of cells surviving exposures to specific concentration of toxin. In our first two experiments we used primary cultures of fibroblasts from

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1028283

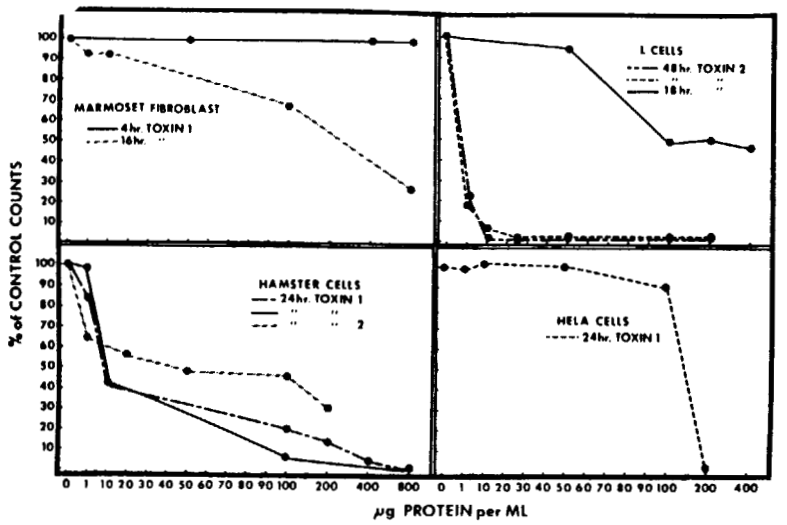


Fig. 54 Percentages of surviving cells in four different cell lines exposed to various concentrations of toxin preparations. Note that the different cell lines exhibited different sensitivities to the toxin and that the length of time of exposure dramatically affected the percentages of cells surviving exposure to specific concentrations of toxin.

marmosets, and exposed the cells to toxin for 4 to 16 hr. In the cells incubated for the shorter period of time, we could not detect a decrease in the number of cells plated in wells containing 200 μg, 400 μg, or 800 μg protein/ml culture medium. The fact that the cells apparently plated normally even after exposure to toxin of extremely high concentrations indicated that this substance had no effect on plating efficiency. However, in cultures exposed to toxin for 16 hr, we observed a 33% reduction in the percentage of surviving cells in wells containing 100 μg protein. This observation indicated that the toxin might have lethal effects during some stages of the cell cycle, and encouraged us to search for other cell lines with a higher degree of sensitivity. We then ran preliminary tests with three continuous cell lines. In a single experiment with HeLa cells exposed to toxin for 24 hr we found virtually no decrease in cell survival in wells containing up to 100 μg protein/ml. However, a much greater sensitivity to the toxin was observed in SV-40 virus transformed hamster cells. In two experiments in which cells were exposed to toxin preparation I for 24 hr, over 50% of the cells were killed after exposure to only 10 μg toxin. A second batch of crude exotoxin was tested in this cell line, and although this preparation appeared to contain less toxic protein than the first, 44% of the cells were killed after exposure to 20 μg protein/ml. The last cell line tested was mouse L cells. After 18 hr incubation, a concentration of 100 μg was required to reduce the number of surviving cells by 50%. However, increasing the exposure time to 48 hr resulted in death of 74 to 78% of the cells in wells containing only 1 μg protein.

Although our studies at this time are quite preliminary, several observations can be made from our data. First, we have had good success with the plating of small numbers of

1028284

cells in Micro-Test wells (100 cells/well). This procedure has several advantages over in vitro titration protocols requiring the evaluation of lethality in large numbers of cells plated in flasks or petri dishes. Using the wells, it is not necessary before counting to use trypsinization to recover the cells remaining attached after exposure to the toxin. The attached cells can be scored directly under the microscope, and simultanously evaluated for gross cytopathic changes. Likewise, by using a total volume of only 0.2 ml of cell suspension, only small quantities of toxic materials are required for several replicate tests. Regarding the toxicity of the exotoxin preparation of *Pseudomonas*, the preparations used in our study were impure, since the exotoxin had not been separated from other protein present in the original precipitate. The fact that 1.0 μg quantities of this crude preparation were lethal to over 70% of mouse L cells exposed for 48 hr suggests that this cell line may have pronounced sensitivity to purified fractions of exotoxin.

Miscellaneous Research Activities

Leukemogenesis as Related to Embryogenesis

Our previous studies showed similarities in isozymes of fetal and leukemic tissues as opposed to the corresponding normal adult tissues. Additionally, serum from tumor-bearing mice had specific alterations in protein profiles that were identical to alterations observed in serum of pregnant mice. Further, gallium-67 citrate was seen to concentrate in both fetal and leukemic murine tissues. Because of these previous studies, and many other recent reports showing similarities between fetal and neoplastic tissues, we have pursued the study of leukemogenesis as related to embryogenesis.

Gallium-67 Citrate Concentration in Pregnant Rabbits and Rats

We (R. L. Tyndall, Stuart Chaskes, J. Daniel*, Bill Nelson, Maril Weber† and J. E. Carlton) have assayed the patterns of ^{67}Ga uptake in pregnant rabbits at various times throughout the gestation period. The most noteworthy observations to date are the marked increase in isotope activity associated with uterine tissue and flushings (Table 57) early in the gestation period (5 days) and with placental and mammary tissue late in pregnancy (26 to 27 days).

Ultrafiltration studies of the uterine fluids indicated that the majority of gallium was bound to fraction(s) greater than 10,000 MW. Gel filtration of uterine flushings and placental extracts showed that the gallium was bound to material that eluted with the void volume indicating that the moiety(ies) binding gallium in these tissues were either particulate or in excess of 200,000 MW.

In contrast to marked concentration of the isotope in selected tissues during pregnancy, both blood and thymus tissues showed a marked inability to bind gallium in pregnant rabbits late in the gestation period.

The lack of gallium binding in thymus tissue of the pregnant rabbit corresponds to

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†Undergraduate research trainee from Southwestern State College, Weatherford, Oklahoma.

TABLE 57
GALLIUM-67 CITRATE UPTAKE* IN TISSUES OF PREGNANT RABBITS

Tissue	Virgin	Gestation Time			
		Pseudopreg	5-day preg	15 to 17-day preg	26 to 27-day preg
Serum	39	73	50	29, 58	8, 11
Liver	69	124	117	60, 74	75, 133
Spleen	79	146	113	81, 126	88, 83
Kidney	198	198	NT	231, 215	130, 120
Thymus (whole)	90	35	124	7, 8	4, 5
Placenta	NA	NA	NA	480, 650	1333, 1700
Uterus	42	19	273	NT	NT
Uterine flushings†	5	NT	325	NA	NA
Mammary gland	9	NT	NT	19	165

* = Percent of inoculated dose/kg of tissue wt, except for thymus.

† = cpm x 10^3 in total uterine flushings.
NA = not applicable
NT = not tested.

our previous report of a similar lack of gallium uptake in thymus tissue from pregnant mice. This effect of pregnancy on gallium binding by thymus tissue is of particular interest because of a similar decline in gallium binding by thymus tissues in tumor-bearing rats. Additionally, the major fraction binding gallium in extracts of rat thymus tissue has a molecular weight (40,000) similar to that in extracts of tumor cells and dissimilar to that found in other normal tissues (R. L. Hayes, et al., companion paper). Accordingly, we (R. L. Tyndall, Stuart Chaskes, R. L. Hayes, and J. E. Carlton) have carried out preliminary studies on thymus tissues of pregnant rats. These showed an approximate 90% reduction in gallium uptake compared to thymus tissue from normal rats.

Esterase and Polymerase Activation in Endometrial Tissues of Pregnant Rabbits.

Esterases have been shown to be good indicators of differentiation in normal or leukemic tissues and RNA-directed DNA polymerases are likewise often indicative of the state of differentiation and/or the presence of leukemia-virus genomes. Relative, in part, to the localization of gallium in uterine tissues and uterine flushings within the first week of pregnancy in rabbits, we (R. L. Tyndall and Shirley Colyer) have undertaken collaborative studies with Dr. J. Daniel, University of Tennessee, and Wen Yang, Oak Ridge National Laboratory, in the esterase and polymerase characterization of such materials. These studies thus far indicate marked activation of a variety of both esterases and DNA polymerases in endometrial tissues and to a lesser degree in uterine flushings during the first week of gestation. Associated with these tissues are a minimum of nine esterases, many of which are activated at specific times during the first week of pregnancy. Correlated with esterase activation is that of DNA polymerase action in that at least three different polymerases are also activated during the first week of pregnancy. The relationship of both the esterase and RNA-directed dependent DNA polymerases to those found in

1028285

neoplasia and/or leukemia virus is presently under study as in the association of these proteins with ^{67}Ga uptake.

Serum Esterases in Tumor-Bearing Mice

Just as tissue esterases are good indicators of differentiation, serum esterases are equally good indicators of hormonal alterations. In collaborative studies with Vivian Smith and Bill Byrd, we (Shirley Colyer and R. L. Tyndall) have analyzed the serum esterase profiles of mice bearing transplantable lymphosarcomas compared with normal mice. The results indicate that mice bearing either of two lymphosarcomas have pronounced alterations in some of the steroid-dependent serum esterases. The alterations are specific and similar in mice bearing either tumor (Fig. 55). Because of the sensitivity of thymocytes or thymus-derived lymphocytes to hormone action, evidence of hormone changes in these tumor-bearing mice together with evidence of reduced thymic weight could be indicative of thymic dysfunction. If tumor-cell growth could directly or indirectly alter the hormone status of the host and thereby affect thymus function, it could favor tumor-cell progression as opposed to regression.

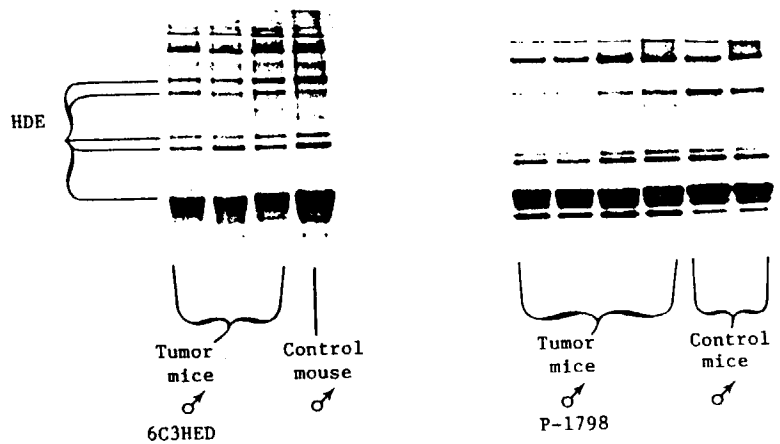


Fig. 55 Alterations in hormone-dependent serum esterases in lymphosarcoma-bearing mice.

1028286

PUBLICATIONS

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JOURNAL ARTICLES

Published

1. Andrews, G. A., Gibbs, W. D., Morris, A. C., Jr., and Ross, D. A. *Whole-body counting.* Semin. Nucl. Med. 3, 367-388, 1973.
2. Beck, W. L., Cloutier, R. J., and Watson, E. E. *Personnel monitoring with film and thermoluminescent dosimeters for high exposures.* Health Phys. 25, 421-425, 1973.
3. Blank, M. L., Wykle, R. L., and Snyder, F. *The retention of arachidonic acid in ethanolamine plasmalogens of rat testes during essential fatty acid deficiency.* Biochim. Biophys. Acta 316, 28-34, 1973.
4. Brown, D. H., Carlton, E., Byrd, B., Harrell, B., and Hayes, R. L. *A rate-zonal centrifugation procedure for screening particle populations by sequential product recovery utilizing edge-unloading zonal rotors.* Arch. Biochem. Biophys. 155, 9-18, 1973.
5. Brown, D. H., Swartzendruber, D. C., Carlton, J. E., Byrd, B. L., and Hayes, R. L. *The isolation and characterization of gallium-binding granules from soft tissue tumors.* Cancer Res. 33, 2063-2067, 1973.
6. Chae, K., Piantadosi, C., and Snyder, F. *An alternate enzymic route for the synthesis of the alkyl analog of phosphatidic acid involving alkylglycerol.* Biochem. Biophys. Res. Commun. 51, 119-124, 1973.
7. Chae, K., Piantadosi, C., and Snyder, F. *Reductase, phosphatase, and kinase activities in the metabolism of alkyldihydroxyacetone phosphate and alkyldihydroxyacetone.* J. Biol. Chem. 248, 6718-6723, 1973.
8. Clapp, N. K., Tyndall, R. L., and Cumming, R. B. *Hyperplasia of hepatic bile ducts in mice following long-term administration of butylated hydroxytoluene.* Fd. Cosmet. Toxicol. 11, 847-849, 1973.
9. Cloutier, R. J., Smith, S. A., Watson, E. E., Snyder, W. S., and Warner, G. G. *Dose to the fetus from radionuclides in the bladder.* Health Phys. 25, 147-161, 1973.
10. Cloutier, R. J., Watson, E. E., Hayes, R. L., Nelson, B., and Smith, E. M. *Summary of current radiation dose estimates to humans from ^{66}Ga-, ^{67}Ga-, ^{68}Ga-, and ^{72}Ga-citrate.* J. Nucl. Med. 14, 755-756, 1973.
11. Cloutier, R. J., Watson, E. E., Rohrer, R. H., and Smith, E. M. *Calculating the radiation dose to an organ.* J. Nucl. Med. 14, 53-55, 1973.
12. Fuhr, J. E. and Gengozian, N. *Coordination of heme and globin synthesis in primate reticulocytes.* Biochim. Biophys. Acta 320, 53-58, 1973.

13. Gengozian, N. and Congdon, C. C. *Immunological memory in radiation chimeras.* Transplantation 16, 32-40, 1973.
14. Gengozian, N., Edwards, C. L. Vodopick, H. A., and Hübner, K. F. *Bone marrow transplantation in a leukemic patient following immunosuppression with anti-thymocyte globulin and total body irradiation.* Transplantation 15, 446-454, 1973.
15. Goh, Kong-oo. *Total-body irradiation and human chromosomes. III. Cytogenetic studies in patients with malignant hematologic diseases treated with total-body irradiation.* Am. J. Med. Sci. 266, 179-186, 1973.
16. Grigor, M. R. and Bell, I. C., Jr. *Synthesis of fatty acid esters of short-chain alcohols by an acyltransferase in rat liver microsomes.* Biochim. Biophys. Acta 306, 26-30, 1973.
17. Grigor, M. R., Blank, M. L., and Snyder, F. *Cholesterol metabolism in rats bearing Morris hepatoma 7777.* Cancer Res. 33, 1870-1874, 1973.
18. Hayes, R. L., and Carlton, J. E. *A study of the macromolecular binding of ^{67}Ga in normal and malignant animal tissues.* Cancer Res. 33, 3265-3272, 1973.
19. Hayes, R. L., Rafter, J. J., Washburn, L. C., and Byrd, B. L. *Affinity of $^{253}einsteinium$ for tumour tissue.* Nat. New Biol. 246, 23-25, 1973.
20. Hofer, K. G. and Swartzendruber, D. C. *^{67}Ga-citrate and ^{125}I-iododeoxyuridine as markers for in vivo evaluation of tumor cell metastasis and death.* J. Natl. Cancer Inst. 50, 1039-1045, 1973.
21. Huisman, T. H. J., Schroeder, W. A., Keeling, M. E., Gengozian, N., Miller, A., Brodie, A. R., Shelton, J. R., Shelton, J. B., and Apell, G. *Search for non-allelic structural genes for γ-chains of fetal hemoglobin in some primates.* Biochem. Genet. 10, 309-318, 1973.
22. Kasama, K., Rainey, W. T., Jr., and Snyder, F. *Chemical identification and enzymatic synthesis of a newly discovered lipid class—hydroxyalkylglycerols.* Arch. Biochem. Biophys. 154, 648-658, 1973.
23. Lee, T-c. and Snyder, F. *Phospholipid metabolism in rat liver endoplasmic reticulum: Structural analyses, turnover studies and enzymic activities.* Biochim. Biophys. Acta 291, 71-82, 1973.
24. Lee, T-c., Stephens, N., Moehl, A., and Snyder, F. *Turnover of rat liver plasma membrane phospholipids: Comparison with microsomal membranes.* Biochim. Biophys. Acta 291, 86-92, 1973.
25. Lee, T-c., Wykle, R. L., Blank, M. L., and Snyder, F. *Dietary control of stearyl CoA and alkylacylglycerophosphorylethanolamine desaturases in tumor.* Biochem. Biophys. Res. Commun. 55, 574-579, 1973.
26. Littlefield, L. G. and Goh, K-o. *Cytogenetic studies in control men and women. I. Variations in aberration frequencies in 29,709 metaphases from 305 cultures obtained over a three-year period.* Cytogenet. Cell Genet. 12, 17-34, 1973.
27. Nelson, B. and Pritchard, B. *Autoradiography with Polaroid film.* Beitr. Pathol. 149, 206-210, 1973.
28. Otten, J. A., Tyndall, R. L., Estes, P. C., Gude, W. D., and Swartzendruber, D. C. *Localization of gallium-67 during embryogenesis.* Proc. Soc. Exp. Biol. Med. 142, 92-95, 1973.
29. Piantadosi, C., Chae, K., Ishaq, K. S., and Snyder, F. *Chemical synthesis of 1-^{14}C-octadecyldihydroxyacetone phosphate and 1-^{14}C-octadecyldihydroxyacetone.* J. Pharm. Sci. 62, 320-322, 1973.

1028287

30. Porter, R. P. and Gengozian, N. *Immunological responsiveness and tolerance of marmoset lymphoid tissue in vitro.* Transplantation 15, 221-230, 1973.
31. Shively, J. M., Ball, F., Brown, D. H., and Saunders, R. E. *Functional organelles in prokaryotes: Polyhedral inclusions (carboxysomes) of* **Thiobacillus neapolitanus.** Science 182: 584-586, 1973.
32. Snyder, C., Malone, B., Nettesheim, P., and Snyder, F. *Urethan-induced pulmonary adenoma as a tool for the study of surfactant biosynthesis.* Cancer Res. 33, 2437-2443, 1973.
33. Snyder, F. *Radioisotopes-Introduction. Detection and chromatographic resolution of labeled lipid intermediates formed in enzyme systems.* J. Chromatogr. 78, 141-147, 1973.
34. Snyder, F. *Thin-layer chromatographic behavior of glycerolipid analogs containing ether, ester, hydroxyl, and ketone groupings.* J. Chromatogr. 82, 7-14, 1973.
35. Snyder, F., Clark, M., and Piantadosi, C. *Biosynthesis of alkyl lipids: Displacement of the acyl moiety of acyldihydroxyacetone phosphate with fatty alcohol analogs.* Biochem. Biophys. Res. Commun. 53, 350-356, 1973.
36. Snyder, F., Malone, B., and Piantadosi, C. *Tetrahydropteridine-dependent cleavage enzyme for O-alkyl lipids: substrate specificity.* Biochim. Biophys. Acta 316, 259-265, 1973.
37. Swartzendruber, D. C. and Hübner, K. F. *Effect of external whole-body x-irradiation on gallium-67 retention in mouse tissues.* Radiat. Res. 55, 457-468, 1973.
38. Urso, P. and Gengozian, N. *T cell deficiency in mouse allogeneic radiation chimeras.* J. Immunol. 111, 712-719, 1973.
39. Watson, E. E., Cloutier, R. J., and Gibbs, W. D. *Whole-body retention of ^{67}Ga-citrate.* J. Nucl. Med. 14, 840-842, 1973.
40. Wykle, R. L., Blank, M. L., and Snyder, F. *The enzymic incorporation of arachidonic acid into ether-containing choline and ethanolamine phosphoglycerides by deacylation-acylation reactions.* Biochim. Biophys. Acta 326, 26-33, 1973.

In Press

41. Blank, M. L. and Snyder, F. *A tissue culture system for studies of the regulation of ether-linked and ester-linked aliphatic moieties in glycerolipids.* Arch. Biochem. Biophys. 160, 100-105, 1974.
42. Blank, M. L. and Snyder, F. *Qualitative and quantitative aspects of thin-layer chromatography in the analysis of phosphorus-free lipids.* J. Am. Oil Chem. Soc.
43. Chaskes, S. and Phillips, A. W. *Pigmentation and autofluorescence of* **Candida** *species after growth on tryptophan or tryptamine media.* Canadian J. Microbiol.
44. Clapp, N. K., Tyndall, R. L., Cumming, R. B., and Otten, J. A. *Effects of butylated hydroxytoluene alone or with diethylnitrosamine in mice.* Fd. Cosmet. Toxicol.
45. DeLand, F. H., Sauerbrunn, B. J. L., Boyd, C., Wilkinson, R. H., Jr., Friedman, B. I., Moinuddin, M., Preston, D. F., and Kniseley, R. M. *^{67}Ga-citrate*

imaging in untreated primary lung cancer: preliminary report of cooperative group. J. Nucl. Med.

46. Gengozian, N., Smith, T. A., and Gosslee, D. G. *External uterine palpation to identify stages of pregnancy in the marmoset, Saguinus fuscicollis ssp.* J. Med. Primatol.
47. Greenlaw, R. H., Weinstein, M. B., Brill, A. B., McBain, J. K., Murphy, L., and Kniseley, R. M. *^{67}Ga-citrate imaging in untreated malignant lymphoma: preliminary report of cooperative group.* J. Nucl. Med.
48. Johnston, G., Benua, R. S., Teates, C. D., Edwards, C. L., and Kniseley, R. M. *^{67}Ga-citrate imaging in untreated Hodgkin's disease: preliminary report of cooperative group.* J. Nucl. Med.
49. Lushbaugh, C. C. and Humason, G., et al. Appendix III. Additional Observations. Part IV. Viscera with continuously replicating cells. W. Haymaker, ed. In: The Effects of Cosmic Particle Radiation on Pocket Mice Aboard Apollo 17, Aerospace Medicine Special Supplement.
50. Morris, A. C., Jr., Goswitz, F. A., Kim, K. H., McDow, A. E., Jr., Aaron, P. A., and Barclay, T. R. *Interface for direct online computer recording of dynamic radionuclide data.* Med. Biol. Eng.
51. Snyder, F., Malone, B., and Piantadosi, C. *Enzymic studies of glycol and glycerol lipids containing O-alkyl bonds in liver and tumor tissues.* Arch. Biochem. Biophys.

51a. Tankersley, W. G. and Woodward, J. M. *Induction and isolation of a minicell-producing strain of* **Salmonella typhimurium.** Proc. Soc. Exp. Biol. Med. 145, 802-805, 1974.

52. Vodopick, H. and Andrews, G. A. *Accidental radiation exposure.* Arch. Environ. Health 28, 53-56, 1974.
53. Wykle, R. L. and Schremmer, J. M. *A lysophospholipase D pathway in the metabolism of ether-linked lipids in brain microsomes.* J. Biol. Chem. 249, 1742-1746, 1974.

CHAPTERS OR ARTICLES IN BOOKS OR SYMPOSIA

Published

54. Akers, L. K., Beck, W. L., Jr., Cloutier, R. J., and Dickson, H. W. *Health physics training at Oak Ridge Associated Universities.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972, 1973, DHEW Publication (FDA) 73-8029, pp. 281-287.
55. Bailey, O. T., Benton, E. V., Cruty, M. R., Harrison, G. A., Haymaker, W., Humason, G., Leon, H. A., Lindberg, R. L., Look, B. C., Lushbaugh, C. C., Philpott, D. E., Samorajski, T., Simmonds, R. C., Suri, K. P., Tremor, J. W., Turnbill, C. E., Vogel, F. S., Winter, D. L., and Zeman, W., Chapter 26. *Biocore Experiment.* In: Apollo 17 Preliminary Science Report, NASA SP-330, Lyndon B. Johnson Space Center, 1973.
56. Barclay, T. R., Morris, A. C., Jr., and Lucas, J. P. *A universal, solid-state attachment for the computer recording of rectilinear scanning data.* In: Proceedings of the Third Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Miami, Fla., 1973, Oak Ridge, Tenn., USAEC Report CONF-730627, 1973, pp. 183-189.

1028288

57. Beck, W. L., Stokes, T. R., and Lushbaugh, C. C. *Dosimetry for total-body irradiation therapy.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972, 1973, DHEW Publication (FDA) 73-8029, p. 423.
58. Cloutier, R. J. and Watson, E. E. *Radiation doses from nuclear medicine procedures.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972, 1973, DHEW Publication (FDA) 73-8029, pp. 71-78.
59. Gengozian, N. and Patton, M. L. *Identification of three blood factors in the marmoset,* **Saguinus fuscicollis ssp.** In: Medical Primatology 1972; Selected papers from the 3rd Conference on Experimental Medicine and Surgery in Primates, Lyon, 1972 (Part I). E. E. Goldsmith and J. Moor-Jankowski, eds., Basel, S. Karger, 1973, pp. 349-360.
60. Hayes, R. L. and Edwards, C. L. *New applications of tumour-localizing radiopharmaceuticals.* In: Medical Radioisotope Scintigraphy, 1972. Proceedings of a Symposium, Monte Carlo, IAEA, 1973, pp. 531-552.
61. Humason, G. *Hemopoietic tissues.* Chapter H. In: The Encyclopedia of Microscopy and Microtechnique, P. Grey, ed. Van Nostrand Reinhold Co., New York, 1973, pp. 248-249.
62. Humason, G. *Leukocytes.* Chapter L. In: The Encyclopedia of Microscopy and Microtechnique, P. Grey, ed. Van Nostrand Reinhold Co., New York, 1973, pp. 289-291.
63. Lombardi, M. H., Beck, W. L., Jr., and Cloutier, R. J. *Survey of radiopharmaceutical use and safety in sixty-nine hospitals.* In: Health Physics in the Healing Arts, Seventh Midyear Topical Symposium, Health Physics Society, San Juan, Puerto Rico, December 1972, 1973, DHEW Publication (FDA) 73-8029, pp. 227-235.
64. Lushbaugh, C. C. *Human radiation tolerance.* Chapter 10. In: Space Radiation Biology and Related Topics. C. A. Tobias and P. Todd, eds., Academic Press, New York, 1973, pp. 475-522.
65. Porter, R. P. and Gengozian, N. *Marmoset bone marrow.* In: Methods and Application of Tissue Culture, P. Krause and M. Patterson, eds. Academic Press, New York, 1973, pp. 93-97.

In Press

66. Edwards, C. L. and Hayes, R. L. *Gallium-67 citrate for tumor scanning.* Proceedings of the Eighteenth Annual Clinical Conference on Radiologic and Other Biophysical Methods in Tumor Diagnosis. Presented at M.D. Anderson Hospital, Houston, Texas, November 8, 1973.
67. Goswitz, F. A. *Radioactive vitamin B_{12} absorption tests and other studies.* In: Diagnostic Radiology, by R. Golden, Section 20, Diagnostic Nuclear Medicine. A. Gottschalk, J. L. Quinn, and E. J. Potchen, eds., Williams and Wilkins, Baltimore.
68. Snyder, F. *Analysis of alkyl and alk-1-enyl ether lipids and their derivatives by chromatographic techniques.* In: Lipid Chromatographic Analysis, G. V. Marinetti, ed. Marcel Dekker, New York.
69. Snyder, F. and Snyder, C. *Glycerolipids and cancer.* In: Progress in Biochemical Pharmacology, Vol. 9 (Lipids in Tumors), K. K. Carroll, ed. S. Karger, New York.

GOVERNMENT DOCUMENTS

70. Barber, L. R., Cloutier, R. J., and Watson, E. E. *Effective absorbed energies.* USAEC Report ORAU-120, Oak Ridge Associated Universities, 1973.
71. Bell, P. R., McClain, W. J., Ross, D. A., McKeighen, R. E., East, J. K., Moore, E. C., Edwards, C. L., and Goswitz, F. A. *A computer-based scanning system.* USAEC Report ORNL-TM-4038, Oak Ridge National Laboratory, 1973.
72. Kniseley, R. M. *Coding index for tumor scanning.* USAEC Report ORAU-119, Oak Ridge, Associated Universities, 1973.
73. Kniseley, R. M. *Cooperative group to study localization of radiopharmaceuticals.* USAEC Report ORAU-118, Oak Ridge Associated Universities, 1973.
74. Stemple, R. *Bibliography of medical uses of technetium-99m 1965-1971,* USAEC Report ORAU-121, Oak Ridge Associated Universities, 1973.
75. Oak Ridge Associated Universities, Medical Division, Research Report, 1972, for the year ending December 31, 1972. USAEC Report ORAU-122, 1973.

MISCELLANEOUS PUBLICATIONS

76. Andrews, G. A. Book Review: *Biochemical Indicators of Radiation Injury in Man.* Proceedings of a Scientific Meeting, Paris—Le Vesinet, 1970. IAEA, 1971. Health Phys. 24, 455-456, 1973.
77. Garcia, Ana Maria. *Cytogenetic Changes in Marrow Cells from Syrian Hamsters Exposed to ^{60}Co Total-Body Radiation.* Master's Thesis, Sao Paulo University, 1973.
78. Gengozian, N. Book Review: *Primates in Medicine.* E. I. Goldsmith and K. J. Moor-Jankowski, eds. Vol. 7, Transplantation in Primates. G. P. Murphy, ed. Basel-Karger, New York, 1972. J. Med. Primatol. 2, 254-255, 1973.
79. Gengozian, N. Book Review: *Transplantation Genetics of Primates.* H. Balner and H. L. van Rood, eds. New York, Grune and Stratton, 1972. (A "Transplantation Proceedings" reprint Vol. 4, No. 1, 1-147, March 1972.) In: J. Med. Primatol. 2, 256, 1973.
80. Hansard, Martha. *Investigation of the Applicability of a Digital Filtering Technique for Electrocardiogram Analysis.* Master's Thesis, University of Tennessee, 1973.
81. Lushbaugh, C. C. Contribution to: *HZE-Particle Effects in Manned Spaceflight,* D. Grahn, ed. Radiobiological Advisory Panel, Committee on Space Biology, Space Science Board, National Research Council, Washington, National Academy of Sciences, 1973.
82. Lushbaugh, C. C. Book Review: *Manual on Radiation Haematology.* A joint undertaking by IAEA and WHO, Technical Report Series No. 123, International Atomic Energy Agency, Vienna, 1971. Health Phys. 24, 456, 1973.

In Press

83. Cloutier, R. J. Book Review: *Nuclear Energy: Its Physics and Its Social Challenge.* D. R. Inglis, ed., Health Phys. 26, 204, 1974.
84. Hodges, H. D., Goswitz, F. A., and Berger, J. D. Letter to the editor: *Defective ^{198}Au Colloid.* J. Nucl. Med. Tech.

1028289

ABSTRACTS

Published

85. Blank, M. L. and Snyder, F. *Ether-linked glycerol lipids: Detection and identification of labeled intermediates and products formed in enzymic systems.* J. Am. Oil Chem. Soc. 50, 91A, 1973. Abstract 111.
86. Brown, D. H., Byrd, B., Rafter, J., and Hayes, R. L. *Subcellular localization of ^{67}Ga in rat hepatoma and normal host liver.* South. Med. J. 66, 1331-1332, 1973.
87. Congdon, C. C., Payne, H. S., Gengozian, N., and Urso, P. *Spleen white pulp blurring in thymectomized radiation chimeras.* Fed. Proc. 32, 870Abs, 1973. Abstract 3666.
88. Gibbs, W. D., Edwards, C. L., and Hodges, H. D. *Linear scanner instrumentation and data handling.* South. Med. J. 66, 1330, 1973.
89. Goswitz, F., Lushbaugh, C., and Vodopick, H. *Marrow changes in chronic granulocytic leukemia after treatment with total-body irradiation.* Blood 42, 984, 1973.
90. Hansard, M. S. and Bishop, A. O., Jr. *P-wave recognition as a function of the R-R interval.* Proceedings of the 26th ACEMB, 1973, p. 230.
91. Hayes, R. L. and Byrd, B. L. *Factors influencing the distribution of rare earth radionuclides in normal and neoplastic tissue.* South. Med. J. 66, 1327-1328, 1973.
92. Hayes, R. L. and Edwards, C. L. *The effect of stable scandium on red blood cells and on the retention and excretion of ^{67}Ga in humans.* South. Med. J. 66, 1339-1340, 1973.
93. Snyder, F., Malone, B., Nettesheim, P., and Snyder, C. *Urethan pulmonary adenomas as a model for studying the biosynthesis of dipalmitoylglycerophosphorylcholine.* Fed. Proc. 32, 561Abs, 1973. Abstract 1938.
94. Swartzendruber, D. C. and Idoyaga-Vargas, N. L. *Localization of gallium-67 in peritoneal cells by electron microscopic autoradiography.* In: Proceedings, Thirty-First Annual Meeting, Electron Microscopy Society of America, New Orleans, 1973. C. J. Arceneaux, ed. Baton Rouge, Claitor's Publishing Division, 1973, pp. 404-405.
95. Swartzendruber, D. C., Wolfe, G., and Hübner, K. F. *Gallium-67 localization in mouse peritoneal cells.* J. Reticuloendothelial. Soc. 13, 379-380, 1973. Abstract 72.
96. Tankersley, W. G. and Woodward, J. M. *Induction and isolation of nonreplicative minicells of* **Salmonella typhimurium** *and their use as immunogens in mice.* Abstracts of the Annual Meeting of the American Society for Microbiology, Abstract M-145, 1973, p. 97.
97. Urso, P. and Gengozian, N. *A, B, and T cell function in mouse allogeneic radiation chimeras.* Fed. Proc. 32, 226Abs, 1973. Abstract 82.
98. Watson, E. E., Cloutier, R. J., Smith, S. A., Snyder, W. S., and Warner, G. G. *Dose to the fetus from radiopharmaceuticals in mother's bladder.* South. Med. J. 66, 1341, 1973.

MEDICAL DIVISION CONFERENCES–1973*

January 9: *CHALONES–A Progress Report.* Helen Vodopick.
January 23: *Urethan-induced Pulmonary Adenoma as a Model for the Study of Surfactant Biosynthesis.* Cathy Snyder, Biology Division, Oak Ridge National Laboratory.
February 6: *Progress Report of Work in the Immunology Group.* N. Gengozian.
February 20: *Progress Report of Work in the Cytogenetics Group.* L. Gayle Littlefield.
March 6: *The Effect of Total-Body Irradiation on Phagocytosis; a Re-examination.* R. L. Tyndall.
March 20: *Experimental Lung Cancer in Mice.* John M. Yuhas, Biology Division, Oak Ridge National Laboratory.
April 3: *The Subcellular Distribution of ^{67}Ga in Normal and Malignant Tissues.* R. L. Hayes and D. H. Brown.
April 17: *Recent Progress in Zonal Centrifugation.* Julian Breillatt, MAN Project of Oak Ridge National Laboratory.
May 1: *Some Trace Element Techniques: NAA, AA, and XRF.* Geoffrey I. Gleason, ORAU Special Training Division.
May 15: *Progress Report of Work in the Ultrastructural Anatomy Group.* Don C. Swartzendruber.
May 29: *Effect of Total-Body Irradiation and Essential Fatty-Acid Deficiency on Lipid Metabolism: Progress Report.* Ten-ching Lee.
June 12: *Progress Report of Work in the Marmoset Research Program.* Donald D. Barnhart.
June 26: *Medical Division Small Computer Capabilities.* Evelyn Watson and Roger Cloutier.
July 10: *Tumor Localization of Rare Earths in Experimental Animals.* R. L. Hayes.
July 24: *Stem Cells in Dog Blood.* Bill Nelson.
August 7: *Report on the Recent Bone-Marrow Transplant Attempt.* C. Lowell Edwards.
August 16: Summer Student Scientific Program:
- *Basic Fortran Language Translation.* Glenda Lenell Baskin.
- *System for Computer Enhancement of Rectilinear Radioisotope Scans.* Dan Cornett.
- *Teeth Development in the Marmoset.* Paul Crawford.
- *Gamma-Ray Measurement with NaI and GeLi Detectors.* Susan Emery.
- *The Effects of Total-Body Irradiation on the Lipid Composition of Adipose Tissue.* Diane Gussler.
- *Some Aspects of the Role of Scandium in Hemolyzing Human Red Blood Cells.* Judith Schartenberg.
- *Studies on "Lyso Phospholipase D" Activity in Rat Tissues.* Rebecca Anne Smith.
- *Computer-Computer Intercommunication.* Richard F. Stadtmiller.
- *The Effect of Antithymocyte Globulin on the Response to* **Pneumococcus** *Polysaccharide Antigen in Mice.* Chrys Ann Synstegard.

* *Presented by Division staff unless otherwise noted.*

1028290

- *The Uptake of Gallium-67 by Adherent and Nonadherent Spleen Cells in the Mouse.* Mollie Ann Walker.
- Pregnancy and Gallium Binding. Maril Joy Weber.
- *Photodensitometric Determination of Ether Lipid in Selected Foods.* Beneta Charlene Wright.

August 21: *Some Maturation Indices of Colony-Bred Marmosets.* C. C. Lushbaugh.
September 4: *Information Retrieval in Toxicology.* Gerald Ulrikson. Environmental Information Systems Office; Helga Gerstner, TOXLINE Data Base; and Kathy Miller, MEDLINE Data Base.
September 18: *P-Wave Recognition as a Function of the R-R Interval.* Martha Hansard.
October 2: *(1) Award-Winning Movie (14 Min) "BIOENGINEERS" Produced at the ORNL/MAN Project. (2) The American Cancer Society in Tennessee.* Francis Goswitz.
October 16: *Gallium-67 Uptake During Pregnancy.* Richard L. Tyndall.
November 6: *Advances in Electrical Safety for the Laboratory and Hospital Environment.* Richard Fowler, Harvey Hubble Corp., Bridgeport, Connecticut.
November 20: *Approaches to Quantitation of Histologic Structures and Alterations.* Bill Nelson.
November 27: *Mechanism of Cell Injury and Death in an Experimental Model.* Emilio Machado, University of Tennessee Memorial Research Hospital, Knoxville.
December 11: *Energy and the Environment (demonstration of energy source simulator).* Jerry B. Minter, ORAU Special Training Division.

LIPID SEMINARS–1973

March 29: *Algal Eyespots: Experimental Studies, with Emphasis on Euglenoid Flagellates.* Patricia L. Walne, Associate Professor of Botany, University of Tennessee, Knoxville.
May 7: *Enzyme Activity and Physiological Reality.* Emile Van Handel, Entomological Research Center, Department of Health and Rehabilitative Services, State of Florida, Vero Beach.
May 17: *The Metabolism of Alkyldihydroxyacetone Phosphate in Rat Brain.* Emad El-Bassiouni, Medical Division staff.
July 12: *Alkylglycerol Kinase in the Pink Harderian Gland.* Charles O. Rock, Medical Division staff.
July 26: *The Molecular Biology of Immunoglobulins.* Alan Solomon, University of Tennessee Memorial Research Center, Knoxville.

EIGHTH ANNUAL REGIONAL LIPID CONFERENCE

Long Beach, North Carolina
October 4-5, 1973

Richard Whorton
Vanderbilt University

Studies on fatty acid synthesis in rat testes.

Carol A. Schiller University of North Carolina at Chapel Hill	*Fatty acid synthesis in white adipose tissue: Coordination between the mitochondrial citrate transporter and pyruvate dehydrogenase.*
Oliver E. Bell, Jr. University of Mississippi	*The formation of long-chain fatty alcohols and fatty acids from long-chain fatty aldehydes by cell-free preparations of brain tissue.*
Michele R. Sullivan and Peter W. Holloway University of Virginia	*The mechanism of stearyl CoA desaturation by hen liver microsomes. The interaction of cytochrome b_5 with phosphatidylcholine vesicles.*
Carol R. Kepler North Carolina State University	*Biohydrogenation of oleic acid.*
John A. Thompson and Ronald C. Reitz University of North Carolina at Chapel Hill	*Studies on the peroxidation of endogenous fatty acids by NADPH oxidase.*
Sharon C. Parker University of North Carolina at Chapel Hill	*Fatty-acid utilization in heart.*
B. Moseley Waite Bowman-Gray School of Medicine	*Hydrolysis and transacylation of glycerides by the phospholipase A_1 from liver-plasma membranes.*
Harold Griffin and Joseph Lumsden Bowman-Gray School of Medicine	*The effect of various inhibitors on the phospholipases of rat-liver lysosomes.*
Roger H. Lumb Western Carolina University	*Microsomal phospholipase activity in Novikoff hepatoma and host liver.*
R. L. Wykle and J. M. Schremmer Oak Ridge Associated Universities	*Hydrolysis of base moieties from ether-containing phospholipids by brain microsomes.*
H. C. Meng and M. C. Wang Vanderbilt University	*The role of mono- and triglyceride lipases in the uptake of the lung.*
Paul Stumpf University of California, Davis	*New ideas and old problems.*
Edward O. Oswald National Institute of Environmental Health Sciences	*Analysis of lipids and lipid soluble environmental agents by combined gas chromatography and mass spectrometry.*

1028291

Claude Piantadosi, Kun Chae, Fred Snyder University of North Carolina at Chapel Hill	*Chemical synthesis of radioactive labeled alk-1-enyl glycerol.*
K. S. Ishaq, Claude Piantadosi, and Fred Snyder University of North Carolina at Chapel Hill	*Synthesis of certain thioethers.*
William M. Grogan Vanderbilt University	*Lipid chemistry and metabolism in male reproductive tissue.*
N. Stephens, A. Moehl, D. Gussler, and T-c. Lee Oak Ridge Associated Universities	*The effect of X irradiation on the lipid metabolism of adipose tissue.*
Daniel H. Albert and Carl E. Anderson University of North Carolina at Chapel Hill	*Glyceryl ethers in human brain tumors.*
Michael L. Bucholtz Florida State University	*Metabolism of extracellular glycolipids from the yeast* **Candida bogoriensis.**
James C. A. Fuchs and Per-Otto Hagen Duke University	*Neutral lipid content of venous arterial bypass grafts.*
M. L. Blank and Fred Snyder Oak Ridge Associated Universities	*A tissue culture system for studies of the regulation of ether-linked and ester-linked aliphatic moieties in glycerolipids.*
B. Malone, C. Snyder, P. Nettesheim, and F. Snyder Oak Ridge Associated Universities	*Synthesis of 1,2-dipalmitoyl-sn-glycero-3-phosphorylcholine by microsomal enzymes in alveolar-type II cells.*
H. J. Fallon University of North Carolina at Chapel Hill	*Diglyceride acyltransferase measured with membrane-bound diglyceride.*
R. G. Lamb University of North Carolina at Chapel Hill	**In vitro** *studies on the incorporation of sn-[1,3-^{14}C] glycerol-3-P into glycerolipids by guinea pig intestinal microsomes.*
W. R. Finnerty University of Georgia	*Membranes of* **Acinetobacter:** *Substrate induction of intracytoplasmic membrane synthesis.*

Iris H. [illegible], G. L. Carlson, and Claude Piantadosi University of North Carolina at Chapel Hill	*Antifertility properties of the a,a-dibenzylcycloalkanones.*
Ronald C. Reitz, Darien Mahaffee, and Robert L. Ney University of North Carolina at Chapel Hill	*Role of mitochondrial cholesterol accumulation in the regulation of steroidogenesis.*
Larry Lee Swift Vanderbilt University	*Studies on sarcoplasmic reticulum from skeletal muscle of normal and myotonic goats.*
Lynn A. LaBounty Vanderbilt University	*An abnormal lipoprotein associated with extraphepatic cholestasis in rats.*
Mary E. Gray Vanderbilt University	*Isolation and characterization of lamellar bodies from rat lung homogenates.*
Zachery Goodman Vanderbilt University	*Exchange of cholesteryl oleate between serum lipoproteins and the liver.*
Gary Powell Clemson University	*Triple-label scintillation counting: Reasonable or unreasonable?*

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1028292

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1028293

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1028294

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	Active Interagency Agreements		
F. Snyder (ORAU) and C. Piantadosi (UNC)	Role of ether- and ketone-containing lipids during brain development	NIH-UNC	9/1/73- 8/31/74
R. Tyndall	A study of infections in irradiated patients	U.S. Army	8/1/73- 6/30/74
R. Cloutier	Center for information on internal dosimetry of radiopharmaceuticals	FDA	5/1/73- 4/30/74
G. A. Andrews	Joint AEC-NIH program for study of gallium 67	NCI	2/1/73- 6/30/74
C. C. Lushbaugh	ORAU study of therapeutic and accidental whole-body irradiation for NASA	NASA	1/1/73-12/31/73
L. G. Littlefield	Chromosome breakages in women taking oral contraceptives	NICHD	7/1/72- 6/30/73
	Active Grants		
C. L. Edwards	Gallium-67 as a tumor-localizing radioindicator	ACS	7/1/71- 6/30/74
F. Snyder	The occurrence, metabolism, and function of ether-linked glycerolipids in neoplasms	ACS	12/1/72-11/30/73
F. Snyder (ORAU) and C. Piantadosi (UNC)	Alkoxyl and alkenyloxy glyceryl ethers and derivatives	NIH-UNC	1/1/73-12/31/73

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AFWL Air Force Weapons Laboratory
AMES Ames Research Laboratory
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NASA National Aeronautics and Space Administration
NCI National Cancer Institute
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F. Snyder	Biosynthetic mechanisms for ether bonds in lipids	NIH	5/1/73- 4/30/74
R. Hayes	Concentration of radio-nuclides by malignant neoplasms	NIH	5/1/73- 4/30/74
R. Wykle	Enzymic synthesis of plasmalogens in heart and brain	NIH	5/1/73- 4/30/74
D. Swartzendruber	Effect of irradiation on gallium-67 localization in animals	NIH	1/1/73-12/31/73
H. Goswitz	Regulation of granulopoiesis	NIH	1/1/73-12/31/73
G. A. Andrews	General research support	NIH	1/1/73-12/31/74
M. Ketchel	Antigen expression on tumor cells	NIH	11/1/73-10/31/74
N. Gengozian	Maintenance of marmoset colony	NCI	7/1/73-12/31/73
C. C. Lushbaugh	Apollo pocket mouse project	NASA-AMES	7/1/73- 6/30/74
N. Gengozian	Potato blight study	UW	8/1/73- 2/28/75
R. Ricks	Psychomotor functions	AFWL	9/1/73- 6/30/74
H. Goswitz	Blood cell separator	ORNL	10/15/73- 9/15/74
C. C. Lushbaugh	Collaborative studies w/ Dr. Yuhas	ORNL	12/1/73- 6/30/74
	Pending Grant and Contract Proposals		**Proposal Dates**
G. A. Andrews	Institutional research grant	ACS	7/1/74- 6/30/75
R. Hayes	ORAU-ORNL study of carbon-11 in nuclear medicine	NIH	5/1/74- 4/30/75
N. Gengozian	Immunologic tolerance in the marmoset	NIH	2/1/74- 1/31/75
F. Goswitz	Southeastern cancer study group protocol	NIH	7/1/74- 6/30/75
N. Gengozian	"Autoimmune" thrombo-cytopenia in the marmoset	NIH	5/1/74- 4/30/75
B. Nelson	Destiny in tissues of stem cells given intravenously	NIH	5/1/74- 4/30/75
R. Tyndall	Minicells of **Salmonella typhimurium** as a vaccine against typhoid	PAHO	Uncertain

R. Tyndall	Gallium-67 uptake in embryogenesis and oncogenesis	NIH	9/1/74- 8/31/75
R. Tyndall	Minicells of **Pseudomonas** and **Klebsiella** as a vaccine	NIH	9/1/74- 8/31/75
L. G. Littlefield	Evaluation of induced cytogenetic lesions in oocytes	NIH	9/1/74- 8/31/75
L. G. Littlefield	Somatic-cell studies	NF	Uncertain
G. A. Andrews	Co-op group—tumor localizing radio-pharmaceuticals	NIH	5/1/74- 4/30/75

1028299

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1974-75

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Research Report

Medical and Health Sciences Division

Oak Ridge Associated Universities

1974-1975

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ORAU-127
Distribution Category: UC–48

Research Report 1974-1975

Medical and Health Sciences Division
Oak Ridge Associated Universities

Foreword

This Research Report encompasses the research progress in 1974 and up through September 1975 of the Medical Division under AEC support and of the new Medical and Health Sciences Division of ORAU under ERDA support. Fiscal year 1975 was transitional from an AEC research program focused largely upon mammalian radiation effects to one under ERDA that will become increasingly concerned with studying the effects in man of toxicants produced by nonnuclear as well as nuclear fuel technologies. Little retooling or restaffing is anticipated for this new direction, since the basic and applied biomedical sciences needed to study the toxic effects of nonnuclear substances are largely the same as those needed in nuclear toxicology and industrial medicine.

The format of this report differs from those of previous years. This change in style reflects our belief that research progress is measured, in large part, by an institution's publications.

C. C. Lushbaugh
Fred Snyder

January 1976

Contents

Page

1 Scope of Research Program 1

2 Publications 3

3 Abstracts of Full-Length Publications 9

4 Collaborative Projects Between Medical and Health Sciences Division Staff and Other Institutions 25

5 Medical and Health Sciences Division Staff and Their Locations 27

This report was prepared under contract with the Energy Research and Development Administration.

Requests for additional information should be directed to:
Medical and Health Sciences Division, Oak Ridge Associated Universities,
P.O. Box 117, Oak Ridge, Tennessee 37830

1028303

Scope of Research Program

During the past year the ORAU Medical and Health Sciences Division (formerly the Medical Division) has redirected its biomedical research efforts to meet the new broadened responsibilities for environmental and safety research under the sponsorship of the Energy Research and Development Administration (ERDA). In the past, under the sponsorship of the Atomic Energy Commission, the Division's emphasis focused to a large extent on clinical applications of nuclear medicine in oncology.

The reorganized research program is designed to support ERDA's newly assigned tasks in Health Studies, Biological Studies, Health Protection, Instrument Technology, Analysis and Evaluations, and Nuclear Education and Training. Our capabilities in Immunology, Biochemistry, Radiopharmaceutical Development, and Radiation Biology provide a four-way approach to the solution of some pressing problems in occupational and preventive medicine related to the development of nonnuclear sources of energy, in addition to those created by nuclear technology. These nonnuclear problems concern principally acute and chronic diseases of the skin, lungs, gastrointestinal tract, and marrow that are induced in man by chemical intoxicants released actively or potentially into the environment by power production and research in its development. Our goal is to enlarge the informational bases that will lead to 1) establishing empirically derived safe-exposure limits and means for their measurements, 2) preventing occupational diseases, 3) early detection of incipient chronic biologic effects of biohazards, 4) improving therapy of acute and chronic occupational diseases, and 5) providing education and training in the biomedical technologies involved.

Although many occupational diseases related to energy-production technologies are well known and many of their causal agents have been identified, the basic mechanisms, dose-response relations, and means for their early detection and specific therapies are still poorly understood or yet to be developed. In addition, the long-term impact of daily exposures to external and internal radiations and chemical intoxicants too small to be acutely effective by themselves, is not known. Therefore, well-planned epidemiologic studies designed for continual human health assessments must be made. These data must be correlated with actual environmental and human concentrations of both nuclear and nonnuclear toxicants and then used in determining the validity of the pathophysiologic endpoints used in morbidity and mortality studies. Gaining this information depends on the development of animal models as surrogates for man under controlled experimental conditions and then using them to elucidate basic mechanisms, and to test the efficacy of methods for prevention and therapy of untoward effects.

Although most of the progress reported here was generated under our former program guidance in the nuclear energy domain, the reader will find much of it relevant to our broadened needs in environmental toxicology, pathology, and medicine. He will also be able to see how the four major programs for FY '76 emerged from these efforts and were identified as Immunobiology, Membrane Biochemistry, Radiopharmaceutical Development, and Radiation Emergency Assistance Center/Training Site (REAC/TS). The four programs are briefly described below.

A. IMMUNOBIOLOGY

The immunobiology program has as one of its major objectives the understanding of the consequences of hemopoietic chimerism on the immunologic competence of an animal. Two animal models are currently being utilized; one the marmoset, a small South American primate that is a natural blood chimera and the second, the mouse, an allogeneic radiation chimera in which chimerism is induced by lethal irradiation and infusion of histoincompatible bone marrow. In the marmoset program, three major problems were undertaken and completed this year: (1) an analysis of the probability of procuring marmosets that are nonchimeric for hemopoietic elements, (2) the identification of T and B lymphocytes, and (3) a characterization of the animal's ability to respond to sheep RBC. This past year we also had the opportunity to examine the ultrastruc-

1028304

ture of colon cancer cells derived from one species of marmoset (*S. oedipus*) that develops adenocarcinoma of the colon spontaneously.

B. MEMBRANE BIOCHEMISTRY

ORAU's membrane biochemistry program is concerned with membrane properties that involve lipids, proteins, and carbohydrates. The focus is aimed at providing a basic understanding of the biochemical mechanisms associated with cancer and lung diseases that result from occupational biohazards such as fossil fuel toxicants and irradiation. Of primary interests are the metabolic pathways, regulation, and function of the ether-linked lipids that we have found to be characteristic of most neoplasms; thus much of our progress reported here for the past year has been made in our studies of the ether lipids. However, an exciting new development resulted from our discovery that the composition of cellular membranes could be manipulated by using unnatural analogs of lipid precursors.

C. RADIOPHARMACEUTICAL DEVELOPMENT

The radiopharmaceutical development program is concerned with the preclinical development and testing of radioisotopic agents for the diagnosis and treatment of disease in humans, particularly those that can arise from energy-related toxicants. Following satisfactory tests of these agents in animals, evaluation of clinical effectiveness is then arranged. Basic studies of the mechanism involved in the action of radiopharmaceuticals provide input into the identification of new agents. During the year several radionuclides have been studied clinically for the first time as tumor-localizing agents. A highlight of this year's preclinical progress has been the development of a method for the production of ^{11}C-labeled 1-amino-cyclopentanecarboxylic acid, a compound that localizes rapidly in malignant tumors.

D. RADIATION EMERGENCY ASSISTANCE CENTER/TRAINING SITE (REAC/TS)

The Radiation Emergency Assistance Center/Training Site program has multiple objectives focused on improving the treatment of radiation-induced injuries in man. These objectives involve the use of a specially designed facility to demonstrate decontamination of persons involved in radiation accidents; to train others—particularly physicians, paramedical personnel, and health physicists in the medical management of radiation accidents; to manage the radiation-exposure problems in an area-wide disaster plan in conjunction with the staff of a community hospital; to develop a group of *Information Registries* concerning human radiation effects for use in assessing radiation effects on human health; and to attempt by experimental pathologic means to answer related pragmatic questions whose solutions are needed in order to improve surgical and medical care of persons contaminated or injured in radiation accidents.

1028305

Publications

From the Medical and Health Sciences Division, Oak Ridge Associated Universities, 1974.

Reprints, when available, and additional information on publications can be obtained from the Publications Office, Medical and Health Sciences Division, Oak Ridge Associated Universities, P. O. Box 117, Oak Ridge, Tennessee 37830.

Allen, E. M. and Gengozian, N. *Effect of antithymocyte globulin pretreatment on immunological reconstitution of lethally irradiated animals.* Transplantation 19, 456-463, 1975.

Alpen, E. L., Bateman, J. L., Blair, H. A., Brennan, J. T., Hasterlik, R. J., Lushbaugh, C. C., Rust, J. H., Leroy, G. V., and Casarett, G. W. (Scientific Committee 14). *Radiological factors affecting decision-making in a nuclear attack.* NCRP Report No. 42, November 15, 1974. National Council on Radiation Protection and Measurements, Washington, D. C. 20014.

Andrews, G. A. Book Review: *Anticancer agents recently developed in the People's Republic of China.* Ann. Intern Med. 81, 863-864, 1974.

Andrews, G. A. *History of radiopharmacy.* In: Radiopharmacy, M. Tubis and W. Wolf, eds., Wiley-Interscience (in press).

Andrews, G. A. *Radiation injury.* In: Encyclopaedia Britannica, 15th edition, Helen Hemingway Benton, Publisher, 1974, pp. 417-421.

Andrews, G. A. and Edwards, C. L. *Tumor scanning with gallium-67.* J. Am. Med. Assoc. 233, 1100-1103, 1975.

Barclay, T. R., Cloutier, R. J., Watson, E. E., and Gilliam, P. L. *Interfacing a multichannel analyzer to a minicomputer.* Nucl. Instrum. Methods 124, 281-283, 1975.

Barnhart, D. D. and Gengozian, N. *An evaluation of the mixed lymphocyte culture reaction in marmosets.* Transplantation 20, 107-115, 1975.

Barnhart, D. D. and Gengozian, N. *Actively induced thrombocytopenia in the marmoset: A possible autoimmune model.* Clin. Exp. Immunol. 21, 493-500, 1975.

Blank, M. L. *Thin-layer chromatography of neutral lipids* (Presented at the American Oil Chemists' Society Conference on the Analysis of Lipids and Lipoproteins, June 12-15, 1974, Washington, D. C.).

Blank, M. L. and Snyder, F. *A tissue culture system for studies of the regulation of ether-linked and ester-linked aliphatic moieties in glycerolipids.* Arch. Biochem. Biophys. 160, 100-105, 1974.

Blank, M. L. and Snyder, F. *Detection of ^{14}C on thin-layer chromatograms by spark chamber analysis.* (Presented at the Qualitative Thin-Layer Symposium Pittsburgh Analytical Meeting, Cleveland, Ohio, 1974.)

Blank, M. L. and Snyder, F. *Qualitative and quantitative aspects of thin-layer chromatography in the analysis of phosphorus-free lipids.* Monograph "Analysis of Lipids and Lipoproteins," E. G. Perkins, ed. American Oil Chemists' Soc., Publisher, 1975, pp. 63-69.

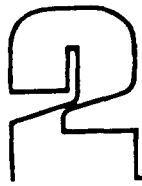
Blank, M. L., Cress, E. A., Piantadosi, C., and Snyder, F. *A method for the quantitative determination of glycerolipids containing O-alkyl and O-alk-1-enyl moieties.* Biochim. Biophys. Acta 380, 208-218, 1975.

Blank, M. L., Piantadosi, C., Ishaq, K. S., and Snyder, F. *Modification of glycerolipid metabolism in L-M fibroblasts by an unnatural amino-alcohol, N-isopropylethanolamine.* Biochem. Biophys. Res. Commun. 62, 983-988, 1975.

Blank, M. L., Wykle, R. L., Alper, S., and Snyder, F. *Microsomal synthesis of the ether analogs of triacylglycerols. Acyl CoA:alkylacylglycerol and acyl CoA: alk-1-enylacylglycerol acyltransferases in tumors and liver.* Biochim. Biophys. Acta 348, 397-403, 1974.

Brown, R. C., Blank, M. L., Kostyu, J. A., Osburn, P., Kilgore, A., and Snyder, F. *Analysis of tumor-associated alkyldiacylglycerols and other lipids during radiation-*

1028306



induced thymic leukemogenesis. Proc. Soc. Exp. Biol. Med. 149, 808-813, 1975.

Byrd, B. L., Watson, E. E., Cloutier, R. J., and Hayes, R. L. *Effect of stable scandium on the long-term whole body retention of intravenously administered ^{46}Sc citrate in the rat.* Health Phys. 29, 375,1975.

Chaskes, S. and Phillips, A. W. *Pigmentation and autofluorescence of Candida species after growth on tryptophan media.* Can. J. Microbiol. 20, 595-603, 1974.

Chaskes, S. and Tyndall, R. L. *Pigment production by Cryptococcus neoformans from para- and ortho-diphenols: Effect of the nitrogen source.* J. Clin. Microbiol. 1, 509-514, 1975.

Chaskes, S., Kingdon, G. C., and Balish, E. *Serum immunoglobulin levels in humans exposed to therapeutic total-body gamma irradiation.* Radiat. Res. 62, 145-158, 1975.

Clapp, N. K., Tyndall, R. L., Cumming, R. B., and Otten, J. A. *Effects of butylated hydroxytoluene alone or with diethylnitrosamine in mice.* Food Cosmet. Toxicol. 12, 367-371, 1974.

Cloutier, R. J. Book Review: *Nuclear energy: Its physics and its social challenge.* D. R. Inglis, Addison-Wesley Publishing Co., Reading, Maine, 1973. Health Phys. 26, 204, 1974.

Cloutier, R. J. and Watson, E. E. *Recent advances in dosimetry for nuclear medicine.* Am. Nuc. Soc. Trans., 19, 43-44, 1974 abstr.

DeLand, F. H., Sauerbrunn, B. J. L., Boyd, C., Wilkinson, R. H., Jr., Friedman, B. I., Moinuddin, M., Preston, D. F., and Kniseley, R. M. *^{67}Ga-citrate imaging in untreated primary lung cancer: Preliminary report of cooperative group.* J. Nucl. Med. 15, 408-411, 1974.

Edwards, C. L. and Hayes, R. L. *Gallium-67 citrate for tumor scanning.* In: Radiologic and Other Biophysical Methods in Tumor Diagnosis, Proceedings of the 18th Annual Clinical Conferences on Cancer, 1973. Year Book Medical Publishers, Inc., Chicago 1975, pp. 39-56.

Edwards, C. L. and Hayes, R. L. *Tumor detection with ^{67}Ga citrate.* In: Proceedings of the First World Congress of Nuclear Medicine, held in Tokyo and Kyoto, Japan, Oct. 1974, World Federation of Nuclear Medicine and Biology, 1974, pp. 120-123.

El-Bassiouni, E. A., Piantadosi, C., and Snyder, F. *Metabolism of alkyl-dihydroxyacetone phosphate in rat brain.* Biochim. Biophys. Acta 388, 5-11, 1975.

Ellis, J. T., Kraft, L. M., Lushbaugh, C. C., Humason, G. L., Hartroft, W. S., Porta, E. A., Bailey, O. T., Greep, R. O., Leach, C. S., Laird, T., Simmonds, R. C., Vogel, F. S., Dennis, R. L., Brashear, H. R., Talmage, R. V., Harrison, G. A., Corbett, R. L., Klein, G., Tilbury, T., Suri, K., and Haymaker, W. *The effects of cosmic particle radiation on pocket mice aboard Apollo XVII: Appendix III: evaluation of viscera and other tissues.* Aviat. Space Environ. Med. 46, 639-654, 1975.

Frenkel, J. K., Nelson, B. M., and Arias-Stella, J. *Immunosuppression and toxoplasmic encephalitis.* Hum. Pathol. 6, 97-111, 1975.

Gengozian, N. and Batson, J. S. *Single-born marmosets without hemopoietic chimerism: Naturally occurring and induced.* J. Med. Primatol. 4, 252-261, 1975.

Gengozian, N. and Bazzell, S. J. *Isoimmunizations among marmosets to identify leucocyte antigens.* In: Advances in Medical Primatology, E. I. Goldsmith and J. Moor-Jankowski, eds., Plenum Press (in press).

Gengozian, N., Salter, B. L., Basford, N. L., and Kateley, J. R. *Characterization of the antibody response of the marmoset to sheep red blood cells.* Clin. Exp. Immunol. (in press).

Gengozian, N., Smith, T. A., and Gosslee, D. G. *External uterine palpation to identify stages of pregnancy in the marmoset, Saguinus fuscicollis ssp.* J. Med. Primatol. 3, 236-243, 1974.

Gibbs, W. D., Edwards, C. L., Hodges, H. D., and Cloutier, R. J. *Measurement of temporal changes in whole-body radionuclide distribution with a profile scanner.* In: Dynamic Studies with Radioisotopes in Medicine, 1974, International Atomic Energy Agency, Vienna, 1974, pp. 75-84.

Goh, K-o. *Exogenous deoxyribonucleoside triphosphates II. Their effect on pulverized human chromosomes at the S phase.* Proc. Soc. Exp. Biol. Med. 145, 938-943, 1974.

Goh, K-o. *Total-body irradiation and human chromosomes. IV. Cytogenetic follow-up studies 8 and 10½ years after total-body irradiation.* Radiat. Res. 62, 364-373, 1975.

Goh, K-o. *Total-body irradiation and human chromosomes. V. Additional evidence of a transferable substance in the plasma of irradiated persons to induce chromosomal breakages.* J. Med. 6, 51-60, 1975.

1028307

Goswitz, F. *Radioactive vitamin B_{12} absorption tests and other studies.* In: Golden's Diagnostic Radiology, Section 20, Diagnostic Nuclear Medicine, A. Gottschalk, J. L. Quinn and E. J. Potchen, eds. Williams and Wilkins (in press).

Goswitz, F. *Radiocolloid spleen scanning in lymphoma and chronic lymphocytic leukemia.* J. Nucl. Med. 15, 496, 1974, abstr.

Goswitz, F., Morris, C., Kim, K. and Hansard, M. *Altered reticuloendothelial (RE) function in hematologic diseases.* J. Nucl. Med. 15, 495-496, 1974, abstr.

Greenlaw, R. H., Weinstein, M. B., Brill, A. B., McBain, J. K., Murphy, L., and Kniseley, R. M. *^{67}Ga-citrate imaging in untreated malignant lymphoma: Preliminary report of cooperative group.* J. Nucl. Med. 15, 404-407, 1974.

Hayes, R. L. *Factors affecting uptake of radioactive agents by tumor and other tissues.* In: Proceedings of IAEA Advisory Group Meeting on Tumor Localization with Radioactive Agents, Vienna, December 1974 (in press).

Hayes, R. L. and Brown, D. H. *Biokinetics of radiogallium.* Proceedings of the International Meeting of the Society of Nuclear Medicine, Munich, West Germany, September 1974 (in press).

Hayes, R. L., Brown, D. H., and Carlton, J. E. *Comparison of the subcellular distribution of the tumor-localizing agents, ^{67}Ga, ^{111}In, ^{206}Bi, and ^{167}Tm.* J. Nucl. Med. 15, 501, 1974, abstr.

Hayes, R. L., Brown, D. H., Byrd, B. L., and Carlton, J. E. *A comparison of the tissue distribution of ^{67}Ga and the rare earth radionuclides.* J. Nucl. Med. 15, 501, 1974, abstr.

Hodges, H. D. *Applications and availability of whole-body counters to the nuclear medicine technologist.* J. Nucl. Med. Technol. 2, 102-104, 1974.

Hodges, H. D., Gibbs, W. D., Morris, A. C., Jr., and Coffey, W. C., II. *An improved high-level whole-body counter.* J. Nucl. Med. 15, 610-612, 1974.

Hodges, H. D., Goswitz, F. A., and Berger, J. D. Letter to the editor: *Defective ^{198}Au-colloid.* J. Nucl. Med. Technol. 2, 18, 1974.

Hodges, H. D., Morris, A. C., Jr., and Gibbs, W. D. *Correlation of thyroid uptake with whole-body retention measurements.* J. Nucl. Med. Technol. 2, 72-75, 1975.

Hübner, K. F. and Littlefield, L. G. *Burkitt lymphoma in three American children.* Am. J. Dis. Child. 129, 1219-1223, 1975.

Hübner, K. F., Gengozian, N., and Gilman, T. A. *Suppression and recovery of the immune response and CFU potential of mice during and after chronic low-dose-rate irradiation.* Int. J. Radiat. Biol. (in press).

Johnston, G., Benua, R. S., Teates, C. D., Edwards, C. L., and Kniseley, R. M. *^{67}Ga-citrate imaging in untreated Hodgkin's disease: Preliminary report of cooperative group.* J. Nucl. Med. 15, 399-403, 1974.

Kateley, J. R. and Gengozian, N. *Rat-mouse radiation chimeras: Characterization of an antibody-mediated graft-vs-host reaction.* J. Immunol. 114, 125-132, 1975.

Kniseley, R. M. *Diseases of the bone marrow.* In: Principles of Nuclear Medicine, 2nd ed., H. N. Wagner, ed. W. B. Saunders, (in press).

Lee, T-c. and Stephens, N. *Dietary effect of stearyl CoA desaturation in Morris hepatomas.* Fed. Proc. 33, 1264, 1974, abstr. 224.

Lee, T-c., Stephens, N., and Snyder, F. *Dietary effects on stearyl coenzyme A desaturase in Morris hepatomas.* Cancer Res. 34, 3270-3273, 1974.

Lee, T-c., Blank, M. L., Piantadosi, C., Ishaq, K. S., and Snyder, F. *Incorporation and subcellular distribution of an unnatural phospholipid base-analog, N-isopropyl-ethanolamine, in rat liver.* Biochim. Biophys. Acta 409, 218-224, 1975.

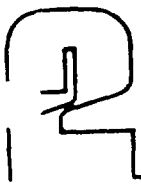
Littlefield, L. G. and Mailhes, J. B. *Comparison of chromosome breakages in lymphocytes and fibroblasts from control women and women taking oral contraceptives.* Fertil. Steril. 26, 828-832, 1975.

Littlefield, L. G. and Mailhes, J. B. *Observations of de novo clones of cytogenetically aberrant cells in primary fibroblast cell strains from phenotypically normal women.* Am. J. Hum. Genet. 27, 190-197, 1975.

Littlefield, L. G. and Vodopick, H. A. *Cytogenetic studies in a patient with acute granulocytic leukemia of 7½ years duration.* Blood 46, 783-789, 1975.

Littlefield, L. G., Lever, W. E., Miller, F. L., and Goh, K-o. *Chromosome breakage studies in lymphocytes from normal women, pregnant women, and women taking*

1028308



oral contraceptives. Am. J. Obstet. Gynecol. 121, 976-980, 1975.

Lushbaugh, C. C. *Human radiation tolerance.* Chapter 10. In: Space Radiation Biology and Related Topics, C. A. Tobias and P. Todd, eds., Academic Press, New York, 1974, pp. 475-522.

Lushbaugh, C. C. and Swartzendruber, D. C. *Histopathologic aspects of radiation effects upon lymphatic tissues and malignancies.* In: Proceedings of the 14th Annual Hanford Biology Symposium, Hanford, Washington, October 1974 (in press).

Lushbaugh, C. C. and Yuhas, J. M. *Pathological features of radiation-induced murine pulmonary carcinomas and their metastases.* Radiat. Res. 59, 222, 1974, abstr. D-28-2.

Mainwaring, H. R., Swartzendruber, D. C., Lushbaugh, C. C., Idoyaga-Vargas, N. L., and Watson, E. E. *Radiation effects on ^{67}Ga retention and distribution in mouse tissues.* In: Proceedings of the 14th Annual Hanford Biology Symposium, Hanford, Washington, October 1974 (in press).

Morris, A. C., Jr. *Advances in nuclear-medical instrumentation.* Cadence (in press).

Morris, A. C., Jr., Goswitz, F. A., Kim, K. H., McDow, A. E., Jr., Aaron, P. A., and Barclay, T. R. *Interface for direct online computer recording of dynamic radionuclide data.* Med. Biol. Eng. 12, 367-369, 1974.

Nelson, B. and Fliedner, T. M. *Lymphocytopoiesis after irradiation.* In: Proceedings of a Symposium on Cellular Aspects of the Effects of Irradiation with Low Doses, Using the Lymphatic System as an Example. Vienna, April 1973, Springer-Verlag (in press).

Niblack, G. D. and Gengozian, N. *Identification of T and B lymphocytes in marmosets.* Clin. Exp. Immunol. (in press).

Poggenburg, J. K. and Hayes, R. L. *Radiopharmaceuticals for function studies present and future.* In: Dynamic Studies with Radioisotopes in Medicine, 1974, International Atomic Energy Agency, Vienna, 1974, pp. 85-96.

Ricks, R. C. and Lushbaugh, C. C. *Quantitation of radiation-induced changes in man.* Radiat. Res. 59, 39, 1974, abstr. A-19-3.

Ricks, R. C. and Lushbaugh, C. C. *The influence of exercise conditioning on the performance decrement effects of total-body irradiation.* Health Phys. 27 (6), 614, 1974, abstr. P/12.

Rock, C. O. and Snyder, F. *A short-chain acyl CoA:1-alkyl-2-acyl-sn-glycerol acyltransferase from a microsomal fraction of the rabbit harderian gland.* Biochim. Biophys. Acta 388, 226-230, 1975.

Rock, C. O. and Snyder, F. *Biosynthesis of 1-alkyl-sn-glycero-3-phosphate via ATP:1-alkyl-sn-glycerol phosphotransferase.* Fed. Proc. 33, 1552, 1974, abstr. 1858.

Rock, C. O. and Snyder, F. *Biosynthesis of 1-alkyl-sn-glycero-3-phosphate via adenosine triphosphate:1-alkyl-sn-glycerol phosphotransferase.* J. Biol. Chem. 249, 5382-5387, 1974.

Rock, C. O. and Snyder, F. *Purification of phospholipase A_2 from venoms by affinity chromatography.* Fed. Proc. 34, 525, 1975, abstr. 1720.

Rock, C. O. and Snyder, F. *Rapid purification of phospholipase A_2 from Crotalus adamanteus venom by affinity chromatography.* J. Biol. Chem. 250, 6564-6566, 1975.

Rock, C. O. and Snyder, F. *Metabolic interrelation of hydroxy-substituted ether-linked glycerolipids in the pink portion of the rabbit harderian gland.* Arch. Biochem. Biophys. (in press).

Snyder, F. *Chromatographic analysis of alkyl and alk-1-enyl ether lipids and their derivatives.* In: Lipid Chromatographic Analysis. G. V. Marinetti, ed. Marcel Dekker, (in press).

Snyder, F. *The use of radioassay techniques in thin-layer and gas liquid chromatography.* (Presented at the American Oil Chemists' Society Conference on the Analysis of Lipids and Lipoproteins, June 12-15, 1974, Washington, D. C.)

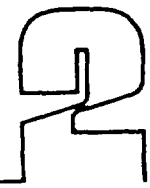
Snyder, F. and Malone, B. *Acyltransferases and the biosynthesis of pulmonary surfactant lipid in adenoma alveolar type II cells.* Biochem. Biophys. Res. Commun. 66, 914-919, 1975.

Snyder, F. and Snyder, C. *Glycerolipids and cancer.* Progr. Biochem. Pharmacol 10, 1-41, 1975.

Snyder, F., Malone, B. and Piantadosi, C. *Enzymic studies of glycol and glycerol lipids containing O-alkyl bonds in liver and tumor tissues* Arch. Biochem. Biophys. 161, 402-407, 1974.

Snyder, F., Blank, M. L., Wykle, R. L., and Lee, T-c. *Ether-linked lipids in cancer cells: Regulation and*

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function. (Presented at the 18th International Conference on the Biochemistry of Lipids, Graz, Austria, 9-11 July 1975.)

Snyder, F. L., Wykle, R. L., Blank, M. L., Lee, T-c., and Malone, B. *Enzymatic interconversions of ether-linked glycerolipids and their precursors.* (Presented Proc. 17th International Conference on the Biochemistry of Lipids, Milan, Italy, Sept. 9-11, 1974.)

Swartzendruber, D. C., Idoyaga-Vargas, N. L., Hubner, K. F., and Urso, P. *Effect of antigenic stimulation on ^{67}Ga uptake in mouse lymphoreticular tissues.* J. Reticuloendothel. Soc. 16, 30A, abstr supplement, 1974, abstr. 59.

Swartzendruber, D. C., Buckland, C. N., Lushbaugh, C. C., Humason, G. L., and Gengozian, N. *Ultrastructural abnormalities of marmoset colon cancer cells.* Fed. Proc. 34, 825, 1975, abstr. 3423.

Tankersley, W. G. and Woodward, J. M. *Induction and isolation of a minicell-producing strain of Salmonella typhimurium.* Proc. Soc. Exp. Biol. Med. 145, 802-805, 1974.

Tyndall, R. L. and Daniel, J. C., Jr. *Alterations in uterine and serum esterases in pregnant mammals.* Fertil. Steril. (in press).

Tyndall, R. L., Colyer, S., and Clapp, N. *Early alterations in plasma esterases with associated pathology following oral administration of diethylnitrosamine and butylated hydroxytoluene singly or in combination.* Int. J. Cancer. 16, 184-191, 1975.

Urso, P. and Gengozian, N. *Variation in T and B cell deficiency in different mouse allogeneic radiation chimeras.* J. Immunol. 113, 1770-1779, 1974.

Vodopick, H. *Cherchez la chienne.* A report of erythropoietic hypoplasia. J. Am. Med. Assoc. 234, 850-851, 1975.

Vodopick, H. and Andrews, G. A. *Accidental radiation exposure.* Arch. Environ. Health 28, 53-56, 1974.

Vodopick, H., Chaskes, S. J., Solomon, A., and Stewart, J. A. *Transient monoclonal gammopathy associated with cytomegalovirus infection.* Blood 44, 189-195, 1974.

Washburn, L. C. and Hayes, R. L. *Importance of excess base in the synthesis of sodium phosphorothioate.* Inorganic Syntheses, Vol 27 McGraw Hill 22 (in press).

Washburn, L. C. and Hayes, R. L. *Thin layer chromatographic and colorimetric methods for monitoring the purity of S-2-(3-aminopropylamino)-ethylphosphorothioic acid (WR-2721).* Anal. Chem. 46, 2231, 1974.

Washburn, L. C., Carlton, J. E., Hayes, R. L., and Yuhas, J. M. *Distribution of WR-2721 in normal and malignant tissues of mice and rats bearing solid tumors: dependence on tumor type, drug dose and species.* Radiat. Res. 59, 475-483, 1974.

Washburn, L. C., Sun, T. T., Wieland, B. W. and Hayes, R. L. *Methods for the rapid synthesis and purification of amino acids labeled with carbon-11.* J. Nucl. Med. 16:579, 1975 (abstr.).

Wykle, R. L. and Schremmer, J. M. *A lysophospholipase D pathway in the metabolism of ether-linked lipids in brain microsomes.* J. Biol. Chem. 249, 1742-1746, 1974.

Wykle, R. L. and Schremmer Lockmiller, J. M. *The biosynthesis of plasmalogens by rat brain: Involvement of the microsomal electron transport system.* Biochim. Biophys. Acta 380, 291-298, 1975.

Wykle, R. L. and Snyder, F. *Microsomal enzymes involved in the metabolism of ether-linked glycerolipids and their precursors in mammals.* Chapter
The Enzymes of Biological Markers, Vol. 2. A. Martonosi, Ed., Plenum Press, New York (in press).

1028310

Abstracts of Full-Length Publications

Allen, E. M. and Gengozian, N. **Effect of antithymocyte globulin pretreatment on immunological reconstitution of lethally irradiated animals.** Transplantation 19, 456-463, 1975.

Clinical and experimental studies have shown that antithymocyte globulin pretreatment will reduce the severity of the graft-versus-host reaction after allogeneic bone marrow transplantation. To determine whether this was attributable to a persisting cytotoxic factor in the recipients' sera or a result of the "masking" of foreign antigens by the antithymocyte globulin, an experimental schema utilizing a syngeneic transfer of immunocompetent cells was devised. Lethally irradiated mice that had been pretreated with rabbit antimouse thymocyte globulin (RAMTG) were injected with syngeneic spleen or bone marrow cells and their immunological competence was measured by the response to a test antigen, sheep red blood cells. It was found that such pretreatment had an adverse effect on the immunological potential of the infused cells. Thus, the plaque-forming cell response to sheep red blood cell antigen in RAMTG-pretreated recipients injected with spleen cells was reduced when compared to the saline or normal rabbit globulin-treated controls. The immunological recovery of lethally irradiated animals protected with syngeneic bone marrow was also delayed, but not permanently impaired, when the recipients had been pretreated with RAMTG. These effects were evident although the spleen or bone marrow cells had been injected into the RAMTG-pretreated recipients at a time when their sera were devoid of any cytotoxic antibodies. It is speculated that two mechanisms may contribute to this alteration in immune function of the infused cells: (1) that RAMTG exists in the serum in amounts sufficient to attach to receptor sites on lymphocytes or their precursors but insufficient to kill the cells, interfering with the antigen recognition mechanism or migration and homing patterns, or (2) that the RAMTG treatment creates a temporary defect in the microenvironment of the spleen and other hemopoietic tissues, thereby affecting the transplantation and proliferation kinetics of the infused cells.

Andrews, G. A. **Radiation injury.** In: Encyclopaedia Britannica, 15th edition, Helen Hemingway Benton, Publisher, 1974, pp. 417-421.

The severity of radiation damage to living tissue depends upon the type of radiation, the size of the exposure, and the rate at which it is experienced. This article discusses the sources, types, terminology, and the injuries produced by ultraviolet and ionizing radiation.

Andrews, G. A. and Edwards, C. L. **Tumor scanning with gallium-67.** J. Am. Med. Assoc. 233, 1100-1103, 1975.

This paper summarizes the current status of gallium-67 citrate as a scanning agent useful in the diagnosis and staging of malignant neoplasms. It describes the history of gallium in medicine, outlines the scanning procedure, and assesses its usefulness. In order of decreasing value gallium is used in (1) assessing patients with recently diagnosed cancer, (2) evaluating patients returning for follow-up, (3) determining the success of a current or recent program of therapy, and (4) looking for cancer in undiagnosed patients with vague symptoms. The last application may become increasingly prominent. We also describe the Cooperative Group to Study Localization of Radiopharmaceuticals, which is sponsored by the Oak Ridge Associated Universities.

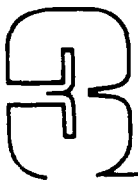
Barclay, T. R., Cloutier, R. J., Watson, E. E., and Gilliam, P. L. **Interfacing a multichannel analyzer to a minicomputer.** Nucl. Instrum. Methods 124, 281-283, 1975.

This report describes the modifications required and the interface circuitry needed to route the output of a Nuclear Data Model 2200 multichannel analyzer directly to a Digital Equipment Corporation PDP 11/20 computer. The analyzer's output then becomes acceptable as input into any computer program written in BASIC language.

Barnhart, D. D. and Gengozian, N. **An evaluation of the mixed lymphocyte culture reaction in marmosets.** Transplantation 20, 107-115, 1975.

Marmosets are unique in that all members may be considered to be natural blood chimeras because of the high frequency of fraternal twinning and placental vascular anastomoses between the fetuses. The mixed lymphocyte culture (MLC) reaction utilizing blood lymphocytes was evaluated to determine whether this in vitro test could detect histocompatibility differences among related and unrelated marmosets. It was found that the responses could be correlated with the probable immunogenetic relationships of these animals. Thus, an allogeneic MLC reaction in which the responding (R) and stimulating (S) cells were obtained from unrelated animals within one subspecies of marmosets (*Saguinus fuscicollis illigeri*) yielded a lower response than a semi-xenogeneic reaction involving R and S cells from two different subspecies of marmosets. (*S.f. illigeri* (R) versus *S.f. lagonotus* (S)). In contrast, MLC reactions between cells from chimeric marmoset co-twins were essentially negative, indicating specific immune tolerance. Under certain experimental conditions, however, it was suggested that the dual lymphocyte cell population from a chimeric marmoset

1028311



could undergo "autostimulation" as measured by label incorporation studies. The necessity of further experiments was recognized before the data could be truly accepted as reflecting an in vitro *manifestation* of incompatibility between the two different genetic populations of blood lymphocytes from a chimera.

Barnhart, D. D. and Gengozian, N. **Actively induced thrombocytopenia in the marmoset: A possible autoimmune model.** Clin. Exp. Immunol. 21, 493-500, 1975.

Inoculation of *S. oedipus* marmosets with white blood cells and platelets from another species of marmosets, *S. fuscicollis,* led to an acute and fatal thrombocytopenia. Antibody in the recipients to the immunizing platelets was found by agglutination and indirect immunofluorescence; also, serum from the afflicted animals passively induced thrombocytopenia in normal recipients. Similar inoculations of *S. oedipus* marmosets with platelet-free WBC preparations from the same donors were without any clinical effect; also, immunization with autologous, allogeneic (unrelated *S. oedipus* marmosets) or human platelets, did not cause the disease, even though antibody to the latter species of platelets, human, was found in the recipients. Failure to detect antibody to autologous (or allogeneic) platelets in the afflicted animals suggests induction of a disease process similar to that of *post-transfusion purpura,* where it has been speculated that the thrombocytopenia results from the adherence of antigen (donor)-antibody complexes to host platelets leading to their destruction by the reticuloendothelial system. The severity and consistency of inducing the disease in the marmoset by an active immunization process suggests this system may serve as an excellent animal model for autoimmune diseases falling within the syndrome of idiopathic thrombocytopenic purpura in man.

Blank, M. L. and Snyder, F. **A tissue culture system for studies of the regulation of ether-linked and ester-linked aliphatic moieties in glycerolipids.** Arch. Biochem. Biophys. 160, 100-105, 1974.

The incorporation of [1-^{14}C]hexadecanol and [1-^{14}C]palmitic acid into ether- and ester-linked lipids was investigated using suspension and monolayer cultures of L-M fibroblasts. Cells maintained in suspension cultures incorporated relatively high amounts of [1-^{14}C]hexadecanol into alkyldiacylglycerols, whereas the same cells grown as monolayers did not. The suspension cultures also synthesized small amounts of ethanolamine plasmalogens when the cells were incubated for 6 hr; none of the other lipid classes contained ^{14}C in the *O*-alk-1-enyl moieties. In the monolayer cultures most of the label from the hexadecanol was incorporated into the acyl moieties, indicating that the enzyme responsible for oxidation of the alcohol is extremely active under these conditions.

Of the total activity incorporated into the lipids of the suspension cultures, 2-5% of the ^{14}C was present in the alkyldiacylglycerols when palmitic acid was the precursor and 5-15% when hexadecanol was the precursor. Approximately 1% of the incorporated ^{14}C was in the alkyldiacylglycerol fraction from the monolayer cultures. Analysis of the alkyldiacylglycerols isolated from the suspension cultures incubated with hexadecanol demonstrated that approximately 60% of the ^{14}C was in the alkyl moieties, whereas the alkyldiacylglycerols isolated from the samples incubated with palmitic acid contained about 35% of the ^{14}C in the alkyl moieties. The incorporation of label from the palmitic acid into *O*-alkyl moieties shows that acyl-CoA reductase activities are present at a much higher level in the L-M fibroblasts maintained in suspension cultures, than in the monolayer cultures.

In some experiments, the *O*-alkyl moieties of cells maintained in suspension cultures were prelabled with [1-^{14}C]hexadecanol so that the turnover of the *O*-alkyl moieties could be traced when the cells were transferred back to monolayer cultures. After these cells grew overnight as monolayers, they contained essentially no labeled alkyldiacylglycerols although substantial amounts of labeled acyl groups were still present. The incorporation of [1-^{14}C]hexadecanol into the alkyl moieties of the glycerolipids in the cells maintained in suspension culture could also be altered by pH of the growth media. At a pH of 6.2, the ^{14}C associated with the alkyldiacylglycerols was 40-50% lower than that observed at pH 8.0. These results demonstrate that the metabolism of ether and ester groupings of glycerolipids in L-M fibroblasts can be manipulated and that the tissue culture systems described should be useful for exploring regulatory mechanisms involving glycerolipids that contain these groupings.

Blank, M. L. and Snyder, F. **Qualitative and quantitative aspects of thin-layer chromatography in the analysis of phosphorus-free lipids.** Monograph: *"Analysis of Lipids and Lipoproteins,"* E. G. Perkins, ed., 1975, American Oil Chemists' Society, pp. 63-69.

Methods are reported for the quantitative and qualitative analysis of non-phosphorus-containing lipids. Specific problems associated with the resolution of closely related lipid structures are also discussed.

Blank, M. L., Cress, E. A., Piantadosi, C., and Snyder, F. **A method for the quantitative determination of glycerolipids containing *O*-alkyl and *O*-alk-1-enyl moieties.** Biochim. Biophys. Acta 380, 208-218, 1975.

We have developed a spectrophotometric procedure, based on a combination of established methods, for the quantitative determination of alkyl and alk-1-enyl (plasmalogens) ether-linked glycerolipids. It depends upon the release of alkylglycerols and alk-1-enylglycerols from phospholipids by phospholipase C (*Bacillus cereus*) followed by saponification or by Vitride reduction of the phospholipids; aldehydes are subsequently formed and measured colorimetrically after reacting them with a fuchsin reagent.

The total alkyl and alk-1-enyl content of glycerolipids is determined by oxidation of the sample with periodate to form aldehydes and alkylglycolic aldehydes. The *O*-alk-1-enyl lipid content is determined on a separate sample by measuring the aldehydes produced after acid hydrolysis. The quantity of *O*-alkyl lipids is calculated from the difference between the values obtained for the total ether-lipid content and that of the *O*-alk-1-enyl lipid content. Alternately, direct determination of alk-1-enylglycerols and alkylglycerols can be made if these hydrolytic products are first separated by thin-layer chromatography.

1028312

Blank, M. L., Piantadosi, C., Ishaq, K. S., and Snyder, F. **Modification of glycerolipid metabolism in L-M fibroblasts by an unnatural amino-alcohol, N-isopropylethanolamine.** Biochem. Biophys. Res. Commun. 62, 983-988, 1975.

The unnatural amino-alcohol, N-isopropylethanolamine, is incorporated into a phospholipid by monolayers of L-M fibroblasts. This phospholipid was identified as 1,2-diacyl-*sn*-glycero-3-phosphoisopropylethanolamine by using chemical and enzymatic procedures combined with thin-layer and gas-liquid chromatography. Since the phospho-N-isopropylethanolamine moiety is removed by phospholipase C, the stereochemistry of the phospholipid analog is identical to naturally occurring phosphoglycerides. Incubation of cells in 10 mM N-isopropylethanolamine inhibited the incorporation of [^{14}C]choline and [^{14}C]ethanolamine into phospholipids and stimulated the incorporation of [1-^{14}C]palmitic acid and [1-^{14}C]hexadecanol into triacylglycerols and alkyldiacylglycerols. These results indicate that N-isopropylethanolamine affects glycerolipid synthesis at the diradylglycerol branch point.

Blank, M. L., Wykle, R. L., Alper, S., and Snyder, F. **Microsomal synthesis of the ether analogs of triacylglycerols. Acyl CoA:alkylacylglycerol and acyl CoA:alk-1-enylacylglycerol acyltransferases in tumors and liver.** Biochim. Biophys. Acta 348, 397-403, 1974.

Our results show that acyl CoA:1-alkyl-2-acyl-*sn*-glycerol acyltransferase activity, which catalyzes the formation of 1-alkyl-2,3-diacyl-*sn*-glycerols, is present in rat livers, 7777 Morris hepatomas and preputial gland tumors. We also demonstrated that a related enzyme activity, acyl CoA:1'-enyl-2-acyl-*sn*-glycerol acyltransferase, occurs in the microsomal fraction of preputial gland tumors; in this system the product was 1-alk-1'-enyl-2,3-diacyl-*sn*-glycerols. Since microsomes from rat livers were capable of synthesizing a product that does not occur in the liver lipids from a precursor that is also not normally present in this tissue, we conclude that the enzyme responsible is probably acyl CoA:1,2-diacyl-*sn*-glycerol acyltransferase, which catalyzes the synthesis of triacylglycerols. Because alkydiacylglycerols are synthesized by both liver and tumor preparations, it would appear that the control point for the synthesis of the higher levels of alkyldiacylglycerols found in tumors is not associated with the acyltransferase that accounts for the terminal acylation of alkylacylglycerols.

Brown, R. C., Blank, M. L., Kostyu, J. A., Osburn, P., Kilgore, A., and Snyder, F. **Analysis of tumor-associated alkyldiacylglycerols and other lipids during radiation-induced thymic leukemogenesis.** Proc. Soc. Exp. Biol. Med. 149, 808-813, 1975.

Lipid composition of thymuses investigated during the development of thymic leukemogenesis induced by exposing C57BL/6J mice to gamma radiation led to the following conclusions.

1. Alkyldiacylglycerols, a class of lipids that are generally elevated in most neoplastic tissues, occurred only in small quantities (less than 1% of the total lipids) in the thymuses of both control and irradiated mice. However, we found a 3- to 8-fold increase in this fraction in thymic tumors of mice at 5 months after irradiation when compared to controls of similar age. However, the small quantity of this lipid class in thymic leukemia and the fact that similar levels were found in some samples of involuted thymuses of mice 3 days after irradiation, suggests to us that the level of alkyldiacylglycerols is not sufficiently specific or sensitive for detecting early stages of thymic leukemogenesis.

2. Thymuses 3 days after irradiation and leukemic thymuses contain 2- to 3-fold greater quantities of cholesterol esters than control thymuses. No major differences were found in the distribution of acyl moieties in the cholesterol esters of the various thymus samples from the same aged mice, except that in thymic tumors the quantity of 18:1 esters was increased by about 25% over that of the controls. The apparent lack of specificity of increased cholesterol esters for neoplasia indicates that its measurement would not provide a suitable indicator of early neoplastic transformation.

3. Acyl composition of the triacylglycerols for thymuses revealed an increase in the 18:1 and a decrease in the 18:2 acids at 3 days after irradiation when compared to the same aged controls. However, thymic tumors occurring at 5 months after irradiation contained less 14:0, 16:0, and 16:1 acids and increased amounts of 18:1 and 18:2 acids esterified as triacylglycerols compared to controls. The fatty acid distribution in the phospholipid fraction of thymuses was not altered by the appearance of leukemia.

Byrd, B. L., Watson, E. E., Cloutier, R. J., and Hayes, R. L. **Effect of stable scandium on the long-term whole body retention of intravenously administered ^{46}Sc in the rat.** Health Phys. 29:375, 1975.

The effect of stable scandium on the whole-body retention and tissue distribution of ^{46}Sc has been studied in the rat. In one group of animals high specific activity ^{46}Sc was administered intravenously; in an another group ^{46}Sc with added stable scandium (0.5 mg/kg) was administered. The retention was followed for 333 days at which time the animals were killed and the distributions of the ^{46}Sc in various tissues determined. In general, administration of stable scandium produced pronounced increases in tissue concentrations, particularly in liver, marrow, intestine, and brain. A least-squares analysis indicated that three exponential components were required to fit the whole-body retention data from both groups of animals. For "carrier-free" ^{46}Sc the three compartments observed were: (1) 21% with a biological half-time of 1.0 day (turnover rate constant, $\lambda = 0.69\ day^{-1}$); (2) 22% with a biological half-time of 7.1 days ($\lambda = 0.098\ day^{-1}$); and (3) 56% with a biological half-time of 485 days ($\lambda = 0.0014\ day^{-1}$). For ^{46}Sc with stable scandium, the three compartments observed were: (1) 23% with a biological half-time of 1.3 days ($\lambda = 0.53^{-1}$); (2) 16% with a biological half-time of 7.3 days ($\lambda = 0.095\ day^{-1}$); and (3) 62% with a biological half-time of 1200 days (λ 0.00058 day^{-1}). Stable scandium thus has a pronounced effect on the long-term whole-body retention of ^{46}Sc. The observed long-term biological half-times in rats for both ^{46}Sc and ^{46}Sc plus carrier were far in excess of that given in IRCP Publication 2, 1959, for ^{46}Sc (30 days).

Chaskes, S. and Phillips, A. W. **Pigmentation and autofluorescence of Candida species after growth on tryptophan media.** Can. J. Microbiol. 20, 595-603, 1974.

Candida albicans and six other medically important *Candida* species were cultured on glucose-salts-biotin media containing tryptophan as the major ni-

1028313

trogen source. *Candida albicans, C. tropicalis,* and *C. parapsilosis* produced a pink pigment; *C. krusei, C. pseudotropicalis,* and *C. gulliermondii* formed brown pigments. Pigmentation of *C. stellatoidea* was variable, some strains producing brown pigments and others a pink pigment. Production of pink pigment was catalyzed by light. Cultures incubated in the dark produced only trace amounts of the pink pigment. Iron enhanced pigment production. Intense autofluorescence was observed in strains producing the brown pigment and weak autofluorescence in strains producing the pink pigment.

Chaskes, S. and Tyndall, R. L. **Pigment production by *Cryptococcus neoformans from para- and* ortho-diphenols: Effect of the nitrogen source.** J. Clin. Microbiol. 1, 509-514, 1975.

Cryptococcus neoformans produced pigments when *p*-diphenols were substrates in a glucose-amino acid-salts medium. The best substrates were 2,5-dihydroxybenzoic acid and 2,5-dihydroxybenzenesulfonic acid. In contrast to the cellular pigment production from *o*-diphenols (hyroxyl groups in the 2,3- or 3,4-position of phenyl ring), the *p*-diphenols (1,4- or 2,5-positions for the hydroxyl groups) produced large amounts of soluble pigments that diffused into the medium. When an optimal source of nitrogen (glutamine, glycine, and asparagine) was used, 89% of the *C. neoformans* strains produced pigments from *p*-diphenols. In contrast, 0 to 67% of the strains produced pigments when a suboptimal nitrogen source (proline, ammonium sulfate, ornithine, and methionine) was used. When glutamine-glycine-asparagine was the nitrogen source, 100% of the *C. neoformans* strains produced pigments from *o*-diphenols, whereas 77 to 100% of the strains produced pigment when proline-ammonium sulfate-ornithine-methionine was the nitrogen source. *Cryptococcus* species other than *C. neoformans* and all tested *Candida* species failed to produce pigments from any of the substrates except when hydroquinone was used. A combination of glutamine-glycine-asparagine and 3,4-dihydroxyphenylalanine allowed differentiation of colonies of *C. neoformans* from *C. albicans* in 3 to 6 days. These data showed that pigment production from *o*- and *p*-diphenols served as an excellent biochemical test for the identification of *C. neoformans.*

Chaskes, S., Kingdon, G. C., and Balish, E. **Serum immunoglobulin levels in humans exposed to therapeutic total-body gamma irradiation.** Radiat. Res. 62, 145-158, 1975.

Reduced serum immunoglobulin (IgA, IgG, IgM) levels developed in the majority of 27 patients with hematologic disorders after treatment with 100-350 R total-body gamma-ray exposures at a dose rate of either 1.5 R/min or 1.5 R/hr. A reduction in IgA of 20% or more was found in 66% of the cases, while 56% showed an IgM decrease, and 49% an IgG decrease of 20%. The severity of immunoglobulin depression was influenced by the total radiation dose and the patient's primary disease. The occurrence of IgG and IgM depression was greater when the radiation was given at 1.5 R/hr than when the dose rate was 1.5 R/min. Substantial but incomplete recovery toward preirradiation immunoglobulin levels was found for most patients by 7 wk after total-body irradiation (TBI).

Clapp, N. K., Tyndall, R. L., Cumming, R. B., and Otten, J. A. **Effects of butylated hydroxytoluene alone or with diethylnitrosamine in mice.** Food Cosmet. Toxicol. 12, 367-371, 1974.

Mice (18 months old) given butylated hydroxytoluene (BHT) at a dietary level of 0·75% for 16 months had 63·6% of lung tumours compared with 24·0% in controls. Mice given dietary BHT as well as diethylnitrosamine (DENA) in the drinking-water (average total intake 330 mg DENA/kg) had more lung tumours per mouse (4·0) than did the controls or those given DENA alone or BHT alone (1·4-2·2). The incidence of reticulum-cell sarcomas in mice killed at 12 months of age in the group treated with BHT plus DENA exceeded control values (52·6 and 22·7%, respectively), while the incidence in groups receiving DENA alone or BHT alone was not significantly different from that in controls. At 18 months, the incidence of reticulum-cell sarcoma in both groups given BHT was significantly less than in the groups not receiving BHT. At 18 months of age, a higher percentage of squamous-cell carcinomas was seen in the forestomach of mice given BHT plus DENA than in mice receiving DENA alone. The production of cysts within the liver parenchyma after DENA treatment also appeared to be potentiated by BHT treatment.

DeLand, F. H., Sauerbrunn, B. J. L., Boyd, C., Wilkinson, R. H., Jr., Friedman, B. I., Moinuddin, M., Preston, D. F., and Kniseley, R. M. **^{67}Ga-citrate imaging in untreated primary lung cancer: Preliminary report of cooperative group.** J. Nucl. Med. 15, 408-411, 1974.

An interinstitutional cooperative study has been undertaken to evaluate ^{67}Ga as a tumor-localizing agent. A uniform protocol and computer handling of data have been used. In 172 untreated patients with primary lung cancer, approximately 84% had one or more sites demonstrated on scanning with ^{67}Ga; 80% of individual lesions histologically verified had positive scans. It is occasionally possible to detect rather small lesions and, conversely, on occasion to miss some large lesions (5 cm or larger). Negative scans were obtained in 16% of lesions histologically proven. In the present analyses, rates of detection differ somewhat according to histologic type but not strikingly. With further developments in tumor-localizing agents and instruments, the agent may eventually be useful in the initial diagnostic workup of suspected lung carcinoma in certain clinical situations. As a single screening scan procedure, it should prove helpful in assessing the extent of disease before surgery or other type of therapy.

Edwards, C. L. and Hayes, R. L. **Gallium-67 citrate for tumor scanning.** In: Radiologic and Other Biophysical Methods in Tumor Diagnosis, Proceedings of the 18th Annual Clinical Conferences on Cancer, 1973. Year Book Medical Publishers, Inc., Chicago 1975, pp. 39-56.

The poorly understood affinity of tumors for ^{67}Ga has been widely but empirically applied to the clinical problem of detecting cancers. Gallium-67 scans are often difficult to interpret; the results are often ambiguous and have led to contradictory reports of clinical experience. Depending somewhat on histological type, most untreated primary lung cancers, untreated Hodgkin's tumors, and malignant lymphomas will be detected on scanning with ^{67}Ga. The incidence of false-positive results depends to a large extent on the skill of the interpreter at recognizing radioactivity in normal or non-neoplastic tissue. The clinical applications for which ^{67}Ga is

1028314

best suited are: (1) the staging of Hodgkin's disease and certain other lymphomas that might be treated with radiation therapy; (2) the detection of early occult recurrences after therapy in Hodgkin's disease and other lymphomas; (3) the detection of occult metastases of lung and breast cancer (possibly others) prior to extensive surgical procedures aimed at curing the patient; and (4) the detection of metastases of known cancers in patients with symptoms not attributable to known sites of tumor.

Negative ^{67}Ga scans do not rule out cancer, not even the presence of additional metastases in a patient in whom the primary cancer showed ^{67}Ga uptake. Likewise, ^{67}Ga uptake in a lesion neither proves nor rules out malignancy of the lesion, nor can one reliably predict the histopathologic classification of a tumor on the basis of the scan findings.

With a better understanding of the mechanisms responsible for the uptake of gallium in normal and neoplastic tissues, additional clinical applications for ^{67}Ga scanning, such as the early assessment of therapy, may take on even greater importance.

Edwards, C. L. and Hayes, R. L. **Tumor detection with ^{67}Ga citrate.** In: Proceedings of the First World Congress of Nuclear Medicine, held in Tokyo and Kyoto, Japan, Oct. 1974, World Federation of Nuclear Medicine and Biology, 1974, pp. 120-123.

For optimum contrast, the clinician must wait ~2 days or more after administering ^{67}Ga before scanning. This is often inconvenient and has been a deterent to the greater clinical application of this radiopharmaceutical. Another problem encountered in using ^{67}Ga for tumor detection is the tendency for ^{67}Ga to be retained in the bowel. It also lacks specificity. Many tumors fail to take up ^{67}Ga while a variety of non-neoplastic lesions do.

One approach to the problem has been to look for another nuclide with greater tumor specificity. Indium is chemically similar to gallium and ^{111}In is clearly a superior nuclide as far as its emissions are concerned. Photoscans showing the distribution of both ^{111}In and ^{67}Ga in 6 patients were made simultaneously by using two photorecorders and two spectrometers, each with a narrow window setting (20Kev). We observed more ^{67}Ga than ^{111}In in all cases where there was significant concentration of either nuclide in tumor tissue. This was in agreement with animal findings.

A second approach for circumventing the shortcomings of ^{67}Ga scanning is the use of an agent to block the initial binding of ^{67}Ga to plasma proteins. The simultaneous injection of 0.5 mg Sc/kg with ^{67}Ga (both as citrate) in tumor-bearing animals causes a marked increase in the tumor uptake of the ^{67}Ga relative to that in the liver, spleen and most other organs except the kidneys and skeleton. With the plasma protein binding sites blocked the ^{67}Ga is rapidly excreted while the tumor uptake is unaffected. Enhanced tumor to non-tumor ratios become maximum by ~ 4 hours, permitting scanning within a few hours of injection. Unfortunately, in human trials Sc produced a brisk intravascular hemolysis. Apparently Sc affects only human erythrocytes.

Our experience with patients has led us to make ^{67}Ga scans a regular part of the routine followup evaluation of patients having Hodgkin's disease and other malignant lymphomas. Perhaps in those patients whose primary tumors show a good concentration of ^{67}Ga the radiotherapist could confine his treatments to known sites of tumor, thus avoiding some of the serious side-effects resulting from total lymph node irradiation.

El-Bassiouni, E. A., Piantadosi, C., and Snyder, F. **Metabolism of alkyldihydroxyacetone phosphate in rat brain.** Biochim. Biophys. Acta 388, 5-11, 1975.

Alkyldihydroxyacetone-*P* is the first detectable product in the biosynthetic pathway for ether-linked glycerolipids that eventually leads to the formation of ethanolamine plasmalogens, a major constituent of myelin. During early postnatal development, the specific activity of $NADPH_2$:alkyldihydroxyacetone-*P* oxidoreductase in microsomes from rat brain is maximum at 4-5 days after birth, the time when the specific activity of the enzymes that synthesize alkyldihydroxyacetone-*P* also peaks. For the oxidoreductase assay, we developed a thin-layer chromatographic method that separates alkyldihydroxyacetone-*P* as the dinitrophenylhydrazine derivative from its reduced product (alkylglycerol-*P*), with excellent resolution.

Phosphohydrolases associated with brain microsomes exhibit optimal pH maximums at 5.2–5.6 and 7.5–7.8 for all three substrates tested – alkyldihydroxyacetone-*P*, alkylglycerol-*P* and alkylacylglycerol-*P*. Alkylglycerol-*P* was most readily dephosphorylated under all experimental conditions. The enzyme(s) that dephosphorylates alkyldihydroxyacetone-*P* and alkylglycerol-*P* have similar properties with respect to Mg^{2+} or EDTA; with both substrates, Mg^{2+} had no effect and EDTA was highly stimulatory. In contrast, EDTA strongly inhibited the dephosphorylation of alkylacylglycerol-*P* and although Mg^{2+} (1 mM) appeared to be required for optimal activity, higher levels inhibited the reaction.

Ellis, J. T., Kraft, L. M., Lushbaugh, C. C., Humason, G. L., Hartroft, W. S., Porta, E. A., Bailey, O. T. Greep, R. O., Leach, C. S. Laird, T., Simmonds, R. C., Vogel, F. S., Dennis, R. L., Brashear, H. R., Talmage, R. V., Harrison, G. A., Corbett, R. L., Klein, G., Tilburg, T., Suri, K., and Haymaker, W. **The effects of cosmic particle radiation on pocket mice aboard Apollo XVII: Appendix III: evaluation of viscera and other tissues.** Aviat. Space Environ. Med. 46, 639-654, 1975.

Histopathological findings in the lungs, livers, bone marrows, small intestines, gonads, kidneys, and other tissues of the four pocket mice (*Perognathus longimembris*) that survived the Apollo XVII flight were evaluated in the light of their immediate environment and as targets of HZE cosmic ray particles. Results of this study failed to disclose changes that could be ascribed to the HZE particle radiation. Decreased numbers of erythropoietic cells in the bone marrow of the flight mice were probably related to the increased oxygen pressure. The small intestine showed no changes. Ovaries and testes appeared normal. Two of the three surviving male flight mice displayed early stages of spermatogenesis, just as ground-based controls did at the same season. Abnormalities were also not found in the thyroid, parathyroids, adrenals, or kidneys. The status of the juxtaglomerular apparatus could not be evaluated. The lungs exhibited nonspecific slight reactions. A variety of incidental lesions were noted in the livers of both the flight mice and their controls. The heart muscle showed nothing that could be regarded as pathological. Sections of skeletal muscle examined were free from significant change.

1028315

Frenkel, J. K., Nelson, B. M., and Arias-Stella, J. **Immunosuppression and toxoplasmic encephalitis.** Hum. Pathol. 6, 97-111, 1975.

Encephalitis developing after prolonged antineoplastic therapy in two patients with Hodgkin's disease and in one with multiple myeloma was found at autopsy to be caused by toxoplasmosis. To better understand the pathogenesis of the brain lesions, ranging from microscopic foci to some having a diameter of 6 cm. and characterized by proliferation of the organisms at the margins of expanding necrosis, an animal model was studied. Similar lesions were produced in hamsters by inducing relapse of chronic latent toxoplasmosis through administration of cortisone, cyclophosphamide, or whole body irradiation, but toxic doses of nitrogen mustard and urethane did not precipitate relapse. Notably, relapsing toxoplasmosis generally involves the brain exclusively, suggesting a special susceptibility related to immune mechanisms. The roles of cells and of antibodies in immune surveillance against this chronic infection in otherwise normal hosts are considered. In man the suppression of cellular immunities by certain antineoplastic agents would seem to be decisive in causing relapse of toxoplasmosis, rather than the replacement of immunologically active cells by neoplasm. Because the infection can be controlled with sulfadiazine and pyrimethamine, a high index of suspicion is essential to detect incipient cerebral toxoplasmosis. Serial serologic testing is helpful by demonstrating titer elevations; however, poor antibody production or transferred antibody may be misleading clinically when single tests are evaluated. Similarly, a poor inflammatory cell response can make it difficult for the histopathologist to detect small lesions in these patients.

Gengozian, N. and Batson, J. S. **Single-born marmosets without hemopoietic chimerism: Naturally occurring and induced.** J. Med. Primatol. 4, 252-261, 1975.

Marmosets have a high frequency of fraternal twinning, and placental vascular anastomoses between the twin fetuses invariably lead to hemopoietic chimerism. The occasional finding of chimerism in single-born marmosets suggested that in a twin pregnancy one fetus had undergone resorption after contributing hemopoietic stem cells to its twin. In this study non-chimeric single-born marmosets were produced by fallopian tube ligation or surgical relocation of one ovary in breeding females. Further, in an examination of hemopoietic cells from over 50 single-born young from nonoperated females, chimerism occurred less frequently than what one would expect if resorption of a co-twin had occurred after a functional anastomosis had been established.

Gengozian, N. and Bazzell, S. J. **Isoimmunizations among marmosets to identify leucocyte antigens.** In: Advances in Medical Primatology, E. I. Goldsmith and J. Moor-Jankowski, eds., Plenum Press (in press).

S. fuscicollis ssp., *S. oedipus,* and *S. nigricollis* marmosets were immunized with white blood cells (WBC) from *S.f. illigeri* marmosets. Of the three genetic donor-host combinations attempted, only an interspecies immunization of *S. oedipus* marmosets resulted in antibody formation which was detectable by agglutination or cytotoxicity tests. Analysis of one of the antisera with a panel of WBC from *S.f. illigeri* marmosets indicated varying specificities, reactions ranging from strong, intermediate, to completely negative. That the serum was detecting genotypic as well as phenotypic markers was suggested by the similarity of reactions of WBC from co-twin pairs and in preliminary testing of individual families.

Gengozian, N., Salter, B. L., Basford, N. L., and Kateley, J. R. **Characterization of the antibody response of the marmoset to sheep red blood cells.** Clin. Exp. Immunol. (in press).

The immune competence of two species of marmosets, *S. fuscicollis* and *S. oedipus*, was evaluated by the intravenous (i.v.) and intramuscular (i.m.) injection of sheep red blood cells. In *S. fuscicollis* marmosets, 1 ml of a 50% suspension yielded titers of hemolysin and agglutinating antibodies equal to or greater than 1 ml of a 10% dose of antigen. In both species, the i.v. route, while resulting in formation of 19S and 7S agglutinins, yielded only 19S hemolysins, even after multiple antigen injections. Repeated i.v. injections resulted in a progressive decrease in peak titers, in contrast to the i.m. route where booster inoculations gave a typical anamnestic response. Jerne plaque-forming cells (PFC) in the spleens of *S. oedipus* marmosets showed predominately 19S plaques after a primary i.v. challenge; only 19S PFC were detected in the spleen of an animal that had been given multiple inoculations, the type of antibody produced reflecting that found in the serum. 19S but not 7S hemolysins of both species were sensitive to heating at 56°C for ½ hr. The serum titers and splenic PFC data from the marmosets suggest these animals, particularly *S. oedipus*, respond poorly to sheep RBC when a comparison is made to similar studies in mice and rats.

Gengozian, N., Smith, T. A., and Gosslee, D. G. **External uterine palpation to identify stages of pregnancy in the marmoset, Saguinus fuscicollis ssp.** J. Med. Primatol. 3, 236-243, 1974.

External palpation of the uterus has provided a simple yet relatively accurate means of identifying pregnant marmosets (*S. fuscicollis ssp.*) in different stages of gestation. Estimates of uterine size and their relation to days to delivery have allowed us to formulate an equation which now permits prediction of parturition time. The data have also provided an estimate of a gestation period of approximately 159 days in the marmoset.

Gibbs, W. D., Edwards, C. L., Hodges, H. D., and Cloutier, R. J. **Measurement of temporal changes in whole-body radionuclide distribution with a profile scanner.** In: Dynamic Studies with Radioisotopes in Medicine, 1974, International Atomic Energy Agency, Vienna, 1974, pp. 75-84.

Our linear scanner has been instrumented to acquire data in digital form that are entered easily into a computer for quantitative analysis of linear distribution of activity and whole-body retention. The instrumentation consists of an external amplifier, a single-channel analyzer, a multichannel analyzer operated in multiscale mode, and paper tape punch. When a scan table speed of 8 in/min (other speeds are available) and analyzer timing of 3 s/channel (also variable) are used, a total-body linear scan requires 10 min and 200 channels of analyzer memory. Each channel thus represents about 1 cm of body length. A

1028316

camera attached to the multichannel analyzer oscilloscope provides a record of the linear scan. The linear scan data stored in the multichannel analyzer are transferred to paper tape. With information on patient identification, anatomical landmarks, etc., the scan data are entered into the computer and stored on magnetic tape. Our computer analysis program calculates the amount of activity in the whole-body and the fraction of activity in each of eight sections of the body defined by the landmarks. The program also can be used to compare the results with earlier scans and calculate changes of distribution with time.

Goh, K-o. **Exogenous deoxyribonucleoside triphosphates II. Their effect on pulverized human chromosomes at the S phase.** Proc. Soc. Exp. Biol. Med. 145, 938-943, 1974.

Sixteen peripheral blood leukocyte cultures were made from a patient with pulverized chromosomes. To 15 of his cultures, different and combinations of different deoxyribonucleotides were added 24 hr (S phase) before termination of the cultures. No deoxyribonucleotide was added to the sixteenth culture (experimental control). Seventy-nine of the one-hundred metaphases from the experimental control and 217 of the 1500 metaphases from the experimental cultures were pulverized. Most pulverized chromosomes in the experimental cultures belonged to two cultures. The nucleotides added were dATP and dCTP in one and dGTP and TTP in the other. The mitotic rates were within normal range in all cultures. This S phase experiment confirmed our G_2 phase experiment in that exogenous deoxyribonucleoside triphosphates can restore pulverized chromosomes. However, in two S phase experimental cultures, further pulverization was seen. This is thought to be due to the imbalance of the metabolic end-products of these nucleosides.

Goh, K-o. **Total-body irradiation and human chromosomes. IV. Cytogenetic follow-up studies 8 and 10½ years after total-body irradiation.** Radiat. Res. 62, 364-373, 1975.

Chromosomal breaks and abnormal chromosomes were found in the peripheral blood and bone marrow cultures from six persons who were accidentally exposed to total-body irradiation 8 and 10½ yr previously. These type of abnormalities were found in these same patients by Bender and Gooch 2½ yr and 3½ yr after the radiation exposure and by us 7 yr after the accident. The frequencies of the abnormalities remain higher than the normal controls. There is good evidence that there is an increased incidence of cancer and leukemia among populations exposed to radiation. It has been suggested that chromosomal aberrations are involved in the development of some cancer or leukemia in irradiated persons. However, none of our patients has these diseases. It is possible that the development of clinical cancer or leukemia (phenotype) depends not only on the presence of abnormal cells (genotype), but also, on factors that give advantage to these abnormal cells.

Goh, K-o. **Total-body irradiation and human chromosomes. V. Additional evidence of a transferable substance in the plasma of irradiated persons to induce chromosomal breakages.** J. Med. 6, 51-60, 1975.

A substance (or substances) produced or activated by total-body irradiation in man and capable of affecting the chromosomes for an extended time has been postulated. Leukocytes from normal persons were cultured with autologous plasma, homologous plasma or serum from patients irradiated 10.5 years previously. Thirty (5.5%) chromosomal breaks were found in the 550 metaphases from the control cultures, 152 (13%) in the 1,175 metaphases from the irradiated plasma cultures and 130 (12%) in the 1,100 metaphases from the irradiated serum cultures. This finding confirms and extends our previous observation that plasma from total-body irradiated persons in capable of inducing chromosomal breakage in normal leukocytes even 10.5 years after the irradiation.

Goswitz, F. **Radioactive vitamin B_{12} absorption tests and other studies.** In: Golden's Diagnostic Radiology, Section 20, Diagnostic Nuclear Medicine, A. Gottschalk, J. L. Quinn, and E. J. Potchen, eds. Williams and Wilkins (in press).

The greatest advantage of the ^{57}Co-B_{12} absorption test and related tests evaluating B_{12} metabolism is in the evaluation of patients with neurologic symptoms affecting primarily the posterior column and in analysing hematologic changes which may first manifest themselves by macrocytic red cells.

Greenlaw, R. H., Weinstein, M. B., Brill, A. B., McBain, J. K., Murphy, L., and Kniseley, R. M. **^{67}Ga-citrate imaging in untreated malignant lymphoma: Preliminary report of cooperative group.** J. Nucl. Med. 15, 404-407, 1974.

An interinstitutional cooperative study has been undertaken to evaluate ^{67}Ga as a tumor-localizing agent. A uniform protocol and computer handling of data have been used. In 167 cases of previously untreated lymphoma (other than Hodgkin's disease) scanned with ^{67}Ga, approximately 78% had one or more positive sites demonstrated on scan. Of histologically proven sites, 51% had a positive scan and with those sites apparent or suspected but not biopsied, 54% were positive. Thus a negative scan does not exclude the presence of disease. A low "false-positive" rate was encountered; if a lesion is demonstrated by scan, it is highly likely that disease is present. Disease, unsuspected or believed absent, was first known at the time of scan in 28 sites. An additional 42 positive scan sites as yet unconfirmed by other evidence may also represent lesions discovered de novo by ^{67}Ga. The percent of positive scans is highest in the thorax. Histiocytic types, including mixed cell types, of lymphoma have higher rates of positive scans than lymphocytic types. Overall, the rate of positive scans is lower in lymphoma than in Hodgkin's disease.

Hayes, R. L. **Factors affecting uptake of radioactive agents by tumor and other tissues.** In: Proceedings of IAEA Advisory Group Meeting on Tumor Localization with Radioactive Agents, Vienna, December 1974 (in press).

The subject of factors that affect the uptake of radioactive agents by tumor and other tissues is a broad and complicated one, but one that does strike at the heart of these discussions. Factors that may have considerable bearing on the relative affinity of tumor tissue for such agents are, in general, the following: (1) vascularity, blood flow, and interstitial fluid space; (2) uptake of

1028317

a protein-bound form of the agent; (3) cell proliferation; (4) presence of carrier; (5) capillary and cell permeability; (6) presence of inflammation; and (7) pH. Certain tumor-localizing processes are based on the specific properties of the tissues in question, i.e., radioiodine for functioning thyroid carcinoma, bone seekers for bone lesions, etc., but there is as yet still no clear indication of the basic process(es) involved in the tumor-localization of agents such as ^{67}Ga and others on which to base projections as to new materials that might be more specific for tumor tissue in general.

Hayes, R. L. and Brown, D. H. **Biokinetics of radiogallium.** Proceedings of the International Meeting of the Society of Nuclear Medicine, Munich, West Germany, September 1974 (in press).

Gallium-67 would seem to be unique in many ways as a diagnostic agent, since it not only can be used as such for detection of cancer, but can also be of potential use in a number of other fields as well. An understanding of the biokinetics of this element, because of its diverse in vivo involvement in physiologic processes, may lead to the development of more sophisticated uses for this agent in nuclear medicine and in other fields as well.

Hodges, H. D. **Applications and availability of whole-body counters to the nuclear medicine technologist.** J. Nucl. Med. Technol. 2, 102-104, 1974.

The responsibility of nuclear safety is a part of each individual's daily routine. Even though precautionary measures are observed, there is always the possibility that radioactive contamination to some hospital personnel can occur. This paper briefly reviews some of these aspects and suggests the potential role of low-level whole-body counters to help eliminate some of the problems.

Hodges, H. D., Gibbs, W. D., Morris, A. C., Jr., and Coffey, W. C., II. **An improved high-level whole-body counter.** J. Nucl. Med. 15, 610-612, 1974.

This whole-body counter is relatively inexpensive to build and provides quantitative whole-body retention values from 0.01 to 100 mCi. Two NaI(Tl) detectors suspended from the ceiling are positioned so that a point source anywhere on the bed gives a uniform counting response (±5%). Subjects are counted on a floor-level pad 1.9 meters under the detectors. The pad is hinged and can be fastened flat against the wall of the counting room when not in service. Optimum-window counting techniques provide a highly quantitative response regardless of the distribution of activity within the subject. A 1-min count of a person with more than 0.1 mCi results in an expected standard deviation of less than ±5%; 10 min are required for similar statistical results in the 0.01-0.1 mCi range.

Hodges, H. D., Morris, A. C., Jr., and Gibbs, W. D. **Correlation of thyroid uptake with whole-body retention measurements.** J. Nucl. Med. Technol. 2, 72-75, 1975.

The use of conventional thyroid measurement is not practicable in a number of clinical situations. We investigated the correlation of 48-hr whole-body retention to thyroid uptake results at 4, 24, and 48 hr after administration. The 48-hr count gives the best results, confirmed in studies on 69 patients. Equations computed to fit our data show high levels of confidence with administered doses as low as 50 nCi. The technique can be used in infants, in pregnant women when there is a massive goiter, or in other clinical situations in which a conventional thyroid measurement is contraindicated or technically unsatisfactory.

Hübner, K. F. and Littlefield, L. G. **Burkitt lymphoma in three American children.** Am. J. Child. Dis. 129, 1219-1223, 1975.

The clinical aspects and cytogenetic findings of Burkitt lymphoma in three American children are discussed. The great variability of the clinical manifestations and importance of early diagnosis are emphasized.

Hübner, K. F., Gengozian, N., and Gilman, T. A. **Suppression and recovery of the immune response and CFU potential of mice during and after chronic low-dose-rate irradiation.** Int. J. Radiat. Biol. (in press).

Chronic exposure of mice to ^{60}Co γ rays at rates of 1.6 R/hr and 6 R/hr leads to profound immunosuppression as demonstrated with the Jerne hemolysis-in-agar technique. The immunosuppressive effect is dose-rate dependent. Thus, significant reduction of the number of splenic antibody plaque-forming cells (PFC) to sheep red blood cells is seen after exposure to 1005 R at 1.6 R/hr; at the higher exposure rate of 6 R/hr, PFC suppression is seen after a total dose of only 468 R. Although recovery of the PFC response is possible after accumulated doses of 1005, 1579, and 2138 R at 6 R/hr and 5256 R at 1.6 R/hr, a return to normal immunologic capacity was not obtained for a period of 120 days postirradiation. In contrast, recovery of splenic colony-forming units (CFU) as expression of the hematological potential of chronically irradiated mice is found to be much more rapid than the repair of the immune system. Thus, 10 days postirradiation, at a time when little or no recovery in immune competence is apparent, the spleen of the chronically irradiated animal has a CFU capacity approximately 50-fold that found in the spleen of a normal, unirradiated mouse. The CFUs in the chronically irradiated spleens were also shown to be functional by virtue of their ability to protect lethally irradiated animals from hemopoietic death.

Johnston, G., Benua, R. S., Teates, C. D., Edwards, C. L., and Kniseley, R. M. **^{67}Ga-citrate imaging in untreated Hodgkin's disease: Preliminary report of cooperative group.** J. Nucl. Med. 15, 399-403, 1974.

An interinstitutional cooperative study has been undertaken to evaluate ^{67}Ga as a tumor-localizing agent. A uniform protocol and computer handling of data have been used. In 151 untreated patients with Hodgkin's disease, approximately 90% had one or more positive sites demonstrated on scan. Of histologically proven or clinically evident sites of tumor known by means other than scanning, 73% were positive. A "false-positive" rate of less than 5% was discovered. Of 249 lesions proven histologically or obviously apparent by other

1028318

means, 21% were first detected by ^{67}Ga, although the sequence of procedures in the patient's workup may have some influence on this number. The percentage of positive scans is highest in the neck and chest. Gallium-67 imaging appears to have a place in staging Hodgkin's disease but a negative scan does not exclude the presence of disease. In comparison with lymphangiography it appears to be slightly more sensitive but each of these procedures found lesions missed by the other.

Kateley, J. R. and Gengozian, N. **Rat-mouse radiation chimeras: Characterization of an antibody-mediated graft-vs-host reaction.** J. Immunol. 114, 125-132, 1975.

A modification of the Jerne hemolysis-in-agar technique was used to demonstrate antibody-mediated graft-vs-host (GvH) hemolytic activity in lymphoid tissues of rat-mouse radiation chimeras. Initial foci of hemolytic activity were detected on the 3rd day in spleens of lethally x-irradiated mice infused with rat spleen cells (950 R-RSp chimeras). The peak GvH foci response occurred near the end of the 1st week when 70% of 950 R-RSp chimera spleens examined contained an average of 18 to 21 foci per spleen. GvH hemolytic activity was also detected in mice reconstituted with rat bone marrow (950 R-RBM chimeras); however, only 40% of these chimeras contained splenic foci (nine foci per spleen) on day 8, the peak day of response. Mesenteric lymph nodes from 950 R-RSp chimeras, but not 950 R-RMB chimeras, also contained GvH hemolytic foci. Specificity experiments demonstrated that the GvH hemolytic reaction could be inhibited with rabbit anti-rat IgM serum and host ($C3BF_1$) antigenic cell fragments. Indirect (IgG) hemolytic activity was also observed in spleens of 950 R-RSp chimeras 6 to 10 days after treatment. At least 90% of indirect foci appeared coincident with direct foci. Rat IgM and IgG containing cells beneath hemolytic foci were observed by combining the fluorescent antibody and foci techniques. To determine whether the foci response reflects an in vivo sensitization of donor cells, spleen cell transfer experiments were done. The results indicate that spleen cells from 950 R-RSp chimeras were more effective in accelerating mortality and anemia and induced a higher incidence of Coombs' positive red cells in lethally irradiated isogeneic recipients than spleen cells from 950 R-RBM chimeras; spleen cells from either chimera were more effective in accelerating GvH indices than spleen and marrow cells from normal rats.

Lee, T-c., Stephens, N., and Snyder, F. **Dietary effects on stearyl coenzyme A desaturase of Morris hepatomas.** Cancer Res. 34, 3270-3273, 1974.

In the present study, we determined the effect of dietary changes on the activity of stearyl coenzyme A desaturase in two transplantable Morris hepatomas (5123C and 7777) and their host livers; the activity of fatty acid synthetase was also measured in the same preparations. Stearyl coenzyme A desaturase activities of the hepatomas were increased, whereas the fatty acid synthetase activities remained the same when tumor-bearing rats maintained on laboratory chow were fasted for 48 hr and refed for 48 hr. Similar results were obtained when rats were fed a fat-free diet for 2 months before transplanting the hepatomas and then continued on the same diet for 1 month (7777 tumors) or 2 months (5123C tumors). We conclude that acyl coenzyme A desaturase is regulated in hepatomas as it is in normal liver, and therefore it does not exhibit the lack of regulation that is characteristic of the enzymes that synthesize fatty acids and cholesterol in hepatomas.

Lee, T-c., Blank, M. L., Piantadosi, C., Ishaq, K. S., and Snyder, F. **Incorporation and subcellular distribution of an unnatural phospholipid base-analog, N-isopropylethanolamine, in rat liver.** Biochim. Biophys. Acta 409, 218-224, 1975.

An unnatural phospholipid, phosphatidyl-N-isopropylethanolamine, was isolated from rat liver after intraperitoneal injections of N-isopropylethanolamine; it was identified on the basis of enzymic, chemical, and chromatographic analyses. Although this phospholipid was formed at the expense of phosphatidylcholine and phosphatidylethanolamine, its fatty acid composition did not resemble either of these lipids. Microsomes, mitochondria, and plasma membranes contained significant amounts (up to 9%) of this unusual phospholipid. Radioisotope incorporation experiments suggest that the N-isopropylethanolamine containing phospholipid is rapidly equilibrated between microsomes and mitochondria and more slowly with surface membranes.

Littlefield, L. G. and Mailhes, J. B. **Comparison of chromosome breakages in lymphocytes and fibroblasts from control women and women taking oral contraceptives.** Fertil. Steril. 26, 828-832, 1975.

To determine whether exposure to synthetic hormones resulted in increased chromosome damage in cells other than lymphocytes, we evaluated series of lymphocyte and fibroblast cultures from five control women and five women taking oral contraceptives (OC). The results of this study showed: (1) no difference in chromosome breakages between lymphocytes and fibroblasts; (2) no differences in breakages in replicate fibroblast cultures exposed to fetal calf serum, autologous serum, homologous serum, or homologous serum from OC users; (3) no difference in breakages between lymphocyte and fibroblast cultures from OC users, compared with lymphocyte and fibroblast cultures from control women; and (4) no increase in the frequency of cytogenetically aberrant stem-lines in fibroblast cultures from women taking OC. These findings suggest that synthetic hormones do not cause increased chromosome breakages in fibroblasts from women taking OC.

Littlefield, L. G. and Mailhes, J. B. **Observations of de novo clones of cytogenetically aberrant cells in primary fibroblast cell strains from phenotypically normal women.** Am. J. Hum. Genet. 27, 190-197, 1975.

In a recent study of chromosome breakage frequencies in 36 primary fibroblast cell strains derived from skin from 10 phenotypically normal women, we observed seven different clones of cells having consistent chromosomal abnormalities. Five of the stem lines were noted in cultures from "control" women and two in fibroblasts from women taking oral contraceptives. We observed aneuploid clones as well as stem lines bearing structural abnormalities (e.g., translocations, inversions). The various aberrant clones were found in cultures ranging in age from 41 to 144 days and comprised varying percentages of the cell populations ranging from 0.8% to virtually 100%. The possible evolution in culture of clones of cells having aberrant

1028319

karyotypes should be considered in interpreting findings from fibroblast cultures initiated for clinical evaluations.

Littlefield, L. G. and Vodopick, H. A. **Cytogenetic studies in a patient with acute granulocytic leukemia of 7½ years duration.** Blood 46, 783-789, 1975.

During a 7½-yr period we monitored a chromosomally aberrant cell-line in a woman with actue granulocytic leukemia whose disease followed a rather unusual course. Her initial remission induced with 6-mercaptopurine (6-MP) and prednisone was maintained for 52 months with biweekly doses of methotrexate (MTX) given orally. Because signs of liver dysfunction occurred, maintenance therapy was stopped. After 15 mo without chemotherapy she suffered her first relapse (5 yr and 7 mo after the initial diagnosis). A second remission, again induced with 6-MP and prednisone, was maintained for 1 yr after which a second relapse occurred. Another remission lasting for only 4 mo was followed by a relapse of the leukemic process which led to her death. Cytogenetic studies of marrow cells and peripheral blood at the time of her initial diagnosis showed abnormal stem-lines with characteristic chromosome markers. During the years of her prolonged remission a small percentage of malignant cells bearing these markers persisted in her marrow, but at the time of her death comprised virtually 100% of her marrow population.

Littlefield, L. G., Lever, W. E., Miller, F. L., and Goh, K-o. **Chromosome breakage studies in lymphocytes from normal women, pregnant women, and women taking oral contraceptives.** Am. J. Obstet. Gynecol. 121, 976-980, 1975.

Cytogenetic studies of 977 lymphocyte cultures from ten normal men and 54 normal women have shown: (1) variations in the frequencies of chromosome breakages among consecutive cultures from the same person and among cultures from different people, indicating that many extraneous factors may affect lymphocyte chromosomes, and (2) significantly higher average breakages in cultures from nulligravidas taking oral contraceptives (OC) and in pregnant women who had had previous pregnancies than in nulligravidas who had never taken the pill. The increase in breakages in cultures from the OC users was not observed in all cultures and could not be correlated with length of time on pill or stage of the pill cycle, suggesting that synthetic hormones do not directly damage lymphocyte chromosomes. Since estrogens and progestogens are known to affect many metabolic and biochemical systems, it is possible that these substances may induce in OC users and pregnant women a slightly altered in vivo condition in which increased chromosome breakages may be expressed.

Lushbaugh, C. C. and Swartzendruber, D. C. **Histopathologic aspects of radiation effects upon lymphatic tissues and malignancies.** In: Proceedings of the 14th Annual Hanford Biology Symposium, Hanford, Washington, October 1974 (in press).

Morphologic study using the light microscope remains our most facile and rapid means of tissue identification, diagnosis and staging of diseases and the demonstration of radiation-induced and other toxic effects. The inadequacy of its use alone, however, for the solution of biologic problems is nowhere better illustrated than in such studies on lymphatic tissues as are being reported in this symposium. Nearly every classical concept concerning lymphocyte biology and disease derived by morphologic methods has been challenged or disproven in recent years by applications of non-morphologic technologies. Studies using light and electron microscopy in combination with cell-labeling techniques, histochemical methodology, virology, immunology, and radiation biology, have corrected many of our misconceptions and provided unifying concepts of lymphatic tissue structure and function that explain anew our observations of the past. For example, nearly everyone now accepts the biologic role of viruses in what once were considered radiation-caused neoplasms in rodents, although whether the role of radiation and other physical and chemical insults in human carcinogensis is direct or indirect is still to be elucidated. Also, the exact relations that obtain between radiation and cancer induction via viruses even in well-studied rodent systems remain to be determined and here morphologic studies continue to play an important integrating role for the multidisciplinary studies that are required.

Mainwaring, H. R., Swartzendruber, D. C., Lushbaush, C. C., Idoyaga-Vargas, N. L., and Watson, E. E. **Radiation effects on ^{67}Ga retention and distribution in mouse tissues.** In: Proceedings of the 14th Annual Hanford Biology Symposium, Hanford, Washington, October 1974 (in press).

We have studied the effect of external X-irradiation on the whole-body retention and tissue distribution of gallium-67 (^{67}Ga) in mice over a period of 20 days. Adult male C57BL/6 Cum mice were injected intraperitoneally with 5 μCi ^{67}Ga 24 hr before, immediately before, immediately after, or 24 hr after whole-body X-ray exposure to 450 R. Control mice received ^{67}Ga injections and sham irradiation. Increased excretion and reduced soft-tissue concentrations of ^{67}Ga occurred in irradiated mice. The greatest effect was observed when the radionuclide was injected 24 hr after irradiation and assayed 24 hr later. An analysis of variance of the retention data showed that the differences between the groups were significant at the 99% confidence level.

Exponential least-squares analysis of whole-body counting results revealed three compartments in the biological retention data. The long-lived compartment possibly corresponds to ^{67}Ga bound relatively tightly in certain body tissues and lost slowly. The two remaining compartments have been tentatively identified as circulating unbound ^{67}Ga that is lost rapidly, and circulating bound ^{67}Ga that is lost less rapidly.

Morris, A. C., Jr. **Advances in nuclear-medical instrumentation.** Cadence (in press).

Recent advances in nuclear-medical instrumentation have improved the diagnosis and treatment patients receive. This progress includes more sensitive detectors, improved electronic circuitry, faster data processing, and better readout imaging methods. Nowadays, such advances in instrumentation are being sought by both the medical and scientific professions, plus a very competitive nuclear-medicine industry. This lively search for better technical capability has resulted in new developments reaching the user's hands in very short periods of time. Recent instrument successes are centered largely around four major areas: (1) probe counters for ventilation and dynamic function studies; (2) high-

1028320

sensitivity rectilinear scanners for whole-body and area scans; (3) gamma cameras with improved spatial resolution and counting-rate specifications; and (4) new data recording and analysis capabilities.

Future possibilities in instrumentation for nuclear medicine can now only whet the imagination. These most certainly will include: (a) general incorporation of semiconductor detectors, with their improved energy resolution, into scanning devices; (b) scanners and cameras that can give planar cross-sections of a patient's activity distribution; (c) improved computer programs for the technical augmentation of scan quality; (d) increasing numbers of nuclear-counting instruments with some built-in computing capability; (e) faster gamma-cameras for better imaging of cardiac and brain-blood-flow studies; (f) other exotic instruments such as xenon cameras with an inherent resolution of 1.0 mm or less.

The present accomplishments in nuclear-medical instruments are successes in which we can all take justifiable pride — new concepts now coming into sight promise a future of mind-boggling innovation.

Morris, A. C., Jr., Goswitz, F. A., Kim, K. H., McDow, A. E., Jr., Aaron, P. A., and Barclay, T. R. **Interface for direct online computer recording of dynamic radionuclide data.** Med. Biol. Eng. 12, 367-369, 1974.

This paper describes the development of a ratemeter-digital interface to transmit data from a 4 probe external counting system to an IBM 1800 computer and from the computer to a 4 channel ratemeter at the site of data origination. This system is useful in collecting and analysing dynamic radioisotope data such as in iron kinetics or kidney function studies.

Nelson, B. and Fliedner, T. M. **Lymphocytopoiesis after irradiation.** In: Proceedings of a Symposium on Cellular Aspects of the Effects of Irradiation with Low Doses, Using the Lymphatic System as an Example. Vienna, April 1973, Springer-Verlag (in press).

1. Lymph nodes of dogs kept alive 10 days after exposure to 1200 rads are devoid of germinal centers and mitoses, and are greatly depleted of the small round cells called lymphocytes.

2. Varying degrees of a lymph-node regeneration are seen if such dogs are given mononuclear blood cells shortly after irradiation.

3. The pattern of lymph node regeneration is consistent with participation of two types of lymphocytes: thymus-derived "T cells" and thymus-independent "B cells", although this is a conjecture requiring confirmation in the dog.

4. This dog model could be adapted for characterizing separated subpopulations of blood lymphocytes, with the goal of developing clinical methods for restoring immunopoietic and hemopoietic functions. It could also be used for determining the radiosensitivity of subpopulations of mononuclear blood cells, which is surely pertinent to the theme of this session. (*Cellular Aspects of the Effects of Irradiation with Low Doses, Using the Lymphatic System as an Example*).

Niblack, G. D. and Gengozian, N. **Identification of T and B lymphocytes in marmosets.** Clin. Exp. Immunol. (in press).

The thymus-derived (T) lymphocyte and bone marrow-derived (B) lymphocyte populations of the marmoset were characterized using specific cell surface markers. Approximately 85% of the thymocytes formed rosettes with neuraminidase-treated sheep erythrocytes (E_n). The percentage (~69%) of peripheral blood lymphocytes (PBL) forming rosettes with E_n was the same as that which stained with fluorescently labeled goat anti-marmoset thymocyte serum (ATS). These two assays identified the same cell population since treatment of cells with ATS and complement resulted in a concomitant decrease in E_n rosette formation. Marmoset PBL also formed rosettes with human erythrocytes sensitized with antibody and complement (HEAC); since the percentage (~20%) HEAC rosette was the same as that of cells stained with fluorescently-labeled goat anti-marmoset IgG, these cells were considered to be B cells. A small percentage of cells (~1.5%) possessed both types of receptors. The mean percentages of T and B cells present in PBL of single-born, presumably non-chimeric animals, were the same as that of isosexual and heterosexual chimeras.

Poggenburg, J. K. and Hayes, R. L. **Radiopharmaceuticals for function studies present and future.** In: Dynamic Studies with Radioisotopes in Medicine, 1974, International Atomic Energy Agency, Vienna, 1974, pp. 85-96.

The paper reviews present radiopharmaceutical agents and techniques in function studies and attempts an assessment of future developments in this field. Rapid and slow function studies each have their own demands and accordingly are treated separately. Advances in instrumentation and computational techniques, coupled with new production capabilities now coming into existence, can be expected to play important roles. Potassium-43 appears to have particular promise as a myocardial scanning agent. Iodine-123 and ^{97}Ru will undoubtedly find increasing use, and short half-life positron emitters and spallation-produced radionuclides should find increased use in dynamic studies in nuclear medicine and in clinical research applications.

Rock, C. O. and Snyder, F. **A short-chain acyl CoA:1-alkyl-2-acyl-*sn*-glycerol acyltransferase from a microsomal fraction of the rabbit harderian gland.** Biochim. Biophys. Acta 388, 226-230, 1975.

The 3-position of the alkyldiacylglycerols from the pink portion of the rabbit harderian gland is occupied exclusively by isovaleric acid. We describe a microsomal 1-alkyl-2-acyl-*sn*-glycerol acyltransferase from this gland which specifically incorporates short-chain acyl-CoA's into the 3-position of alkylacylglycerols. The enzyme is most active in the presence of CoA esters with chain lengths similar to isovaleric acid and is inactive in the presence of acetyl CoA and long-chain acyl-CoA's. No evidence was found for an enzyme that would transfer long-chain acyl-CoA's to the same substrate. The specificity of this acyltransferase can account for the exclusion of long-chain acyl moieties from the 3-position of the alkyldiacylglycerols in the harderian gland of rabbits.

1028321

Rock, C. O. and Snyder, F. **Biosynthesis of 1-alkyl-*sn*-glycero-3-phosphate via adenosine triphosphate: 1-alkyl-*sn*-glycerol phosphotransferase.** J. Biol. Chem. 249, 5382-5387, 1974.

The pink portion of the rabbit harderian gland was found to be a rich source of ATP:1-alkyl-*sn*-glycerol phosphotransferase. The enzyme, associated with the microsomal fraction of the gland, exhibits a pH optimum of 7.1. ATP and Mg^{2+} are absolute requirements for enzymatic activity; the apparent K_m for Mg^{2+}ATP is 1.6 mM. Manganese partially substituted for Mg^{2+}, but the measured rate in the presence of Mn^{2+} was only one-half of the rate determined in the presence of Mg^{2+}. We identified the product of the reaction by chromatographic, chemical, and enzymatic techniques as 1-alkyl-*sn*-glycero-3-phosphate. In incubations supplemented with coenzyme A, 1-alkyl-2-acyl-*sn*-glycero-3-phosphate was identified as the major product.

High levels of monoacylglycerols, acylglycerols, and their alkyl analogs did not inhibit phosphorylation of alkylglycerols. Attempts were made to show the phosphorylation of monoacylglycerols, but due to the high lipase activity in harderian gland microsomes, the results were inconclusive.

The transfer of the terminal phosphate of ATP to position 3 of a 1-alkyl-*sn*-glycerol was stereoselective; other isomeric alkylglycerols, as well as alkylethylene glycol and *S*-alkylglycerol were not phosphorylated by this system. These data demonstrate a way in which 1-alkyl-*sn*-glycerols may enter the biosynthetic pathway that forms complex ether-linked glycerolipids.

Rock, C. O. and Snyder, F. **Rapid purification of phospholipase A_2 from Crotalus adanamteus venom by affinity chromatography.** J. Biol. Chem. 250, 6564-6566, 1975.

We have used alkyl ether analogs of ethanolamine and choline phospholipids as ligands to purify phospholipase A_2 (EC 3.1.1.4) from *Crotalus adamanteus* venom by affinity chromatography. One of the affinity columns was prepared with *rac*-1-(9-carboxy)nonyl-2-hexacylglycero-3-phosphocholine linked to -Sepharose 4B via the carboxyl group. Specific adsorption of phospholipase A_2 to this column was achieved in buffer containing Ca^{2+}, and the enzyme was eluted in buffer containing EDTA. The two enzymes from this venom were prepared in good yield (>90%), and were homogeneous as judged by polyacrylamide gel electrophoresis. Retention of phospholipase A_2 did not occur when the initial irrigant was devoid of Ca^{2+}. These results support the compulsory ordered mechanism for this enzyme proposed by Wells (1972), *Biochemistry* 11, 1030-1041) on the basis of kinetic considerations. The second affinity support was prepared with 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine attached through the amine moiety to CH-Sepharose 4B. Specific adsorption of phospholipase A_2 to this column did not occur. These data indicate that the phospholipid base group must be accessible to the enzyme for optimal binding, and that modifications in the alkyl side chains are more desirable when designing affinity matrices for purification of enzymes involved in phospholipid metabolism.

Rock, C. O. and Snyder, F. **Metabolic interrelation of hydroxy-substituted ether-linked glycerolipids in the pink portion of the rabbit harderian gland.** Arch. Biochem. Biophys. (in press).

The pink portion of the rabbit harderian gland is known to contain a preponderance of ether-linked glycerolipids consisting primarily of 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols and smaller amounts of 1-alkyl-2,3-diacyl-*sn*-glycerols. In the present study, we have used a combination of chemical, enzymatic, and chromatographic techniques to identify two minor lipid components in the gland as 1-hydroxyalkyl-2-acyl-*sn*-glycerols and 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols. The long-chain acyl groups occurring in the 1-hydroxyalkyl-2-acyl-*sn*-glycerols and 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols are almost exclusively hexadecanoic acid, whereas the 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols have a ratio of hexadecanoic acid to octadecanoic acid of 2/1. The 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols and the 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols also contain a short-chain acyl moiety identified as 3-methylbutanoic acid (isovaleric acid). This acid was found to occupy the 3-position of the glycerol backbone in these lipid classes.

Metabolic experiments demonstrate that 3-methylbutanoic acid in the lipids of the gland is derived from the catabolism of L-leucine. Pulse-chase data show a precursor-product relation between the 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols and 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols as a possible biosynthetic route to the 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols.

Characterization of the alkyl and acyl groups and the positional distributions of the acyl moieties in combination with the metabolic information indicated the acylation sequence involved in the formation of 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerol is 1-hydroxyalkyl-2-diacyl-*sn*-glycerols → 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols → 1-(*O*-acyl)-hydroxyalkyl-2,3-diacyl-*sn*-glycerols. The data also suggest that hydroxylation of the alkyl side-chain occurs before or at the alkylacylglycerol stage.

Snyder, F. and Malone, B. **Acyltransferases and the biosynthesis of pulmonary surfactant lipid in adenoma alveolar type II cells.** Biochem. Biophys. Res. Commun. 66, 914-919, 1975.

We have examined the in vivo labeling of lipids after a single intraperitoneal injection of the carcinogen, [^{14}C]dimethylnitrosamine, into rats. Liver was most active in incorporating [^{14}C]-methyl groups into lipids (0.91% of the injected dose) and 80% of the activity appeared in *sn*-3-phosphatidylcholine. Chromatographic analysis of the products (and derivatives) formed after treatment of the [^{14}C]phosphatidylcholine with phospholipase A_2 (EC 3.1.1.4) and phospholipase C (EC 3.1.4.3) demonstrated that 89% of the radioactivity was in the choline moiety. These results indicate the transfer of methyl groups to lipids occurred via the lipid methylation pathway that converts phosphatidylethanolamine to phosphatidylcholine.

Snyder, F., Malone, B., and Piantadosi, C. **Enzymic studies of glycol and glycerol lipids containing *O*-alkyl bonds in liver and tumor tissues.** Arch. Biochem. Biophys. 161, 402-407, 1974.

The utilization of [1-^{14}C]hexadecyl-[2-^{3}H]ethyleneglycol and [1-^{14}C]hexadecyl-[2-^{3}H]glycerol as substrates for acyltransferase, phosphotransferase, phosphorylcholine, and phosphorylethanolamine transferase, and *O*-alkyl cleavage activities in cell-free preparations from normal rat liver and preputial gland tumors of mice was investigated. Our studies demonstrate

1028322

that alkylethyleneglycols, like alkylglycerols, can serve as substrates for acyltransferases in both the liver and tumor microsomes; the product alkylacylethyleneglycerol can be readily deacylated by pancreatic lipase. A polar lipid was formed from the alkylethyleneglycol by the tumor homogenates in the presence of ATP and Mg^{2+}; although the small quantities formed precluded absolute identification, its thin-layer chromatographic behavior in acidic and basic solvent systems indicated that a free phosphate group was present. As expected, phosphorylbase transferases in these preparations did not utilize either the alkylethyleneglycol or alkylglycerol as substrates. The *O*-alkyl moiety of hexadecylethyleneglycol was oxidized to hexadecanal by a tetrahydropteridine-dependent cleavage enzyme in rat liver microsomes, whereas in the tumor microsomes this activity was not present. We conclude that alkylethyleneglycols are metabolized in a manner similar to alkylglycerols and perhaps by identical enzymes.

Tankersley, W. G. and Woodward, J. M. **Induction and isolation of a minicell-producing strain of Salmonella typhimurium.** Proc. Soc. Exp. Biol. Med. 145, 802-805, 1974.

A mutant of *S. typhimurium* which produces small spherical anucleate bodies (minicells) was induced and isolated after treatment of a normal strain of *S. typhimurium* with increasing amounts of triethylenemelamine (TEM).

No differences were observed in growth characteristics or colonial morphology between the minicell-producing strain and the normal strain of *S. typhimurium*. The minicells were shown to be approximately one-tenth the size of normal cells of *S. typhimurium* and were easily isolated from the larger parent cells by a combination of differential and density-gradient centrifugations. Purified minicell preparations contained about 0.007 the amount of DNA per cell as normal cells. They utilized glucose linearly with time, and were serologically indistinguishable from normal cells.

Tyndall, R. L. and Daniel, J. C., Jr. **Alterations in uterine and serum esterases in pregnant mammals.** Fertil. Steril. (in press).

Uterine flushings, endometrial extracts and serum from estrous and pregnant rabbits were analyzed by acrylamide gel electrophoresis for esterase activity. Two bands of enzyme activity in uterine flushings were detectable on days four through seven after coitus. No activity was detectable in washings from nonpregnant or pregnant animals on days 1-3 of pregnancy. Esterase activity was detectable in endometrial extracts of all rabbits. The intensity and complexity of the esterase activity markedly increased on day three and persisted through day seven after coitus during which time nine to ten bands were apparent compared to four bands in extracts from estrous animals. Serum from pregnant rabbits showed two basic patterns. High esterase activity concentrated in four major bands was seen in some sera while other serum samples showed little esterase activity. No consistent correlation was apparent, however, between the stage of gestation and the serum esterase pattern. In contrast analysis of mouse serum prior to and after mating showed consistent, reproducible changes in esterase profiles first noticeable about 7 days after coitus and becoming increasingly pronounced by parturition. Changes in mouse serum included both marked enhancement of some esterases and concomitant diminution of others with increasing gestation time.

Tyndall, R. L., Colyer, S., and Clapp, N. **Early alterations in plasma esterases with associated pathology following oral administration of diethylnitrosamine and butylated hydroxytuluene singly or in combination.** Int. J. Cancer 16, 184-191, 1975.

Plasma esterase changes are apparent in BALB/c mice within 3 weeks after feeding of laboratory chow with 0.75% of the antioxidant BHT added. Other esterase changes, different from those in BHT-treated animals, are also apparent in plasma of mice during exposure to the carcinogen DENA. Interference with these DENA esterase alterations is apparent in plasma of mice treated concomitantly with both DENA and BHT. Esterase changes resulting from either the carcinogen or antioxidant exposure precede the overt histologically detected changes induced by these compounds. The early esterase changes and subsequent tumorigenesis resulting from DENA exposure are more severe in female mice. Some of the esterases altered by DENA or BHT exposure are testosterone-related.

Urso, P. and Gengozian, N. **Variation in T and B cell deficiency in different mouse allogeneic radiation chimeras.** J. Immunol. 113, 1770-1779, 1974.

The immunologic deficiency of allogeneic radiation chimeras was examined in vitro by culturing spleen cells of the chimera with allogeneic or syngeneic (to donor-type cells of the chimera) activated thymus-derived (T) cells (ATC) or bone marrow-derived (B) cells. The plaque-forming cell (PFC) response to sheep RBC of spleen cells from 100% of the $CD2F_1 \rightarrow C3BF_1$ chimeras was improved considerably (27-fold) with allogeneic ATC but syngeneic ATC gave only a 2-fold increase. Allogeneic B cells induced a moderate increase (4-fold) in the response of spleen cells from only 19% of the chimeras while syngeneic B cells were ineffective. With spleen cells from the reciprocal $C3BF_1 \rightarrow CD2F_1$ chimera there was only a 7-fold increase in the response with allogeneic ATC in 38% of the chimeras and only a 2- to 3-fold increase in 18% with syngeneic ATC. In contrast, there was a 27-fold increase in PFC in about 45% of these latter chimeras with allogeneic B cells while syngeneic B cells failed to improve the response. The PFC generated in mixtures involving both types of chimera spleen cells cultured together were typed with specific isoantiserum and shown to be of $CD2F_1$ orgin. These results demonstrate that deficiency in the interacting cellular components of the spleen will vary in different chimeras. Thus, $CD2F_1 \rightarrow C3BF_1$ chimeras are primarily T cell deficient while those of the reciprocal combinations, $C3BF_1 \rightarrow CD2F_1$, are deficient primarily in the B cell population.

1028323

Vodopick, H. **Cherchez la chienne.** A report of erythropoietic hypoplasia. J. Am. Med. Assoc. 234, 850-851, 1975.

A two-year-old boy exposed to benzene hexachloride developed erythropoietic hypoplasia. The covert source of exposure proved to be a pet dog which had been treated repeatedly with the chemical. Prompt recovery of the patient's erythropoietic marrow function occurred after use of the chemical was stopped.

Vodopick, H. and Andrews, G. A. **Accidental radiation exposure.** Arch. Environ. Health 28, 53-56, 1974.

We report a radiation accident in which a technologist was in close proximity for 40 seconds to a source containing 7,700 curies of cobalt 60. The average bone marrow dose was estimated at 118 rads. Within the first 24 hours postexposure, lymphopenia developed d after two weeks, the platelet and anulocyte count began to fall and reached nadir at approximately four weeks. During the phase of granulocyte depression, the protective environment of a laminar airflow facility was believed to have lessened the risk of infection. This patient's hematological recovery was complete in six weeks.

After exposure to higher doses of radiation than this patient received, recovery can occur if appropriate supportive care, ranging from rest and observation to marrow transplantation, is employed. The clinical course, laboratory data, and estimated dose-exposure will dictate what type of therapy is needed. Each case must be individually evaluated.

Vodopick, H., Chaskes, S. J., Solomon, A., and Stewart, J. A. **Transient monoclonal gammopathy associated with cytomegalovirus infection.** Blood 44, 189-195, 1974.

A transient monoclonal gammopathy, IgA-lambda light chain, associated with a cytomegalovirus (CMV) infection occurred in a patient with acute leukemia in remission. Evidence of CMV infection was confirmed by indirect hemagglutination titers, initially being 1:4096 and gradually falling to 1:64. During the infection, serum and salivary IgA levels rose to 2450 mg/100 ml and 72 mg/100 ml, respectively. Bence Jones proteinuria, lambda type, (0.1 g/24 hr) occurred during the period of excessive immunoglobulin synthesis. Antibody activity against CMV was detected in serum IgA and IgG. Also unusual were the histologic changes indistinguishable from malignant lymphoma seen in a cervical lymph node. After withholding for 1 wk the immunosuppressive drug (methotrexate) she had been receiving, symptoms and signs of the CMV infection and of the monoclonal gammopathy disappeared without recurrence after maintenance methotrexate was resumed.

Washburn, L. C. and Hayes, R. L. **Thin layer chromatographic and colorimetric methods for monitoring the purity of S-2-(3-aminopropylamino)-ethylphosphorothioic acid (WR-2721).** Anal. Chem. 46, 2231, 1974.

Because of a proposed clinical investigation of the use of WR-2721 in the radiotherapy of solid tumors, we sought methods for quality control to allow the detection of impurities in WR-2721 preparations. The major impurities commonly found are the analogous mercaptan and symmetrical disulfide, which can be formed by hydrolysis of WR-2721 and a resultant oxidation of the mercaptan so formed. Colorimetric and TLC methods were developed for this purpose. Using the colorimetric procedure, one can easily and quickly monitor a WR-2721 preparation for impurities at intervals during its storage and use. The TLC method, although requiring a longer time for the analysis, is a valuable supplementary technique.

Washburn, L. C., Carlton, J. E., Hayes, R. L., and Yuhas, J. M. **Distribution of WR-2721 in normal and malignant tissues of mice and rats bearing solid tumors: dependence on tumor type, drug dose and species.** Radiat. Res. 59, 475-483, 1974.

S-2-[3-Aminopropylamino]ethylphosphorotioic acid (WR-2721) preferentially protects the normal, as opposed to the malignant, tissues of tumor-bearing mice from radiation injury. The major factor responsible for this difference in response appears to be the relatively poor absorption of the drug by the tumor. In order to determine whether deficient drug absorption was characteristic of solid tumors and to extend the study of the absorption patterns to the problems which might be encountered in clinical radiotherapy, normal and tumor tissue concentrations of ^{35}S-labeled WR-2721 were determined 30 min after injection of graded doses of the drug (1, 50, 100, or 300 mg/kg) in two species (mice and rats) bearing 4 different solid tumors (P-1798 lymphosarcoma and CA-755 adenocarcinoma in mice and the RFT tumor and Morris 7777 hepatoma in rats). It has been demonstrated that: (1) 3 of the 4 tumors studied evidenced deficient drug absorption, but the Morris 7777 hepatoma absorbed WR-2721 as readily as the host's normal tissues; (2) the relative concentration of the drug in normal and

1028324

tumor tissues was independent of the injected dose between 50 and 300 mg/kg; (3) the injection of tracer levels of WR-2721 (1 mg/kg) can be used to identify those tumors which show a deficient absorption, but not to quantitate the degree of this deficiency; and (4) the relationship between the injected dose and tissue concentration, after correction for species differences in surface area, was species independent for the lungs, small intestine and spleen, but species dependent for kidney and liver. We conclude therefore that WR-2721 is a promising agent for increasing the efficiency of solid tumor radiotherapy, but that each tumor must be considered individually.

Wykle, R. L. and Schremmer, J. M. **A lysophospholipase D pathway in the metabolism of ether-linked lipids in brain microsomes.** J. Biol. Chem. 249, 1742-1746, 1974.

Microsomal preparations from rat brain in the presence of Mg^{2+} hydrolyzed the ethanolamine, choline, and phosphate moieties from 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphorylethanolamine, 1-[1-^{14}C]hexadec-1-enyl-*sn*-glycero-3-phosphorylethanolamine, and 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphorylcholine. Studies of the hydrolysis of 1-[1-^{14}C] hexadecyl-*sn*-glycero-3-phosphorylethanolamine in this system indicated that the ethanolamine moiety was first removed by a phosphodiesterase to form 1-[1-^{14}C]-hexadecylglycero-3-phosphate which was then dephosphorylated to form 1-[1-^{14}C]hexadecylglycerol. Only minimal hydrolysis occurred when the 2-positions of the substrates were acylated; otherwise the phosphodiesterase activity was similar to that of phospholipase D from plants. The occurrence of such a lysophospholipase D pathway has not been previously reported. When Ca^{2+} (5 mM) was added to the system instead of Mg^{2+} (5 mM), little, if any, stimulation occurred; higher concentrations of Ca^{2+} (25 mM) inhibited the reaction. Therefore, this reaction does not appear to be related to the Ca^{2+}-stimulated base exchange reaction found in the brain by others.

Wykle, R. L. and Schremmer Lockmiller, J. M. **The biosynthesis of plasmalogens by rat brain: Involvement of the microsomal electron transport system.** Biochim. Biophys. Acta 380, 291-298, 1975.

The biosynthesis of 1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (ethanolamine plasmalogens) was studied using 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphoethanolamine as the substrate and EDTA-washed microsomes from brains of 14-day-old rats. It was found that the 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphoethanolamine was first acylated to form 1-[1-^{14}C]hexadecyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, then was desaturated to form 1-[1-^{14}C]hexadec-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine. The desaturation required O_2 and NADH or NADPH and was inhibited by KCN but not by CO. The data indicated that the desaturation is carried out by a mixed-function oxidase system similar to that involved in the desaturation of fatty acids and that the pathway for the biosynthesis of plasmalogens in brain is similar to that previously found in other tissues. The desaturase was not stimulated by ATP and Mg^{2+} nor inhibited by EDTA. The specific activity of microsomes from brains of rats of different ages was determined; the activity decreased with age until in adults the activity was only 15% that of the 12–14-day-old rats.

1028325

Collaborative Projects Between Medical and Health Sciences Division Staff and Other Institutions

Project	Other Institution[1] and Staff	Division Staff
In-patient Radiopharmaceuticals trials	CRC/UT; Girardi/Patterson	Andrews, G.
Radiation sensitivity (cytogeneic) of the marmoset	ORNL; Brewen, J.G.	Gengozian, N.
Microbiology of fecal flora of marmoset	Memphis SU; Peterson, G.	Gengozian, N.
^{11}C in Nuclear medicine	ORNL; Butler, T.A. and Poggenburg, K.	Hayes, R.L.
Radioprotective drug, WR-2721, for possible use in radiotherapy	ORNL; Yuhas, J.	Hayes, R.L.
Production and assessment of various radionuclides as imaging agents	ORNL; Poggenburg, K.	Hayes, R.L.
T and B cells in cancer patients	UT MRC; Solomon, A.	Hübner, K.
Anti-cancer (human) antibody proteins	UT MRC; Solomon, A.	Hübner, K.
Pulmonary disease detection	ETCDH; Winebrenner, J.	Hübner, K.
Histopathology of primary and metastatic pulmonary adenocarcinoma induced in mice by radiation exposure	ORNL; Yuhas, J.	Lushbaugh, C.
ET Tumor registry	CRC; Girardi, A.	Lushbaugh, C.
π Meson dosimetry	ORNL/LASL; Alsmiller, R.	Lushbaugh, C.
US Transuranium registry	Hanford Environmental Health Foundation; Fuqua, P.	Lushbaugh, C.
Trace metals in man	LASL; Campbell, E.	Lushbaugh, C.
Metabolism of alkoxy and alkenyloxy glyceryl ethers and derivatives	UNC; Piantadosi, C.	Snyder, F.
Ether and ketone lipids during brain development	UNC; Piantadosi, C.	Snyder, F.

[1] *Abbreviations:*
ORNL—Oak Ridge National Laboratory
CRC—Cancer Research Center
UT—University of Tennessee
SU—State University
UTMRC—University of Tennessee Memorial Research Center
ETCDH—East Tennessee Chest Disease Hospital
LASL—Los Alamos Scientific Laboratory
UNC—University of North Carolina

1028326

Project	Other Institution[1] and Staff	Division Staff
Analysis of tumor-associated alkyl diacylglycerols and other lipids in the thymus during irradiation-induced thymic leukemogenesis	Rutherford Hospital Rutherfordton, N.D.; Brown, R. C.	Snyder, F.
Ether lipids in preneoplastic states	Bruce Lyon Memorial Research Laboratory, Childrens Hospital, Oakland, Calif.; Abraham, S.	Snyder, F.
Role of lipids in cell cycle and cell transformation	ORNL; Hsie, A.	Snyder, F.
High resolution electron microscopy of chromatin	ORNL; Olins, A.	Swartzendruber, D.C.

[1] *Abbreviations:*
ORNL—Oak Ridge National Laboratory
CRC—Cancer Research Center
UT—University of Tennessee
SU—State University
UTMRC—University of Tennessee Memorial Research Center
ETCDH—East Tennessee Chest Disease Hospital
LASL—Los Alamos Scientific Laboratory
UNC—University of North Carolina

1028327

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1028328

1975-76

1028329

ORAU-128

Oak Ridge Associated Universities

Medical and Health Sciences Division

Research Report

1975-1976

1028330

Oak Ridge Associated Universities (ORAU) is a private, nonprofit corporation sponsored by 43 colleges and universities. Established in 1946, it was one of the first university-based, science-related, corporate management groups. ORAU conducts diverse programs of scientific research, education, information, and training for the Energy Research and Development Administration (ERDA) and other public and private agencies.

The **Medical and Health Sciences Division** is one of the nation's principal centers of nuclear medicine. It conducts an interrelated program of clinical and preclinical research, medical physics, and training. Facilities include REAC/TS, the ERDA radiation-accident treatment center where national training programs are conducted; the division also operates a radioisotope-dosimetry information center and an outstanding marmoset research center.

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Microfiche $3.00 (domestic)
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1028331

Printed in the United States of America
USERDA Technical Information Center, Oak Ridge, Tennessee

ORAU-128
Distribution Category: UC–48

Research Report 1975-1976

Medical and Health Sciences Division
Oak Ridge Associated Universities

Contents

Foreword

It has been a little over a year since the Medical and Health Sciences Division initiated a major reorganization of its research efforts to meet the newly broadened responsibilities of the environmental and safety research programs of ERDA, our primary source of support. In addition to ERDA-sponsored projects, the Division has considerable financial support from the National Institutes of Health and the American Cancer Society. The Division consists of five sections: Radiation Emergency Assistance Center/Training Site (REAC/TS), Biochemistry, Immunology, Radiopharmaceuticals, and Epidemiology. Although organized in this fashion for the purpose of administrative efficiency, budget control, and peer review of scientific excellence, there is a close problem-oriented approach in pursuing the various research projects. The scope of the different programs is described in Section 1 of this report.

Our capabilities in immunology, biochemistry, radiopharmaceuticals, epidemiology, and radiation biology provide a five-way approach to the solution of pressing problems in occupational and preventive medicine related to nuclear and nonnuclear energy. These problems concern principally acute and chronic diseases of the skin, lungs, and gastrointestinal tract induced by radiations and chemical toxicants released into the environment by power production. Our goal is to obtain the informational bases that will lead to (1) establishment of safe-exposure limits and means for their measurement, (2) prevention of occupational disease, (3) earlier detection of incipient chronic biological effects, (4) improvement in therapy of acute and chronic occupational diseases, and (5) education and training of others in the biomedical technologies involved.

This report provides an account of all published papers from the Division for the period September 1975–August 1976.

C. C. Lushbaugh
Fred Snyder

October 1976

Page

1 Scope of Research Program 1

2 Publications 5

3 Abstracts of Full-Length Publications 8

4 Collaborative Projects Between Medical and Health Sciences Division Staff and Other Institutions 13

5 Medical and Health Sciences Division Staff . . . . . Inside back cover

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1028332

Scope of Research Program

The scope of the biomedical research efforts of the ORAU Medical and Health Sciences Division has remained essentially unchanged since the program was initially redirected toward the needs of occupational medicine related to energy production. The year was spent working to attain our goals that had been set up for the four major program divisions: Immunobiology, Membrane Biochemistry, Radiopharmaceutical Development, and Radiation Emergency Assistance Center/Training Site (REAC/TS). As can be seen in the following program summaries, our progress is reflected in increased research momentum that in turn has generated increased financial support. This is presently enabling us to integrate our staffs geographically in renovated laboratories centered around a new research library and an animal care facility that is based on the best principles of veterinary practice.

Occupational epidemiology represents a new area of research at the division. The objective of this program is to use modern epidemiological technology as a means for monitoring large populations of workers in industries related to fuel and energy production to detect any harmful effects of these occupations as soon as possible so that health protection can be evaluated and constantly improved. A major project being pursued on a continuing basis by the epidemiology group is evaluation of the disability and mortality experience of persons with a history of employment in selected installations associated with the nuclear industry. The other primary project concerns a prospective study of health hazards associated with newly emerging or rapidly expanding industries related to energy production from nonnuclear fuels.

A. IMMUNOBIOLOGY

The immunology program is focusing its efforts on analyzing at the cellular level the effects of irradiation and chemical environmental pollutants on the immune system of mammals. Two experimental animal models are being developed and utilized in these studies, each of which provides a rather unique physiological state, blood chimerism, with which to evaluate the immunological consequences of external insults. One model concerns the marmoset, a small primate which by virtue of its high frequency of fraternal twinning and placental vascular anastomoses is a natural blood chimera. The second utilizes mice which have been lethally irradiated and transplanted with allogeneic bone marrow, yielding animals that are also chimeras but with the significant difference that all blood elements are of donor origin. Exploitation of these two animal models for the objectives stated above has necessitated a greater understanding of the chimeric phenomenon itself, particularly as it relates to the immune system. The studies undertaken the past year have therefore focused on some basic questions relative to this phenomenon.

We had established some time ago that the immune system of murine allogeneic radiation chimeras was abnormal, even though by other physiological criteria these animals compared favorably to syngeneic chimeras and normal, untreated littermates. Extensive in vitro studies have established that the T and B lymphocytes of allogeneic chimeras cannot function properly in the initiation and maintenance of an in vitro antibody response. The specific cellular deficiency, T or B cell, has varied for different radiation chimeras with no apparent predilection for abnormality in either population. Current studies have now revealed that the T cells in some chimera strain combinations are functioning as suppressor cells in the maintenance of tolerance with respect to host antigens. Thus, T lymphocytes from the chimera, of donor origin, have abrogated the ability of normal donor-type cells to express a cell-mediated immune function in an appropriate assay system. What relationship, if any, this suppressor activity has to a helper function of T cells in antibody formation is not known and is currently under investigation.

Our studies in the marmoset this year have centered on two facets of the chimerism phenomenon and on the immunological capacity of these animals. The first question concerns the feasibility of identifying histocompatibility antigens on the white blood cells of various marmoset species. Using an isoimmunization approach to this problem, we have succeeded in developing

1028333

reagents capable of distinguishing between two species, *S. fuscicollis* and *S. o. oedipus*, and furthermore have developed one reagent capable of identifying individual marmosets within one particular species. The latter reagent is now being applied to the problem of white blood cell chimerism, which heretofore could be demonstrated only by sex chromosome analyses of animals having a cotwin of the opposite sex. The other facet of chimerism concerns the relative proportions of cotwin cells in the T and B lymphocyte populations of these animals. Utilizing reagents developed in this laboratory for T and B cell identification, the techniques of cell separation, mitogen activation, and chromosome analyses have now revealed that the percentage of chimerism between the T and B cells is the same. This information supports a fundamental concept in developmental immunology, i.e., that the thymocytes arise from hemopoietic stem cells migrating from the bone marrow. Thus, in situ development of thymocytes in the thymic organ would not have led to a chimeric population of T cells.

The in vitro utilization of tissues such as lymph nodes and spleen for antibody formation is limited with the marmoset because of the difficulty of procuring these routinely. Therefore, our demonstration this year that primary antibody formation could be achieved in vitro with blood lymphocytes from these animals represents a minor breakthrough in our approach to some of the more fundamental aspects of chimerism in the marmoset. An interesting facet to these studies has been the species difference in the in vitro responses with blood lymphocytes. Thus, the cotton-top marmoset (*S. o. oedipus*) has consistently failed to yield a response while cells from the white-lipped marmoset (*S. fuscicollis*) have shown a high probability of responding to the antigen (sheep RBC). The significance of this species difference is not known, but parallel studies on in vivo challenges with a variety of antigens, including sheep RBC, have revealed the white-lipped marmoset to be far superior to the cotton-top. Of further interest, however, is that neither of the two species appears to respond with titers comparable to those obtained in other laboratory animals, such as the rat and mouse, when challenged with the same antigens. Whether this relative immunological incompetence of the marmoset reflects a physiological state consequent to the chimerism of these animals, as in the mouse radiation allogeneic chimera, is not known.

B. MEMBRANE BIOCHEMISTRY

Research in Biological Chemistry concentrates primarily on fundamental studies of membrane properties and lipid metabolism in cancer and respiratory diseases induced by chemical toxicants (irritants, carcinogens, and mutagens). Recent results in our studies of membrane modifications and their effect on cellular function have demonstrated that membrane lipids can be manipulated by employing unnatural polar base analogues of normal lipid precursors (e.g., *N*-isopropylethanolamine). Moreover, these alterations have been found to elicit highly specific functional responses in the modified cells. For example, only a single component (the terminal cyanide-sensitive protein) of an integrated multienzyme complex, acyl-CoA desaturase, was affected in one such modified membrane system. The high degree of selectivity in response to the membrane lipid alterations stresses the potential of this technique in controlling functional activities of membranes at the molecular level. Also, these observations point out that environmental toxicants or protective agents could be incorporated into cellular membrane constituents by mechanisms similar to those involving the unnatural lipid precursor analogues.

In studies of the metabolism of lipids in cancer cells, our results document that harderian glands are extremely rich in the enzymes that synthesize lipids with ether linkages, a class of lipids that is characteristically elevated in most cancers. These data indicate that the gland can be used as an appropriate model system for studying the enzymes that metabolize the tumor-associated lipids. For example, one advantage of the glands over the tumor systems is the ease with which the enzyme activities are solubilized from the membranes with detergents in order that the enzymes can be purified for investigation of their chemical and physical properties. The purified enzymes will be used for investigation of their mechanisms of action and for the development of inhibitors of the synthesis of the tumor-associated lipids. Another aspect of this work shows that catalase, an enzyme that attacks hydrogen peroxide, plays a major function as a modifier of membrane-bound enzyme activities (alkyl and acyl desaturases) associated with lipid metabolism in cancer cells; this appears to be related to its ability to prevent oxidative damage to the polyunsaturated components of the membrane-bound complexes and to the enzyme itself.

In our studies of chemical toxicants, we demonstrated that a metabolite of dimethylnitrosamine, a potent carcinogen, is incorporated into phosphatidylcholine, an important lipid constituent of all membranes. These results indicate that dimethylnitrosamine could markedly alter both the lipid composition and the functional properties of membranes. In related studies of

1028334

other energy-derived pollutants, a dramatic increase in pulmonary surfactant lipids was observed in rats exposed to nitrogen dioxide, a major toxic byproduct in the use of coal as fuel. These results are of special significance, since pulmonary surfactant lipids are absolutely essential for lung function, preventing alveolar collapse. The data also reveal that surfactant lipids undoubtedly play a crucial role as a protective barrier against toxicants that would otherwise penetrate surface membranes.

Research aimed at answering basic questions on the biochemistry of lung surfactants has continued with a pulmonary adenoma that consists of a homogeneous population of cells, which appear to be identical to the surfactant-producing type II cells found in normal lung. The tumor is proving to be an ideal model for our lung metabolic studies, since studies with normal lung are complicated because of the more than 40 different cell types present. A significant breakthrough with the tumor system has been the determination of the primary metabolic pathway (deacylation-reacylation of phosphatidylcholine) responsible for the formation of lung surfactant lipid.

C. RADIOPHARMACEUTICAL DEVELOPMENT

The Medical and Health Sciences Division's Radiopharmaceutical Development Program aims specifically at the ultimate testing of new or improved radiopharmaceuticals in man. Such tests are arranged for at the division on an outpatient basis or by using inpatients at other cooperating institutions. Preliminary to these tests in humans, extensive preclinical studies are required which involve the identification of possible new agents, preparation and purification of the agents, testing of their effectiveness in animal models, performance of necessary toxicity studies in animals, and establishment of quality control procedures. All studies in humans are then carried out after approval by the Food and Drug Administration. In addition, basic studies of the mechanisms involved in the uptake of radiopharmaceuticals of proven usefulness are a vital part of the development program. These shed light on the reasons for the effectiveness of radiopharmaceutical agents, and lead to the identification of new agents. Close ties are maintained with Oak Ridge National Laboratory as well as other cooperating institutions.

During the past year considerable progress has been made in the preparation of carbon-11-labeled radiopharmaceuticals for use in the external visualization of various organs and in the detection of cancer. An unnatural amino acid (1-aminocyclopentanecarboxylic acid) (ACPC) has been labeled with carbon-11 in 100 mCi amounts. Carbon-11 has a half-life of only 20.4 min, but unlike carbon-14 (a pure beta emitter with a 5730-year half-life) carbon-11 emits a positron which in turn yields high-energy photons capable of being detected externally in a tomographic (three-dimensional) mode. The radiopharmaceutical ^{11}C-ACPC is now undergoing preliminary trials in humans as a cancer detection agent and shows considerable promise. Another amino acid, DL-valine, labeled in the carboxyl group with carbon-11 (produced in 200 mCi amounts) is also undergoing clinical trials as a visualizing agent for the pancreas, a difficult organ to detect. Carbon-11-labeled tryptophan and various analogues of ACPC are also being produced as possible agents for pancreas imaging and tumor detection.

Preliminary clinical studies of dysprosium-157 and erbium-171 as possible tumor-localizing agents have been completed. These two radionuclides, administered in the citrate form, were in general found to be inferior to gallium-67 citrate, but they appear to show potential in the detection of abdominal cancers where gallium-67 detection is poor because of the accumulation of gallium-67 activity in the bowel.

A study of the clinical use of a high-resolution position-sensitive line proportional counter developed by Oak Ridge National Laboratory in the scanning of the thyroid has been completed. Clinical experience gained with the instrument justifies further study with advanced designs.

In studies of the mechanism of gallium and indium uptake by tumor tissue it has been shown that the in vitro uptake of these two radionuclides leads to entirely different subcellular distributions than those seen with in vivo uptake. Subcellular fractionation of in vitro tumor tissue indicates no association of gallium or indium with organelles while tumor tissue exposed in vivo shows a major association of these two elements with lysosomes and smaller lysosome-like particles that have not yet been fully characterized. For these studies a new in vitro technique involving a multichamber interconnecting tissue (MIT) culture system has been developed. The 40,000 dalton protein found in tumor tissue extracts that is a specific binder of gallium and indium has been purified and identified as a glycoprotein.

D. RADIATION EMERGENCY ASSISTANCE CENTER/TRAINING SITE (REAC/TS)

The Radiation Emergency Assistance Center/Training Site program achieved reality in a practical sense on the completion this year of its facility in the

1028335

new emergency care wing of the Oak Ridge Hospital of the United Methodist Church. It is now rapidly nearing complete readiness to handle any kind of radiation accident that involves problems ranging from questionable radiation exposure and damage to extreme levels of radiation contamination and supralethal irradiation. The new site gave increased impetus to the continuing development of specialty courses in radiation-accident management and expansion of the radiation-accident registries to include an overexposure registry and greater in-depth clinical and laboratory studies following up the progress of former radiation-accident victims. This program was broadened by staff additions in epidemiology and biostatistics that will in time enable the activities of the ERDA Health and Mortality Studies to be headquartered here. These studies, collaborative with others at Hanford, Los Alamos, Oak Ridge National Laboratory, Mound Laboratory, and the University of Pittsburgh, have required the establishment of a data depository and epidemiological study center in our Tyrone Road Building. The staff has continued to provide medical consultation in cases of radiation burns, to work on the development of an animal model for studying radiation gangrene, to improve the use of cytogenetics as a biological indicator of radiation exposure, and to help improve chelation therapy of plutonium-contaminated wounds through the management of the drug diethylenetriaminepentaacetic acid.

1028336

Publications

This section lists abstracts (all papers presented at meetings, regardless of whether they are published), journal articles, review articles, books, chapters, and doctoral dissertations. All "in press" material is included. For abstracts of full-length publications, see Section 3.

Andrews, G. A. *History of radiopharmacy.* In: Radiopharmacy, M. Tubis and W. Wolf, eds., Wiley-Interscience, New York, 1976, pp. 3–23.

Baker, R. C., Wykle, R. L., and Lockmiller, J. S. *Identification of a soluble protein stimulator of microsomal cytochrome b_5-dependent desaturation reactions.* Fed. Proc. 35, 1625, 1976, abstr. 1357.

Baker, R. C., Wykle, R. L., Lockmiller, J. S., and Snyder, F. *Effect of a soluble protein factor on plasmalogen biosynthesis.* (Presented at the 10th Annual Lipid Conference, Gatlinburg, Tennessee, 1975.)

Baker, R. C., Wykle, R. L., Lockmiller, J. S., and Snyder, F. *Identification of the soluble protein stimulator of plasmalogen biosynthesis and stearoyl-CoA desaturase.* Arch. Biochem. Biophys. (in press).

Blank, M. L., Lee, T.-c., Piantadosi, C., Ishaq, K. S., and Snyder, F. *Membrane lipid modification and stearoyl-CoA desaturase activity in L-M cells.* Arch. Biochem. Biophys. (in press).

Blank, M. L., Rainey, W. T., Jr., Christie, W. H., Piantadosi, C., and Snyder, F. *Mass spectral analysis of a homologous series of S- and O-alkylglycerols.* Chem. Phys. Lipids (in press).

Brown, D. H., Byrd, B. L., Carlton, J. E., Swartzendruber, D. C., and Hayes, R. L. *A quantitative study of the subcellular localization of ^{67}Ga.* Cancer Res. 36, 956–963, 1976.

Dosado, E. A., Hsie, A. W., and Snyder, F. *Effect of 1,2-dimethylxanthine on lipid metabolism of Chinese hamster ovary cells.* (Presented at the 10th Annual Lipid Conference, Gatlinburg, Tennessee, 1975.)

Dosado, E. A., Hsie, A. W., and Snyder, F. *Rapid screening of lipid metabolism in monolayer cell cultures.* J. Lipid Res. 17, 285–288, 1976.

Frome, E. L., Frederickson, E. L., and Lushbaugh, C. C. *Power spectrum of the respiratory system.* Biometrics 32, 423–428, 1976.

Gengozian, N., and Bazzell, S. J. *Isoimmunizations among marmosets to identify leucocyte antigens.* J. Med. Primatol. (in press).

Gengozian, N., and Urso, P. *Status of T and B cell cooperation in radiation chimeras: Evidence for a suppressor effect.* Transplant. Proc. (in press).

Gengozian, N., Batson, J. S., and Smith, T. A. *Breeding of the marmoset in the laboratory.* In: The Biology and Conservation of the Callithricidae, D. G. Kleiman, ed., Smithsonian Press, Washington, D.C. (in press).

Gengozian, N., Bazzell, S. J., and Nickerson, D. *Histocompatibility antigens and antibody forming cells in the marmoset.* Fed. Proc. 35, 353, 1976, abstr. 831.

Gengozian, N., Salter, B. L., Basford, N. L., and Kateley, J. R. *Characterization of the antibody response of the marmoset to sheep red blood cells.* Clin. Exp. Immunol. 23, 525–535, 1976.

Goswitz, F. *Vitamin B_{12} absorption test and other studies (Nuclear hematology).* In: Diagnostic Nuclear Medicine, A. Gottschalk and E. J. Potchen, eds., Williams and Wilkins, Baltimore, pp. 186–190, 1976.

Hayes, R. L. *Factors affecting uptake of radioactive agents by tumor and other tissues.* In: Proceedings of the International Atomic Energy Agency Advisory Group Meeting on Tumor Localization with Radioactive Agents, Vienna, December 1974 (in press).

Hayes, R. L., and Brown, D. H. *Biokinetics of radiogallium.* In: Nuklearmedizin: Fortschritte der Nuklearmedizin in klinischer und technologischer Sicht, 12th Annual Meeting, Gesellschaft für Nuklearmedizin München, 1974, H. W. Pabst, ed., Schattauer Verlag, Stuttgart-New York, 1975, pp. 837–848.

1028337

Hayes, R. L., Washburn, L. C., Wieland, B. W., Sun, T. T., Turtle, R. R., and Butler, T. A. *Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid, a potential agent for cancer detection.* J. Nucl. Med. 17, 748–751, 1976.

Hilenski, L. L., Walne, P. L., and Snyder, F. *Aliphatic chains of esterified lipids in isolated eyespots of Euglena gracilis var. bacillaris.* Plant Physiol. 57, 645–646, 1976.

Hodges, H. D., and Gibbs, W. D. *The participation of nuclear medicine technologists in radiation accident management.* J. Nucl. Med. Technol. 4, 95, 1976, abstr.

Hodges, H. D., and Yaden, S. L. *Restraining rats with radiation-induced tail damage.* Lab. Anim. Sci. 26, 244, 1976.

Hübner, K. F., Gengozian, N., and Gilman, T. A. *Suppression and recovery of the immune response and CFU potential of mice during and after chronic low-dose-rate gamma irradiation.* Int. J. Radiat. Biol. 28, 301–312, 1975.

Kniseley, R. M. *Diseases of the bone marrow.* In: Principles of Nuclear Medicine, 2nd ed., H. N. Wagner, ed., Saunders, Philadelphia (in press).

Lee, T-c., and Snyder, F. *Metabolism of lipids and sterols in cancer cells.* In: Lipid Metabolism in Mammals, F. Snyder, ed., Plenum Press, New York (in press).

Lee, T-c., Blank, M. L., Piantadosi, C., Ishaq, K. S., and Snyder, F. *Effect of an unnatural base analog, N-isopropylethanolamine, on the fatty acid desaturation system in L-M cells.* (Presented at the 10th Annual Lipid Conference, Gatlinburg, Tennessee, 1975.)

Lushbaugh, C. C., and Casarett, G. W. *The effects of gonadal irradiation in clinical radiation therapy: a review.* Cancer 37, 1111–1120, 1976.

Lushbaugh, C. C., and Swartzendruber, D. C. *Histopathologic aspects of radiation effects on lymphatic tissues and malignancies.* In: Radiation and the Lymphatic System, Proceedings of the 14th Annual Hanford Biology Symposium, 1974, US ERDA Report CONF-740930, 1976, pp. 203–208.

Lushbaugh, C. C., Cloutier, R. J., Andrews, G. A., Beck, W. L., Hübner, K. F., and Berger, J. D. *"REACTS": A pragmatic approach for providing medical care and physician education for radiation emergencies.* In: Proceedings of the International Atomic Energy Agency Symposium on Diagnosis and Treatment of Incorporated Radionuclides, Vienna, 1975, pp. 565–577, 1976.

Machlin, L. J., Garcia, F., Kuenzig, W., Richter, C. B., Spiegel, H. E., and Brin, M. *Lack of antiscorbutic activity of ascorbate 2-sulfate in the rhesus monkey.* Am. J. Clin. Nutr. (in press).

Mainwaring, H. R., Swartzendruber, D. C., Lushbaugh, C. C., Idoyaga-Vargas, N. L., and Watson, E. E. *Radiation effect on ^{67}Ga retention and distribution in mouse tissues.* In: Radiation and the Lymphatic System, Proceedings of the 14th Annual Hanford Biology Symposium, 1974, US ERDA Report CONF-740930, 1976, pp. 28–35.

Morris, A. C., Jr., Barclay, T. R., Akin, T. E., Hansard, M. C., Gibbs, W. D., and Modzelewski, C. U. *14" X 17" film recorder for computer-enhanced scans.* In: Proceedings of the Sixth Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Atlanta, Georgia, 1976, pp. 401–411, 1976. (Also published as: US ERDA CONF-760119.1, 1976, pp. 1–20.)

Nelson, B., and Andrews, G. A. *Autologous stem-cell banks for restoring hemopoiesis.* Adv. Radiat. Biol. 6, 325–338, 1976.

Newsome, Y. L., and Littlefield, L. G. *Adriamycin-induced chromosomal aberrations in human fibroblasts.* J. Natl. Cancer Inst. 55, 1061–1064, 1975.

Niblack, G. D., and Gengozian, N. *Identification of T and B lymphocytes in the marmoset; a natural haemopoietic chimera.* Clin. Exp. Immunol. 23, 536–543, 1976.

Niblack, G. D., Kateley, J. R., and Gengozian, N. *T and B lymphocyte chimerism in the marmoset.* Immunology (in press).

Nickerson, D. A., and Gengozian, N. *Primary in vitro antibody formation by blood leucocytes of a sub-human primate.* Cell. Immunol. (in press).

Palisano, J. R., Walne, P. L., and Brown, D. H. *Physicochemical and ultrastructural characterization of isolated developing plastids of Euglena gracilis.* J. Cell Biol. 70, 166a, 1976, abstr. 498.

Ricks, R. C., Cloutier, R. J., Berger, J. D., Beck, W. L., Jr., and Lushbaugh, C. C. *REAC/TS: A radiation emergency assistance center/training site.* In: Program of the Twenty-first Annual Meeting of the Health Physics Society, San Francisco, 1976, p. 44, abstr. Ja-9.

Rock, C. O. *Harderian glands: A model organ for the study of ether lipid metabolism in cancer cells.* Ph.D. dissertation, University of Tennessee, 1976.

1028338

Rock, C. O. *The harderian gland.* In: Lipid Metabolism in Mammals, F. Snyder, ed., Plenum Press, New York (in press).

Rock, C. O., and Snyder, F. *Metabolic interrelations of hydroxy-substituted ether-linked glycerolipids in the pink portion of the rabbit harderian gland.* Arch. Biochem. Biophys. 171, 631-636, 1975.

Rock, C. O., and Snyder, F. *Stimulation of alkyldihydroxyacetone phosphate synthesis by detergents.* Fed. Proc. 35, 1724, 1976, abstr. 1861.

Rock, C. O., Fitzgerald, V., Rainey, W. T., Jr., and Snyder, F. *Mass spectral identification of 2-(O-acyl)-hydroxy fatty acid esters in the white portion of the rabbit harderian gland.* Chem. Phys. Lipids (in press).

Sherman, M. I., Miller, R. A., and Richter, C. B. *Analysis of tumors developing from mouse blastocyst derived cell lines.* J. Natl. Cancer Inst. (in press).

Snyder, F. *Chromatographic analysis of alkyl and alk-1-enyl ether lipids and their derivatives.* In: Lipid Chromatographic Analysis, Vol. 1, G. V. Marinetti, ed., Marcel Dekker, New York, 1976, pp. 111–148.

Snyder, F., ed. Lipid Metabolism in Mammals, Plenum Press, New York (in press).

Snyder, F., and Malone, B. *Transfer of methyl groups from N-dimethylnitrosamine to glycerolipids in rat liver.* Chem.-Biol. Interact. 13, 237–241, 1976.

Snyder, F., Lee, T -c., Blank, M. L., Ishaq, K. S., and Piantadosi, C. *Modification of membrane phospholipids and metabolic consequences.* (10th International Congress of Biochemistry, Hamburg, Germany, July 1976, in press.)

Swartzendruber, D. C., and Lushbaugh, C. C. *Intracellular microvilli in cystic colonic cancer cells.* J. Cell Biol. 67, 423a, 1975, abstr. 846.

Tyndall, R. L., and Daniel, J. C., Jr. *Alterations in uterine and serum esterases in pregnant mammals.* Fertil. Steril. 26, 1098–1104, 1975.

Tyndall, R. L., Chaskes, S. J., Carlton, J. E., Nelson, B., and Daniel, J. C. *Gallium-67 distribution in pregnant mammals.* J. Exp. Zool. 195, 417–424, 1976.

Urso, P., and Gengozian, N. *Status of T and B cell cooperation in radiation chimeras: evidence for a suppressor effect.* Fed. Proc. 35, 788, 1976, abstr. 3206.

Voelker, D. R., and Lee, T -c. *Fatty acid biosynthesis in pulmonary type II cell adenomas.* Fed. Proc. 35, 1625, 1976, abstr. 1354.

Voelker, D. R., Lee, T -c., and Snyder, F. *Biosynthesis of fatty acids in pulmonary adenomas.* (Presented at the 10th Annual Lipid Conference, Gatlinburg, Tennessee, 1975.)

Voelker, D. R., Lee, T -c., and Snyder, F. *Fatty acid biosynthesis and dietary regulation in pulmonary adenomas.* Arch. Biochem. Biophys. (in press).

Washburn, L. C., and Hayes, R. L. *Importance of excess base in the synthesis of sodium phosphorothioate.* Inorganic Syntheses, Vol. 17 (in press).

Washburn, L. C., Rafter, J. J., Hayes, R. L., and Yuhas, J. M. *An interspecies comparison of the tissue distribution of WR-2721, a radioprotective drug.* IRCS Med. Sci. Suppl. 3, 28, 1975.

Washburn, L. C., Rafter, J. J., Hayes, R. L., and Yuhas, J. M. *Prediction of the effective radioprotective dose of WR-2721 in humans through an interspecies tissue distribution study.* Radiat. Res. 66, 100–105, 1976.

Washburn, L. C., Sun, T. T., Rafter, J. J., and Hayes, R. L. *C-11-labeled amino acids for pancreas visualization.* J. Nucl. Med. 17, 557-558, 1976. (abstr.)

Washburn, L. C., Coffey, J., Watson, E. E., Sun, T. T., and Hayes, R. L. *Radiation dosimetry of some ^{11}C-labeled amino acid radiopharmaceuticals.* In: Symposium on Radiopharmaceutical Dosimetry, Bureau of Radiological Health, Washington, D.C., 1976 (in press).

Wykle, R. L. *Lipid metabolism in brain.* In: Lipid Metabolism in Mammals, F. Snyder, ed., Plenum Press, New York (in press).

Wykle, R. L., and Snyder, F. *Microsomal enzymes involved in the metabolism of ether-linked glycerolipids and their precursors in mammals.* In: The Enzymes of Biological Membranes, Vol. 2, A. Martonosi, ed., Plenum Press, New York, 1976, pp. 87–117.

1028339

3 Abstracts of Full-Length Publications

This section consists of abstracts of full-length publications. Only published original articles and review articles that contain previously unpublished data are included.

Brown, D. H., Byrd, B. L., Carlton, J. E., Swartzendruber, D. C., and Hayes, R. L. **A quantitative study of the subcellular localization of ^{67}Ga.** Cancer Res. 36, 956–963, 1976.

This paper describes a quantitative procedure for the isolation of ^{67}Ga-binding granules (GBG) from normal rat liver and Morris 5123C hepatoma homogenates by a combination of rate and isopyknic density gradient zonal centrifugation. Another class of GBG has been found that is much smaller than the lysosomal GBG we have previously described. These smaller particles, or microvesicles, bind the largest portion of the ^{67}Ga found in the hepatoma, whereas, in the liver, the GBG lysosomes are the major binding component. Previously, we had shown that considerably more ^{67}Ga is taken up in hepatoma than in liver (as percentage of administered dose per gram of tissue). The preferential association of ^{67}Ga with these microvesicles in the 5123C hepatoma may be indicative of a basic difference between normal and malignant tissue.

Dosado, E. A., Hsie, A. W., and Snyder, F. **Rapid screening of lipid metabolism in monolayer cell cultures.** J. Lipid Res. 17, 285–288, 1976.

Monolayer cell cultures grown on coverslips in the presence of radioactive lipid precursors were embedded in silica gel layers for extraction and resolution of the labeled products directly by thin-layer chromatography. The method permits rapid screening of lipid metabolism in tissue cultures with a small number of cells.

Frome, E. L., Frederickson, E. L., and Lushbaugh, C. C. **Power spectrum of the respiratory system.** Biometrics 32, 423–428, 1976.

Spectrum analysis is proposed as a method for analyzing biological time series data that arise in the study of respiration. A mechanical model of the respiratory system is used to provide a theoretical basis for the interpretation of the air flow spectrum in terms of a physically defined energy concept. We show that under certain conditions the flow variance is proportional to the mechanical work of breathing, and indicate how this new parameter is related to conventional measures of respiratory function. An example is presented to describe the computational procedure and to illustrate the graphical aspects of cross-spectrum analysis.

Gengozian, N., Salter, B. L., Basford, N. L., and Kateley, J. R. **Characterization of the antibody response of the marmoset to sheep red blood cells.** Clin. Exp. Immunol. 23, 525–535, 1976.

The immune competence of two species of marmosets, *Saguinus fuscicollis* and *S. oedipus*, was evaluated by the intravenous (i.v.) and intramuscular (i.m.) injection of sheep red blood cells (RBC). In *S. fuscicollis* marmosets, 1 ml of a 50% suspension yielded titers of hemolysin and agglutinating antibodies equal to or greater than 1 ml of a 10% dose of antigen. In both species, the i.v. route, while resulting in formation of 19 S and 7 S agglutinins, yielded only 19 S hemolysins, even after multiple antigen injections. Repeated i.v. injections resulted in a progressive decrease in peak titers, in contrast to the i.m. route where booster inoculations gave a typical anamnestic response. Jerne plaque-forming cells (PFC) in the spleens of *S. oedipus* marmosets showed predominantly 19 S plaques after a primary i.v. challenge; only 19 S PFC were detected in the spleen of an animal that had been given multiple inoculations, the type of antibody produced reflecting that found in the serum. 19 S but not 7 S hemolysins of both species were sensitive to heating at 56°C for ½ hr. The serum titers and splenic PFC data from the marmosets suggest that these animals, particularly *S. oedipus*, respond poorly to sheep RBC when a comparison is made to similar studies in mice and rats.

Hayes, R. L., and Brown, D. H. **Biokinetics of radiogallium.** In: Nuklearmedizin: Fortschritte der Nuklearmedizin in klinischer und technologischer Sicht, 12th Annual Meeting, Gesellschaft für Nuklearmedizin Müchen, 1974, H. W. Pabst, ed., Schattauer Verlag, Stuttgart-New York, 1975, pp. 837–848.

Since its introduction as a diagnostic agent in 1969, ^{67}Ga has come into rather widespread use for the detection of soft tissue tumors by scanning techniques; more than 50 articles on the clinical diagnostic use of ^{67}Ga have been published during the last year and a half. It is, however, not a tumor-specific agent. For example, recently ^{67}Ga has been used for visualization of abscesses. The basis for its enhanced localization in malignant tissue is not yet known, although considerable work has been carried out by a number of investigators relative to the mechanism involved. It is our purpose in this paper to review the kinetics of ^{67}Ga uptake at the cellular and subcellular levels and to point out some possible pathways that the results of these investigations suggest.

1028340

Hayes, R. L., Washburn, L. C., Wieland, B. W., Sun, T. T., Turtle, R. R., and Butler, T. A. **Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid, a potential agent for cancer detection.** J. Nucl. Med. 17, 748–751, 1976.

Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid (^{11}C-ACPC) has been prepared in multimillicurie amounts. The conversion of H^{11}CN ($T_{1/2}$ ^{11}C = 20.4 min) to ^{11}C-ACPC was accomplished by a rapid (20-min), two-step, high-temperature modification of the Bücherer-Strecker amino acid synthesis technique that should be applicable at other accelerator installations. Purification was by ion exchange techniques. Animal studies have indicated that ^{11}C-ACPC is a potential tumor-localizing agent for the detection of cancer in humans by nuclear medical scanning techniques.

Hilenski, L. L., Walne, P. L., and Snyder, F. **Aliphatic chains of esterified lipids in isolated eyespots of Euglena gracilis var. bacillaris.** Plant Physiol. 57, 645–646, 1976.

Isolated eyespot granules of *Euglena gracilis* Klebs var. *bacillaris* Pringsheim contained approximately 6% lipids (based on protein). Separation of the lipid extracts by thin-layer chromatography revealed four major fractions: wax esters, triacylglycerols, free fatty acids, and phospholipids. Methanolysis of each fraction yielded between 27 and 29 different fatty acids ranging from 12:0 to 22:6. Acetates of the fatty alcohols of the wax fraction consisted of 11:0 to 18:0 carbon chains, with 14:0 being the major component; unsaturated alcohols were not detected.

Hodges, H. D., and Yaden, S. L. **Restraining rats with radiation-induced tail damage.** Lab. Anim. Sci. 26, 244, 1976.

A device to capture and restrain rats without causing further injury to radiation-induced tail damage was designed and constructed. It consists of a 2½- X 12-inch segment of flexible vinyl hose with metal spirals having one end bent at 45° and covered with screen wire. The animal is positioned within the hose, leaving only the tail exposed for experimental study.

Hübner, K. F., Gengozian, N., and Gilman, T. A. **Suppression and recovery of the immune response and CFU potential of mice during and after chronic low-dose-rate gamma irradiation.** Int. J. Radiat. Biol. 28, 301–312, 1975.

Chronic exposure of mice to ^{60}Co γ-rays at rates of 1.6 R/hr and 6 R/hr leads to profound immunosuppression as demonstrated with the Jerne hemolysis-in-agar technique. The immunosuppressive effect is dose-rate dependent. Thus, significant reduction of the number of splenic antibody plaque-forming cells (PFC) to sheep red blood cells is seen after exposure to 1005 R at 1.6 R/hr; at the higher exposure rate of 6 R/hr, PFC suppression is seen after a total dose of only 468 R. Although recovery of the PFC response is possible after accumulated doses of 1005, 1579, and 2138 R at 6 R/hr and 5256 R at 1.6 R/hr, a return to normal immunological capacity is not obtained for a period of 120 days postirradiation. In contrast, recovery of splenic colony-forming units (CFU) as expression of the hematological potential of chronically irradiated mice is found to be much more rapid than the repair of the immune system. Thus, 10 days postirradiation, at a time when little or no recovery in immune competence is apparent, the spleen of the chronically irradiated animal has a CFU capacity approximately 50-fold of that found in the spleen of a normal, unirradiated mouse. The CFU in the chronically irradiated spleens were also shown to be functional by virtue of their ability to protect lethally irradiated animals from hemopoietic death.

Lushbaugh, C. C., and Casarett, G. W. **The effects of gonadal irradiation in clinical radiation therapy: a review.** Cancer 37, 1111–1120, 1976.

Recent improvements in radiation therapy of some malignancies in lower abdominal sites are leading to prolongation of life in persons of childbearing age. These successes require an evaluation of the possible undesirable consequences of the unavoidable gonadal irradiation that occurs in these cases. A review of radiobiological data from experimental animal studies and retrospective clinical studies suggests that in most instances human gonadal exposures in both sexes are insufficient to cause permanent sterility, because the exposures are fractionated and the total gonadal dose is much less than 600 rads. As a consequence, return of fertility must be anticipated, and the worrisome questions of radiation-induced genetic damage is subsequent pregnancies must be addressed. This review did not substantiate this fear, because no case reports could be found of malformed infants among the progeny of previously irradiated parents. Some experimental studies suggest that radiation-damaged spermatogonia are self-destructive, but any evidence for this phenomenon in the ovary is nonexistent. We suggest that the difference between fact and theory here may be the mathematical result of the interplay of low probability for occurrences and the small number of patients who until now have survived long enough for study.

Lushbaugh, C. C., and Swartzendruber, D. C. **Histopathologic aspects of radiation effects on lymphatic tissues and malignancies.** In: Radiation and the Lymphatic System, Proceedings of the 14th Annual Hanford Biology Symposium, 1974, US ERDA Report CONF-740930, 1976, pp. 203–208.

Morphological study with the light microscope remains our most facile and rapid means of tissue identification, diagnosis and staging of diseases, and demonstration of radiation-induced and other toxic effects. The inadequacy of its use alone, however, for the solution of biological problems is nowhere better illustrated than in such studies on lymphatic tissues as are reported in this symposium. Nearly every classical concept concerning lymphocyte biology and disease derived by morphological methods has been challenged or disproved in recent years by applications of nonmorphological technologies. Studies with light and electron microscopy in combination with cell-labeling techniques, histochemical methodology, virology, immunology, and radiation biology have corrected many of our misconceptions and provided unifying concepts of lymphatic tissue structure and function which explain anew our observations of the past. For example, nearly everyone now accepts the biological role of viruses in what once were considered radiation-caused neoplasms in rodents, although whether the role of radiation and other physical and chemical insults in human carcinogenesis is direct or indirect is still to be elucidated. Also, the exact relations that exist between radiation and cancer induction via viruses even in well-studied rodent systems remain to be deter-

1028341

mined; and here morphological studies continue to play an important integrating role for the multidisciplinary studies that are required.

Mainwaring, H. R., Swartzendruber, D. C., Lushbaugh, C. C., Idoyaga-Vargas, N. L., and Watson, E. E. **Radiation effect on ^{67}Ga retention and distribution in mouse tissues.** In: Radiation and the Lymphatic System, Proceedings of the 14th Annual Hanford Biology Symposium, 1974, US ERDA Report CONF-740930, 1976, pp. 28–35.

We have studied the effect of external X-irradiation on the whole-body retention and tissue distribution of gallium-67 (^{67}Ga) in mice over a period of 20 days. Adult male C57BL/6 Cum mice were injected intraperitoneally with 5 μCi ^{67}Ga 24 hr before, immediately before, immediately after, or 24 hr after whole-body X-ray exposure to 450 R. Control mice received ^{67}Ga *injections and sham irradiation. Increased excretion and* reduced soft-tissue concentrations of ^{67}Ga occurred in irradiated mice. The greatest effect was observed when the radionuclide was injected 24 hr after irradiation and assayed 24 hr later. An analysis of variance of the retention data showed that the differences between the groups were significant at the 99% confidence *level.*

Exponential least-squares analysis of whole-body counting results revealed three compartments in the biological retention data. The long-lived compartment possibly corresponds to ^{67}Ga bound relatively tightly in certain body tissues and lost slowly. The two remaining compartments have been tentatively identified as circulating unbound ^{67}Ga that is lost rapidly and circulating bound ^{67}Ga that is lost less rapidly.

Morris, A. C., Jr., Barclay, T. R., Akin, T. E., Hansard, M. C., Gibbs, W. D., and Modzelewski, C. U. **14" X 17" film recorder for computer-enhanced scans.** In: Proceedings of the Sixth Symposium on Sharing of Computer Programs and Technology in Nuclear Medicine, Atlanta, *Georgia, 1976, pp. 401–411, 1976. (Also published as:* US ERDA CONF-760119.1, 1976, pp. 1–20.)

Physician acceptance of computer-enhanced scan results, presented in the form of small Polaroid pictures, has been very *limited, for a number of subjective reasons. We have designed* and constructed a new recorder that presents the results of computer-augmented scans through a medium that is quite familiar to doctors – the standard 14- X 17-inch X-ray film.

In this instrument, each element of a rescan film is exposed to pulsed light from a small argon bulb. The position of this bulb is changed between element exposures by two stepping motors – accuracy of position is held within ±0.002 inch, resulting in a very uniform presentation that is free of the mechanical artifacts so common to "one-pass" scan recording. The maximum lamp-pulse time required for the most dense exposure on the film is 250 msec. Prompt firing of the bulb is assured by supplying background light photons from two light-emitting diodes.

An entire rescanning process is automatic under computer control; once the technician starts a rescan run he is free to do work elsewhere. Films are then processed similar to standard X-rays. Current time to achieve a rescan is 10–20 min, depending on scan size. With uncomplicated mechanical improvements, this time can be reduced to one-half or one-third of these present requirements.

Nelson, B., and Andrews, G. A. **Autologous stem-cell banks for restoring hemopoiesis.** Adv. Radiat. Biol. 6, 325–338, 1976.

Recent developments may modify planning for management of the hemopoietic syndrome after acute total-body irradiation and similar marrow depression from chemotherapy of neoplasms. Despite earlier pessimism about the number of stem cells obtainable from human blood, a 5-hr leukapheresis with the IBM–NIH centrifuge (processing about 10 liters of blood) probably would yield enough cells for autologous hemopoietic restoration. These cells can be preserved at –196°C, although the theoretical expectation that they would survive for many years lacks clinical confirmation. This confirmation is not likely to be obtained quickly from the use of cryopreserved cells for radiation accidents, which are rare. However, leukapheresis and cryopreservation could readily be assessed for autologous hemopoietic restoration in selected patients treated intensively for cancer, thereby obviating the multiple marrow aspirations that now require general or spinal anesthesia. If the efficacy of the administered cells for restoring hemopoiesis in human beings could be correlated with their ability to form colonies in cultures, then in vitro assays could be used to establish the feasibility of long-term cryopreservation.

In any case, the techniques are too expensive to use in preparation for autologous marrow reconstitution after radiation accidents unless high-risk personnel can be identified. Nearly everyone has less risk of serious radiation exposure than of developing a neoplasm that is best managed by chemotherapy *resulting in dangerous marrow injury. Persons with the greatest* likelihood of being helped by prolonged cryopreservation of stem cells are probably those with genetic, or other, predisposition to cancer. The separation and storage of stem cells may someday be an integral part of blood bank services. The use of those cells may provide direct and indirect benefits in patient care that justify the additional expense.

In pointing out clinical applications based in part on anticipated improvements in the techniques for obtaining and separating blood cells into various types, we wish to emphasize the possible benefits to be obtained by collaboration between those scientists interested in cancer and those interested in the better management of radiation accidents.

Newsome, Y. L., and Littlefield, L. G. **Adriamycin-induced chromosomal aberrations in human fibroblasts.** J. Natl. Cancer Inst. 55, 1061–1064, 1975.

Adriamycin (AM) induced chromosome lesions and cell division delay in human foreskin fibroblasts. Cells treated with 0.01, 0.03, and 0.05 μg AM/ml culture medium for 1 hr and evaluated 5–12 hr posttreatment exhibited a wide spectrum of *cytogenetic injuries, ranging from moderately damaged meta*phases with predominantly simple chromatid-type lesions to heavily damaged metaphases with chromosome stickiness and fragmentation. In moderately damaged metaphases that could be scored for specific types of aberrations, we observed a paucity of chromatid exchanges and chromosome-type lesions even in cultures having a very high frequency of breakages. Further, the distribution of breaks among chromosomes within groups A–G appeared to be random, which suggested that the drug does not show breakage specificity in human fibroblasts. The number of heavily damaged metaphases increased with an increase in concentration of AM and with longer periods of recovery.

1028342

Niblack, G. D., and Gengozian, N. **Identification of T and B lymphocytes in the marmoset; a natural haemopoietic chimera.** Clin. Exp. Immunol. 23, 536–543, 1976.

The thymus-derived (T) lymphocyte and bone marrow-derived (B) lymphocyte populations of the marmoset were characterized using specific cell surface markers. Approximately 85% of the thymocytes formed rosettes with neuraminidase-treated sheep erythrocytes (E_n). The percentage (~69%) of peripheral blood lymphocytes (PBL) forming rosettes with E_n was the same as that which stained with fluorescently labeled goat anti-marmoset thymocyte serum (ATS). These two assays identified the same cell population since treatment of cells with ATS and complement resulted in a concomitant decrease in E_n rosette formation. Marmoset PBL also formed rosettes with human erythrocytes sensitized with antibody and complement (HEAC); since the percentage (~20%) of HEAC rosettes was the same as that of cells stained with fluorescently labeled goat anti-marmoset IgG, these cells were considered to be B cells. A small percentage of cells (~1.5%) possessed both types of receptors. The mean percentages of T and B cells present in PBL of single-born, presumably nonchimeric animals were the same as those of isosexual and heterosexual chimeras.

Rock, C. O., and Snyder, F. **Metabolic interrelations of hydroxy-substituted ether-linked glycerolipids in the pink portion of the rabbit harderian gland.** Arch. Biochem. Biophys. 171, 631–636, 1975.

The pink portion of the rabbit harderian gland is known to contain a preponderance of ether-linked glycerolipids consisting primarily of 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols and smaller amounts of 1-alkyl-2,3-diacyl-*sn*-glycerols. In the present study, we have used a combination of chemical, enzymatic, and chromatographic techniques to identify two minor lipid components in the gland as 1-hydroxyalkyl-2-acyl-*sn*-glycerols and 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols. The long-chain acyl groups occurring in the 1-hydroxyalkyl-2-acyl-*sn*-glycerols and 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols are almost exclusively hexadecanoic acid, whereas the 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols have a ratio of hexadecanoic acid to octadecanoic acid of 2/1. The 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols and the 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols also contain a short-chain acyl moiety identified as 3-methylbutanoic acid (isovaleric acid). This acid was found to occupy the 3-position of the glycerol backbone in these lipid classes.

Metabolic experiments demonstrate that 3-methylbutanoic acid in the lipids of the gland is derived from the catabolism of L-leucine. Pulse-chase data show a precursor-product relation between the 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols and 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols and rule out direct hydroxylation of 1-alkyl-2,3-diacyl-*sn*-glycerols as a possible biosynthetic route to the 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols.

Characterization of the alkyl and acyl groups and the positional distributions of the acyl moieties in combination with the metabolic information indicated that the acylation sequence involved in the formation of 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerol is 1-hydroxyalkyl-2-acyl-*sn*-glycerols → 1-hydroxyalkyl-2,3-diacyl-*sn*-glycerols → 1-(*O*-acyl)hydroxyalkyl-2,3-diacyl-*sn*-glycerols. The data also suggest that hydroxylation of the alkyl side-chain occurs before or at the alkylacylglycerol stage.

Snyder, F., and Malone, B. **Transfer of methyl groups from N-dimethylnitrosamine to glycerolipids in rat liver.** Chem.-Biol. Interact. 13, 237–241, 1976.

We have examined the in vivo labeling of lipids after a single intraperitoneal injection of the carcinogen [^{14}C]dimethylnitrosamine into rats. Liver was most active in incorporating [^{14}C]methyl groups into lipids (0.91% of the injected dose) and 80% of the activity appeared in *sn*-3-phosphatidylcholine. Chromatographic analysis of the products (and derivatives) formed after treatment of the [^{14}C]phosphatidylcholine with phospholipase A_2 (EC 3.1.1.4) and phospholipase C (EC 3.1.4.3) demonstrated that 89% of the radioactivity was in the choline moiety. These results indicate that the transfer of methyl groups to lipids occurred via the lipid methylation pathway that converts phosphatidylethanolamine to phosphatidylcholine.

Tyndall, R. L., and Daniel, J. C., Jr. **Alterations in uterine and serum esterases in pregnant mammals.** Fertil. Steril. 26, 1098–1104, 1975.

Uterine flushings, endometrial extracts, and serum from estrous and pregnant rabbits were analyzed by acrylamide gel electrophoresis for esterase activity. Two bands of enzyme activity in uterine flushings were detectable on days 4–7 after coitus. No activity was detectable in washings from nonpregnant or pregnant animals on days 1–3 of pregnancy. Esterase activity was detectable in endometrial extracts of all rabbits. The intensity and complexity of the esterase activity markedly increased on day 3 and persisted through day 7 after coitus, during which time nine to ten bands were apparent compared to four bands in extracts from estrous animals. Serum from pregnant rabbits showed two basic patterns. High esterase activity concentrated in four major bands was seen in some sera while other serum samples showed little esterase activity. No consistent correlation was apparent, however, between the stage of gestation and the serum esterase pattern. In contrast, analysis of mouse serum prior to and after mating showed consistent, reproducible changes in esterase profiles first noticeable about 7 days after coitus and becoming increasingly pronounced by parturition. Changes in mouse serum included both marked enhancement of some esterases and concomitant diminution of others with increasing gestation time.

Tyndall, R. L., Chaskes, S. J., Carlton, J. E., Nelson, B., and Daniel, J. C. **Gallium-67 distribution in pregnant mammals.** J. Exp. Zool. 195, 417–424, 1976.

Studies on the distribution of ^{67}Ga in pregnant rabbits showed a marked concentration of the isotope in uterine tissue and flushings of 5-day pregnant rabbits 24 hr after isotope injection. The isotope no longer localized throughout the uterine tissue and flushings when it was injected into 7- to 8-day pregnant rabbits but was specifically associated with the blastocysts and sites of implantation. As the gestation time progressed, ^{67}Ga increasingly concentrated in placental and mammary tissue while the concentration in serum and thymus tissue decreased. The marked concentration in placental tissue was readily discernible on total-body scans of pregnant rabbits.

Column chromatography of uterine washings and placental extracts from pregnant rabbits and thymus extracts from estrous rabbits showed most of the isotope associated with moieties ≥ 200,000 MW whereas the isotope in "milk" and extracts of mammary tissue was bound to components of 25,000–35,000

1028343

MW. Extracts of thymus from pregnant rats showed a marked, selective loss of ^{67}Ga binding in two peaks of approximately 50,000 and 120,000 MW compared to the chromatographic profile of extracts from normal rat thymus.

Washburn, L. C., Rafter, J. J., Hayes, R. L., and Yuhas, J. M. **Prediction of the effective radioprotective dose of WR-2721 in humans through an interspecies tissue distribution study.** Radiat. Res. 66, 100–105, 1976.

On the basis of earlier tissue distribution data, obtained in mice and rats, we proposed that the tissue distribution of intravenously injected *S*-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) was more closely correlated with the injected dose per unit surface area than it was with the injected dose per unit body weight. To test this proposal, we have compared the tissue distribution of ^{35}S-labeled WR-2721 at 15 and 30 min after intravenous injection in mice, rats, rabbits, and dogs. The results of this study demonstrate that the tissue distribution is not a strict function of either the body weight or the surface area, but rather falls intermediate to the predictions of these parameters. By extrapolation, this observation would predict that a dose of 20 mg of WR-2721 per kilogram of body weight would provide humans with the same amount of protection that 100 mg/kg provides the mouse, i.e., a 50–80% increase in radiation resistance.

1028344

Collaborative Projects Between Medical and Health Sciences Division Staff and Other Institutions

Project	Other Institution[1]; Staff	Division Staff
Radioprotective drug, WR-2721, for possible use in radiotherapy	ETCRC; A. Girardi UTMRC; F. Comas WRAIR; M. Heiffer ORNL; R. Ullrich U Pennsylvania; D. Biery U New Mexico; J. Yuhas	G. Andrews
Radiation sensitivity (cytogenetic) of the marmoset	ORNL; J. 3rewen	N. Gengozian
Fetal hemoglobin synthesis	UTMRC; J. Fuhr	N. Gengozian
Microbiology of fecal flora of marmoset	Memphis St U; G. Peterson	N. Gengozian
Purification of gallium- and indium-binding proteins	Fordham U; DeS. Lawless	R. Hayes
Production and assessment of various radionuclides as imaging agents	ORNL; K. Poggenburg	R. Hayes
Pulmonary disease detection	ETCDH; J. Winebrenner	K. Hübner
US Transuranium Registry	HEHF; P. Fuqua	C. Lushbaugh
Radiotherapy overdose study	U Cincinnati; E. Saenger	C. Lushbaugh
Metabolism of ether-linked glycerolipids	U North Carolina; C. Piantadosi K. Ishaq	F. Snyder
Amine alcohol analogues as membrane modifiers	U North Carolina; C. Piantadosi K. Ishaq	F. Snyder
Effect of NO_2 on lung surfactant metabolism	ORNL; P. Nettesheim	F. Snyder
Mass spectral analysis of unique lipids	ORNL; W. Rainey	F. Snyder
Lipid metabolism in transformed cells	ORNL; A. Hsie	F. Snyder

[1] *Abbreviations:*
ETCRC – East Tennessee Cancer Research Center
UTMRC – University of Tennessee Medical Research Center
WRAIR – Walter Reed Army Institute of Research
ORNL – Oak Ridge National Laboratory
ETCDH – East Tennessee Chest Disease Hospital
HEHF – Hanford Environmental-Health Foundation

1028345


MEDICAL AND HEALTH SCIENCES DIVISION
OAK RIDGE ASSOCIATED UNIVERSITIES
RESEARCH REPORT 1976–1977

1028346

.k Ridge Associated Universities (ORAU) is a private, not for profit association of 45 colleges and universities. Established in 1946, it was one of the first university-based, science-related, corporate management groups. It conducts programs of research, education, information, and training for a variety of private and governmental organizations. ORAU is noted for its cooperative programs and for its influence on the development of science in the South.

The Medical and Health Sciences Division conducts interrelated research programs in immunobiology, biochemistry, radiopharmaceuticals, epidemiology, and radiation biology that focus on chemical toxicants and radiation problems related to energy production and development. Unique facilities include REAC/TS (Radiation Emergency Assistance Center/Training Site—the Department of Energy radiation accident treatment center) and a marmoset research colony. In addition to research, pre- and postdoctoral training is provided in lipid-membrane biochemistry, immunobiology, cellular and radiation biology, and nuclear medicine.

1028347

ERRATUM

Page 4, Epidemiology. The last sentence of the first paragraph under this heading should read: *The mortality experience of these workers is being compared on a year-sex-race-age-specific basis with normal U. S. population mortality.*

Foreword

This report describes research progress in the Medical and Health Sciences Division for the period October 1, 1976, through September 30, 1977. The major research effort with Department of Energy (DOE, formerly ERDA) support continues to be focused on six major program areas: human radiation biology, epidemiologic techniques to identify and lessen occupational dangers related to energy production and radiation, biochemical studies to understand the role of surfactant production in the prevention of pulmonary disease, biochemical techniques for modifying cellular membranes with the aim of altering cellular responses to toxic and carcinogenic compounds, immunological and cellular approaches to understanding cell surveillance systems with the aim of achieving protection against cancer, and development of improved agents for detecting various environmental cancers and chronic lung diseases. The scientific research groups responsible for developing and carrying out these programs are the Radiation Emergency Assistance Center/Training Site (REAC/TS), Immunobiology, Biological Chemistry, Radiopharmaceutical Development, and Epidemiology. These units also participate in various biomedical training programs, which include high school, undergraduate, predoctoral, and postdoctoral participants. The Division also assumes a major responsibility for DOE in maintaining a national radiation accident registry as part of the REAC/TS program and collaborates with other DOE facilities in developing health and mortality studies for DOE.

The reorganization of the Medical and Health Sciences Division in 1975 led to a redirection of our research efforts in response to the broadened role of DOE's responsibilities for environmental and biomedical problems related to the development of new energy supplies from fossil fuels. Since 1975, newly identifiable service units have evolved; these are now emphasized in the new organizational chart of employees (Section 6). Newly identifiable research units and needs are also beginning to surface. For instance, we expect cytogenetics, which in the past was principally concerned with radiation dosimetry in the REAC/TS program, to eventually develop as an independent research unit, apart from the REAC/TS program, emphasizing chemical rather than radiation damage. We are forming the experimental pathology unit, which currently provides service (including veterinary, pathology, and electron microscopy services) and research input into various Division research programs. The need for a toxicologist and a cell culture biologist to augment our research efforts has become increasingly urgent; we envision that this new manpower and these areas of expertise will be assimilated into the administrative structure of the experimental pathology unit.

During 1977, excellent new centralized quarters for experimental animals were completed. Major efforts are now under way to complete the ancillary facilities, such as specific rooms for surgery and laboratory work. Completion of these areas is expected to be the last step in receiving accreditation from the National Institutes of Health (NIH) for the entire ORAU animal facility. The veterinary science program has planned a husbandry program and an expanded facility for marmoset breeding. The facility will be used as an area resource for biomedical research purposes. Funding for the proposed facility at ORAU is currently being considered by NIH and other organizations. In addition to modernizing our animal facilities, a major renovation of research laboratories for the biological chemistry group is now almost complete. This $280,000 project will relocate the biological chemistry program at the Vance Road site.

In conclusion, we want to call attention to the diversified funding sources (see Section 5) for the research programs described in this report. Whereas the primary financial support for the Division comes from DOE, a number of related research projects are funded by other government and private agencies.

C. C. Lushbaugh, *Chairman* / Fred Snyder, *Assistant Chairman* / October 1977

1028348

Contents

Foreword i

1 Scope of Research Programs 1

2 Publications 5

3 Abstracts of Full-Length Publications 10

4 Collaborative Projects 18

5 Grant-Funded Research 19

Organization and Staff 20

1028349

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1028350

1 Scope of Research Programs



A. Immunobiology

The immunology program is concerned with the effects of irradiation and chemical pollutants on immunological processes at the cellular level. In vitro and in vivo studies are conducted in two mammalian systems—the mouse and the marmoset. A primary facet of our models is the phenomenon of hemopoietic chimerism, a physiologic state that occurs naturally in the marmoset and is induced in the mouse by lethal irradiation followed by bone marrow transplantation. The immunologic tolerance in each of these hemopoietic systems provides an excellent background against which subtle alterations induced in the

1028351

immune system can be measured. The allogeneic radiation chimera has proven to be an excellent animal model for studying questions relative to the effect of environmental hazards on the immune system. Although complete immunologic tolerance exists in the marmoset, T and B lymphocyte function can be, and most frequently is, abnormal. A primary question answered this year concerned the possible involvement of T cells in immunologic tolerance. A strong suppressor function was found for this cellular element suggesting, but not proving, that in this model tolerance may be conveyed by T suppressor cells. Surprisingly, however, this suppression was nonspecific, inhibiting cells from mouse strains unrelated to the chimera and also suppressing immune functions unrelated to the transplant phenomenon.

Studies in the marmoset continue to demonstrate a difference in immune competence of the white-lipped marmoset (*S. fuscicollis*) and the cotton-topped marmoset (*S. o. oedipus*). Extensive in vitro studies on the response capacities of the T lymphocytes indicate the T cell compartment of the immune system operates in an equivalent fashion in both species.

Isoimmunizations with white blood cells in the white-lipped and cotton-topped marmosets have now yielded reagents capable of distinguishing among members of each species. The markers currently provide a means of establishing leucocyte chimerism independent of sex. Whether the antigens detected function as histocompatibility antigens is not known and is currently being explored in mixed-leucocyte culture studies.

B. Biological Chemistry

The research program in biological chemistry focuses primarily on membrane properties and lipid metabolism in cancer and in respiratory diseases induced by chemical toxicants derived from fossil fuels. Current projects include the following: (1) the cellular consequences resulting from modifying biological membranes induced by fossil fuel pollutants and unnatural lipid analogs, (2) pulmonary surfactant metabolism in normal lungs and lungs exposed to nitrogen dioxide, (3) the role of ether linkages in lipids of cancer cell membranes, (4) penetration of membrane barriers with lipophilic chelating agents for intracellular removal of plutonium, (5) biochemical mapping of intracellular membranes with respect to the location of enzymes and lipids associated with their inner and outer surfaces, and (6) lipids and their precursors as regulatory molecules in nucleic acid and protein metabolism.

Our studies on the manipulation of membrane properties have shown it is possible to cause drastic modification of membrane phospholipids by incorporating unnatural analogs of amino alcohols (e.g., N-isopropylethanolamine) or fatty acids (e.g., chaulmoogric acid). Such alterations can modify specific biochemical properties of biological membranes; preliminary data indicate cell division and the cell cycle can also be altered by lipid precursor analogs. Toxicants (such as nitrogen dioxide) derived from fossil fuels also modify membrane lipid composition. Our experiments are designed to pinpoint the biochemical and biological consequences of such modifications, particularly in relation to degenerative processes and abnormal cell proliferation. We have found that N-isopropylethanolamine, an analog of choline, is a potent inhibitor of choline-mediated cellular processes, some of which are associated with cell cycle events. The lung studies have continued to concentrate on how and where surfactant is synthesized in alveolar type II cells. The effect of nitrogen dioxide on the pulmonary surfactant system indicates that altered surfactant metabolism is a

1028352

sensitive indicator of nitrogen dioxide exposure. Results of studies on the role of ether lipids in cancer cells have revealed that the enzymes responsible for the biosynthesis of these unique lipids are distributed asymmetrically in membrane vesicles. A new project initiated recently is designed to explore the usefulness of lipid carriers in getting chelating agents across membrane barriers so that plutonium and other toxic metals can be removed from intracellular compartments.

C. Radiopharmaceutical Development

The objective of this program is to develop new radiopharmaceuticals for potential use in man. Most of these agents are intended for diagnostic purposes, but occasionally therapeutic agents are also studied. The identification of new radiopharmaceutical agents is based on established metabolic pathways and biochemical processes occurring in healthy and diseased states. Once a potential agent is identified, it is synthesized in a radiolabeled form and tested in animals for effectiveness and safety. When effectiveness and safety have been proven, the compounds are tested in human beings after approval by the Food and Drug Administration. Experiments are also performed to clarify the mechanisms involved in locating radiopharmaceuticals by disease processes. Collaborative research projects involve Oak Ridge National Laboratory and medical institutions affiliated with ORAU.

A major addition to the resources of the Division this year has been the purchase and installation of a new instrument for positron tomography, the Emission Computerized Axial Tomograph (ECAT) manufactured by ORTEC Inc. When positron-emitting radiopharmaceuticals decay in the body, they produce annihilation photons that travel in opposite directions. These photons can be detected externally by coincidence counting. By viewing these emissions from various angles in a coincidence mode, it is possible to use computer techniques to reconstruct three-dimensional cross-sectional pictures of the tissue distribution of positron-emitting radiopharmaceuticals. The ECAT has been successfully used in patients who received [^{11}C] 1-aminocyclopentanecarboxylic acid ([^{11}C] ACPC) for visualizing tumors and [^{11}C] DL-valine and [^{11}C] DL-tryptophan for imaging the pancreas. Carbon-11 is a positron emitter having a half-life of 20.4 minutes.

Our study of [^{11}C]ACPC using conventional rectilinear scanning techniques has now been expanded to a total of 41 patients. Even without the advantages of the coincidence detection mode (and in spite of the handicaps involved with high energy gamma emissions [511 kev]), [^{11}C] ACPC has been shown to be a good tumor-scanning agent.

The most promising of other radionuclides under investigation is gallium-68, which can be obtained from a radionuclide generator. Germanium-68, the radionuclide parent of gallium-68, has a long half-life (285 days), thereby affording a steady supply of gallium-68. Various chemical forms of gallium-68 radiopharmaceuticals are being considered for positron tomography. Recent data obtained with the ECAT have demonstrated that the location of a brain hemorrhage can be readily detected with [^{68}Ga] ethylenediaminetetraacetic acid; the ECAT has also given excellent visualization of a number of tumors with [^{68}Ga] citrate. A colloid labeled with gallium-68 is now being developed for imaging the normal liver.

1028353

D. Radiation Emergency Assistance Center/Training Site (REAC/TS)

The REAC/TS facility has been fully operational since its completion in June 1976. This past year a lead and steel shielded operating and autopsy table was fabricated and a high-/medium-level whole body counter was installed. The facility has been tested in a number of training courses and in-house accident drills and exercises that established confidence for actual hands-on medical management of radiation accidents. All REAC/TS program activities are now carried out at the new facility.

Medical and paramedical personnel training was conducted exclusively at the new facility during the past year. Approximately 100 persons including physicians, health physicists, and emergency technologists were trained in radiation-accident management.

REAC/TS radiation accident readiness is now a reality through DOE's Radiation Assistance Programs. Specifically, REAC/TS has assigned responsibilities for seven private nuclear power and reprocessing companies, seven installations of non-DOE government contractors, five ORAU-affiliated universities, the Oak Ridge Union Carbide Corporation–Nuclear Division plants, plus four DOE-operated naval reactor programs. Based on these agreements and direct requests by states and private institutions, REAC/TS gave assistance in 18 radiation incidents involving 43 persons this year.

In addition to its service and training commitments, the REAC/TS staff continued research projects in the cytogenetic methods of evaluating radiation exposure and genetic risk estimates as well as exposure to environmental or occupational toxicants. Studies of radiation-induced vascular disease and gangrene in animal models have continued using nuclear medical methods and ultrasound techniques for early detection of radiation burns.

REAC/TS is playing a leading role in quality control and distribution of diethylenetriaminopentaacetic acid (DTPA) for chelation therapy. The work is supported by additional research projects concerning toxic and chemical properties of DTPA and its modification for safer and more effective use in humans.

E. Epidemiology

The Epidemiology program became operational this year and assumed direction of the DOE (formerly ERDA) health and mortality studies on August 1, 1977. Researchers continually evaluate the mortality experience of persons who have been employed in selected installations associated with the nuclear industry (i.e., workers at Oak Ridge; National Lead of Ohio; Mallinckrodt in Missouri; and Mound Laboratory in Ohio). The mortality experience of a year-sex-race-age-specific basis with normal U.S. population mortality.

A special study of the mortality experience of workers with a history of exposure to metallic nickel is being conducted this year at the Oak Ridge Gaseous Diffusion Plant in cooperation, with Union Carbide Corporation–Nuclear Division. The mortality in these workers is being compared to that of other workers at Oak Ridge Gaseous Diffusion Plant without exposure to nickel or its compounds.

The epidemiology program will concentrate on evaluating the effects of occupational exposure to hazards found in energy production facilities. Although the current program is limited to workers in nuclear facilities, plans are being made to evaluate workers in coal conversion plants as these plants come into production.

1028354

2 Publications



All abstracts (papers presented at meetings, regardless of publication), journal articles, review articles, books, chapters, and doctoral dissertations by Division personnel are listed here. All material in press at this time is included and is so indicated. For abstracts of full-length publications, see Section 3.

Andrews, G. A.; Hübner, K. F.; Washburn, L. C.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.; and Butler, T. A. 1976. Clinical tumor scanning with [^{11}C]carboxyl-labeled 1-aminocyclopentanecarboxylic acid ([^{11}C]ACPC). In Proceedings of the 17th Annual Meeting. Southeastern Chapter, Society of Nuclear Medicine, Louisville, Kentucky. Abstract.

Andrews, G. A.; Hübner, K. F.; Washburn, L. C.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.;

1028355

Butler, T. A.; and Collmann, I. R. 1977. Clinical studies of ^{11}C-labeled amino acids. J. Nucl. Med. 18:638. Abstract.

*Armstrong, R. D., and Frome, E. L. 1976. A branch and bound solution of a restricted least-squares problem. Technometrics 18:447-450.

*Armstrong, R. D., and Frome, E. L. 1976. The calculation of least absolute value estimators for two-way tables. In Proceedings of the Statistical Computing Section, American Statistical Association. Boston, Massachusetts, pp. 101-106.

*Armstrong, R. D., and Frome, E. L. 1976. A comparison of two algorithms for absolute deviation curve fitting. J. Am. Stat. Assoc. 71:328-330.

*Armstrong, R. D., and Frome, E. L. 1977. Robust estimation of spline functions in data analysis. Presented at the Joint Central Regional Meeting, American Statistical Association and the Institute of Mathematical Statistics, Madison, Wisconsin.

Baker, R. C.; Wykle, R. L.; Lockmiller, J. S.; and Snyder, F. 1976. Identification of a soluble protein stimulator of plasmalogen biosynthesis and stearoyl-coenzyme A desaturase. Arch. Biochem. Biophys. 177:299-306.

Blank, M. L.; Lee, T-c.; Piantadosi, C.; Ishaq, K. S.; and Snyder, F. 1976. Membrane lipid modification and stearoyl-coenzyme A desaturase activity in L-M cells. Arch. Biochem. Biophys. 177:317-322.

Blank, M. L.; Rainey, W. T., Jr.; Christie, W. H.; Piantadosi, C.; and Snyder, F. 1976. Mass spectral analysis of a homologous series of S- and 0-alkylglycerols. Chem. Phys. Lipids. 17:201-206.

*Cabot, M. C., and Gatt, S. 1977. Rat brain microsomal lipase activity with an acid pH optimum. Presented at the Centre National de la Recherche Scientifique, International Colloquium on Enzymes of Lipid Metabolism. Strasbourg, France.

*Cabot, M. C., and Gatt, S. In press. Rat brain microsomal lipase activity. In Proceedings of the Colloquium on Enzymes of Lipid Metabolism (P. Mandel, L. Freysz, and S. Gatt, eds.). Plenum Press, New York.

*Charnes, A.; Frome, E. L.; and Yu, P. L. 1976. The equivalence of generalized least squares and maximum likelihood estimation in the exponential family. J. Am. Stat. Assoc. 71:169-172.

Dosado, E. A.; Hsie, A. W.; and Snyder, F. In press. Modification of phospholipid metabolism in dibutyryl-cAMP-mediated morphological conversion of CHO cells. Biochem. Biophys. Res. Commun.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L. 1976. Variations in the frequency of induced aberrations in rabbit somatic cells treated with a chemical clastogen. Excerpta Med. Int. Congr. Ser. 397:120. Abstract.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L. In press. Chemically induced cytogenetic damage in germinal and somatic cells from the female rabbit. Mutat. Res.

*Frome, E. L., and Armstrong, R. D. 1977. A robust procedure for estimating the trend-cycle component of an economic time series. In Proceedings of Tenth Annual Symposium on the Computer Science and Statistics Interface. Gaithersburg, Maryland.

*Frome, E. L., and Yakatan, G. J. 1976. Fitting the one compartment open model to bioavailability data. In Ninth International Biometric Conference. Boston, Massachusetts, p. 19. Abstract.

Fuhr, J. E.; Gengozian, N.; and Overton, M. 1977. In vitro stimulation of primate hemoglobin synthesis by L-thyroxine. Blood 49:407-413.

Gengozian, N. In press. Immunology and blood chimerism of the marmoset. In Primates in Medicine (series edited by E. I. Goldsmith and J. Moor-Jankowski). Proceedings of a Conference on Marmosets in Experimental Medicine (N. Gengozian and F. Deinhardt, conference eds.). Oak Ridge, Tennessee. 1977. Karger, Basel, Switzerland.

Gengozian, N., and McLaughlin, C. L. 1977. Actively induced platelet-bound IgG associated with thrombocytopenia in the marmoset. Fed. Proc. 36:379. Abstract 578.

Gengozian, N., and Urso, P. 1976. Status of T- and B-cell cooperation in radiation chimeras: vidence for a suppressor effect. Transplant. Proc. 8:631-635.

*Work done outside ORAU.

1028356

Gengozian, N.; Batson, J. S.; and Smith, T. A. In press. Breeding of the marmoset in the laboratory. In The Biology and Conservation of the Callithricidae (D. G. Kleiman, ed.). Smithsonian Press, Washington, D. C.

Gengozian, N.; Batson, J. S.; and Smith, T. A. In press. Breeding of marmosets in a colony environment. In Primates in Medicine (series edited by E. I. Goldsmith and J. Moor-Jankowski). Proceedings of a Conference on Marmosets in Experimental Medicine (N. Gengozian and F. Deinhardt, conference eds.), Oak Ridge, Tennessee. 1977. Karger, Basel, Switzerland.

Hayes, R. L. 1976. Factors affecting uptake of radioactive agents by tumor and other tissues. In Tumor Localization with Radioactive Agents (Proceedings of the International Atomic Energy Agency Advisory Group Meeting, Vienna, Austria, 1974), pp. 29-45.

Hayes, R. L. 1977. The tissue distribution of gallium radionuclides. J. Nucl. Med. 18:740-742.

Hayes, R. L. In press. Chemistry and radiochemistry of metal ion nuclides commonly employed in radiopharmaceuticals. In Symposium on the Chemistry of Radiopharmaceuticals. Hahnemann Medical College, Philadelphia, Pennsylvania, 1976.

Hayes, R. L., and Byrd, B. L. 1976. A comparison of the deposition of various tumor-localizing agents in inflammatory tissue. In Proceedings of the 17th Annual Meeting. Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures). Louisville, Kentucky. Abstract.

Hayes, R. L.; Washburn, L. C.; Wieland, B. W.; and Sun, T. T. 1976. Carboxyl-labeled [^{11}C]ACBC, a possible new radiopharmaceutical for detection of cancer using positron tomography. J. Nucl. Med. 18:639. Abstract.

Hayes, R. L.; Washburn, L. C.; Sun, T. T.; Rafter, J. J.; and Byrd, B. L. 1977. Factors affecting the tissue distribution of [^{11}C]ACPC, a new tumor-localizing agent. In Proceedings of the 17th Annual Meeting. Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures). Louisville, Kentucky. Abstract.

Hayes, R. L.; Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Anon, J. B.; Butler, T. A.; and Callahan, A. P. In press. Synthesis and purification of [^{11}C]carboxyl-labeled amino acids labeled with carbon-11. Int. J. Appl. Radiat. Isot.

Hodges, H. D.; Holloway, S.; and Gibbs, W. D. 1977. Participation of nuclear medicine technologists in radiation accident management. J. Nucl. Med. Technol. 5:46-49.

Hodges, H. D.; Holloway, S.; and Gibbs, W. D. In press. Reducing CO_2 inhalation in clinical pulmonary investigations. J. Nucl. Med. Technol.

Hübner, K. F.; Andrews, G. A.; Lushbaugh, C. C.; and Tompkins, E. In press. A follow-up study program for persons irradiated in radiation accidents. In Proceedings of the International Atomic Energy Agency Symposium on the Handling of Radiation Accidents, Vienna, Austria, 1977.

Hübner, K. F.; Andrews, G. A.; Hayes, R. L.; Poggenburg, J. K., Jr.; and Solomon, A. In press. Use of rare earth radionuclides and other bone-seekers in evaluating bone lesions in patients with multiple myeloma and solitary plasmacytoma. Radiology.

Hübner, K. F.; Andrews, G. A.; Washburn, L. C.; Wieland, B. W.; Gibbs, W. D.; and Hayes, R. L. In press. Tumor localization with [^{11}C]carboxyl-labeled 1-aminocyclopentanecarboxylic acid ([^{11}C]ACPC); preliminary clinical trials with single-photon detection. J. Nucl. Med.

Johnston, G. S.; Go, M. F.; Benua, R. S.; Larson, S. M.; Andrews, G. A.; and Hübner, K. F. 1977. Gallium-67 citrate imaging in Hodgkin's disease: Final report of cooperative group. J. Nucl. Med. 18:692-698.

Kateley, J. R.; Nickerson, D. A.; and Gengozian, N. In press. Blastogenic response of marmoset lymphocytes: Cytokinetics and identification of responsive cells. Immunology.

Lee, T-c., and Snyder, F. 1977. Cancer cells. In Lipid Metabolism in Mammals (F. Snyder, ed.). Vol. II. Plenum Press, New York. pp. 293-310.

Lee, T-c.; Baker, R. C.; and Stephens, N. 1977. Evidence for participation of cytochrome b_5 in microsomal Δ-6 desaturation of fatty acids. Fed. Proc. 36-672. Abstract 2113.

Lee, T-c.; Baker, R. C.; Stephens, N.; and Snyder, F. In press. Evidence for participation of cytochrome b_5 in microsomal Δ-6 desaturation of fatty acids. Biochim. Biophys. Acta.

Lincoln, T. A., and Lushbaugh, C. C. In press. Organization, planning and training. In Manual on First-Aid Treatment of Radiation Accidents (J. C. Nenot, ed.). Report of a meeting sponsored by the International Atomic Energy Agency, World Health Organization, and International Labor Organization. Geneva, Switzerland. 1976.

Littlefield, L. G., and Joiner, E. E. 1976. Cytogenetic follow-up studies in 6 radiation accident

1028357

victims (16 years postexposure). Excerpta Med. Int. Congr. Ser. 397:137. Abstract 358.

Lushbaugh, C. C.; Humason, G. L.; Swartzendruber, D. C.; Richter, C. B.; and Gengozian, N. In press. Spontaneous colonic adenocarcinoma in marmosets. In Primates in Medicine (series edited by E. I. Goldsmith and J. Moor-Janowski). Proceedings of a Conference on Marmosets in Experimental Medicine (N. Gengozian and F. Deinhardt, conference eds.). Oak Ridge, Tennessee. 1977. Karger, Basel, Switzerland.

*Machlin, L. J.; Garcia, F.; Kuenzig, W.; Richter, C. B.; Spiegel, H. E.; and Brin, M. 1976. Lack of antiscorbutic activity of ascorbate 2-sulfate in the rhesus monkey. Am. J. Clin. Nutr. 29:825-831.

McDermott, C. E. 1977. The effect of chronic irradiation on T and B lymphocytes. Dissertation for Doctor of Philosophy Degree, University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences. N. Gengozian, advisor.

Moore, C.; Blank, M. L.; Lee, T-c.; Benjamin, B.; Piantadosi, C.; and Snyder, F. In press. Membrane lipid modifications: Biosynthesis and identification of phosphatidyl-N-methyl-N-isopropylethanolamine in rat liver microsomes. Chem. Phys. Lipids.

Morris, H. B.; Baker, R. C.; Wykle, R. L.; and Snyder, F. 1976. Effect of an unnatural phospholipid analog in microsomal membranes on cytochrome *P*-450. Presented at the 11th Annual Regional Lipid Conference, Winston-Salem, North Carolina.

Nelson, B. M.; Andrews, G. A.; and Watson, E. E. In press. Histopathologic studies of the human liver after intravenous colloidal ^{198}Au therapy. Radiology.

Nelson, B.; Calvo, W.; Fliedner, T. M.; Herbst, E.; Bruch, C.; Schnappauf, H-P.; and Flad, H-D. 1976. The repopulation of lymph nodes of dogs after 1200 R whole-body X-irradiation and intravenous administration of mononuclear blood leukocytes. Am. J. Pathol. 84:259-282.

Niblack, C. D.; Kateley, J. R.; and Gengozian, N. 1977. T- and B-lymphocyte chimerism in the marmoset. Immunology. 32:257-263.

Nickerson, D. A., and Gengozian, N. 1976. Primary in vitro antibody formation by blood leucocytes of a subhuman primate. Cell. Immunol. 27:171-176.

*Phelps, M. E.; Hoffman, E. J.; Highfill, R. R.; and Kuhl, D. E. 1977. A new emission computed axial tomograph for positron emitters. J. Nucl. 18:603. Abstract.

Richter, C. B., and Humason, G. L. In press. Endemic *Pneumocystis carinii* in a marmoset colony. J. Comp. Pathol.

Richter, C. B.; Humason, G. L.; and Tankersley, B. 1976. Acanthocephaliasis in caged marmosets. Presented at the 27th Annual Session, American Association for Laboratory Animal Science. Houston, Texas.

Richter, C. B.; Humason, G. L.; and Lushbaugh, C. C. 1976. Endemic *Pneumocystis carinii* infection in marmosets. Presented at the 27th Annual Session, American Association for Laboratory Animal Science. Houston, Texas.

Richter, C. B.; Lushbaugh, C. C.; Humason, G. L.; and Swartzendruber, D. C. 1977. Adenocarcinoma of the colon in cotton-topped marmosets. Presented at the 1st Annual Meeting of the American Society of Primatologists. Seattle, Washington.

Rock, C. O. 1977. The harderian gland. In Lipid Metabolism in Mammals (F. Snyder, ed.). Vol. II. Plenum Press, New York, pp. 311-321.

Rock, C. O., and Snyder, F. 1976. Localization of dihydroxyacetone phosphate acyltransferase. Presented at the 11th Annual Regional Lipid Conference. Winston-Salem, North Carolina.

Rock, C. O., and Snyder, F. 1977. Localization and properties of dihydroxyacetone phosphate acyltransferase. Fed. Proc. 36:850. Abstract 3049.

Rock, C. O., and Snyder, F. In press. Asymmetric localization of alkyldihydroxyacetone-P synthase and acyldihydroxyacetone-P acyltransferase in microsomal vesicles. In Proceedings of the Colloquium on Enzymes of Lipid Metabolism (P. Mandel, L. Freysz, and S. Gatt, eds.). Plenum Press, New York.

Rock, C. O.; Fitzgerald, V.; and Snyder, F. 1977. Activation of alkyldihydroxyacetone phosphate synthase by detergents. Arch. Biochem. Biophys. 181:172-177.

Rock, C. O.; Fitzgerald, V.; and Snyder, F. In press. Properties of dihydroxyacetone phosphate transferase in the harderian gland. J. Biol. Chem.

Rock, C. O.; Baker, R. C.; Fitzgerald, V.; and Snyder, F. 1976. Stimulation of the microsomal alkylglycerol monooxygenase by catalase. Biochim. Biophys. Acta. 450:469-473.

*Work done outside ORAU.

1028358

Rock, C. O.; Fitzgerald, V.; Rainey, W. T., Jr.; and Snyder, F. 1976. Mass spectoral identification of 2-(*O*-acyl)hydroxy fatty acid esters in the white portion of the rabbit harderian gland. Chem. Phys. Lipids. 17:207-212.

*Sherman, M. I.; Miller, R. A.; and Richter, C. B. 1977. Histologic analyses of experimental tumors from mouse blastocyst-derived cell lines. J. Natl. Cancer Inst. 58:993-1002.

Scott, C. C.; Heckman, C. A.; Nettesheim, P.; and Snyder, F. In press. Lipid turnover in squamous-cell carcinoma cells and primary tracheal epithelial cells. J. Cell Biol. Abstract.

Snyder, F. (ed.) 1977. Lipid Metabolism in Mammals. Volumes I and II. Plenum Press, New York.

Snyder, F., and Rock, C. O. 1977. Detergent studies of microsomal enzymes involved in the synthesis of ether lipids. Presented at the Centre National de la Recherche Scientifique, International Colloquium on Enzymes of Lipid Metabolism. Strasbourg, France.

Urso, P., and Gengozian, N. 1977. Immune competence of splenic lymphocytes following graft-vs-host disease in mouse allogeneic radiation chimeras. J. Immunol. 118:657-661.

Urso, P., and Gengozian, N. 1977. Suppression of GVH and PFC responses by spleen cells from radiation chimeras. Fed. Proc. 36:1267. Abstract 5302.

Voelker, D. R.; Lee, T-c; and Snyder, F. 1976. Fatty acid biosynthesis and dietary regulation in pulmonary adenomas. Arch. Biochem. Biophys. 176:753-756.

Washburn, L. C., and Hayes, R. L. In press. Importance of excess base in the synthesis of sodium phosphorothioate. Inorganic Syntheses.

Washburn, L. C.; Sun, T. T.; Wieland, B. W.; and Hayes, R. L. 1977. [^{11}C]Tryptophan, a potential pancreas-imaging agent for positron tomography. J. Nucl. Med. 18:638. Abstract.

Washburn, L. C.; Wieland, B. W.; Sun, T. T.; and Hayes, R. L. 1976. Synthesis and preclinical investigations of DL-valine-1-^{11}C, a new agent for pancreatic imaging. In Proceedings of the 17th Annual Meeting. Southeastern Chapter, Society of Nuclear Medicine. Louisville, Kentucky. Abstract.

Washburn, L. C.; Wieland, B. W.; Sun, T. T.; and Hayes, R. L. 1977. ^{11}C-Labeled amino acids as agents for tumor and pancreas visualization. J. Labelled Comp. Radiopharm. 13:203. Abstract.

Washburn, L. C.; Coffey, J. L.; Watson, E. E.; Sun, T. T.; and Hayes, R. L. 1976. Radiation dosimetry of some ^{11}C-labeled amino acid radiopharmaceuticals. In Radiopharmaceutical Dosimetry Symposium. Oak Ridge, Tennessee, 1976. Proceedings of a Conference (R. J. Cloutier, J. L. Coffey, W. S. Snyder, and E. E. Watson, eds.). United States Department of Health, Education, and Welfare, Washington, D. C., HEW Publication (FDA)76-8044, pp. 441-451.

Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Hayes, R. L.; and Butler, T. A. In press. DL-Valine-1-^{11}C, a potential pancreas-imaging agent. J. Nucl. Med.

Wieland, B. W.; Washburn, L. C.; Turtle, R. R.; Hayes, R. L.; and Butler, T. A. 1977. Development of cyclotron targetry and remote radiochemical techniques for the continuous large-scale production of ^{11}C-labeled amino acids. J. Labelled Comp. Radiopharm. 13:202. Abstract.

Wykle, R. L. 1977. Brain. In Lipid Metabolism in Mammals (F. Snyder, ed.). Vol. 1. Plenum Press, New York, pp. 317-366.

Wykle, R. L., and Kraemer, W. F. 1977. Glycerol kinase activity in adenoma alveolar type II cells. FEBS Lett. 78:83-85.

Wykle, R. L.; Kraemer, W. F.; and Schremmer, J. M. In press. Studies of lysophospholipase D of rat liver and other tissues. Arch. Biochem. Biophys.

Wykle, R. L.; Malone, B.; and Snyder, F. 1976. Biosynthesis of dipalmitoyl-*sn*-glycero-3-phosphocholine. Presented at the 11th Annual Regional Lipid Conference. Winston-Salem, North Carolina.

Wykle, R. L.; Malone, B.; and Snyder, F. 1977. Biosynthesis of dipalmitoyl-*sn*-glycero-3-phosphocholine by adenoma alveolar type II cells. Arch. Biochem. Biophys. 181:249-256.

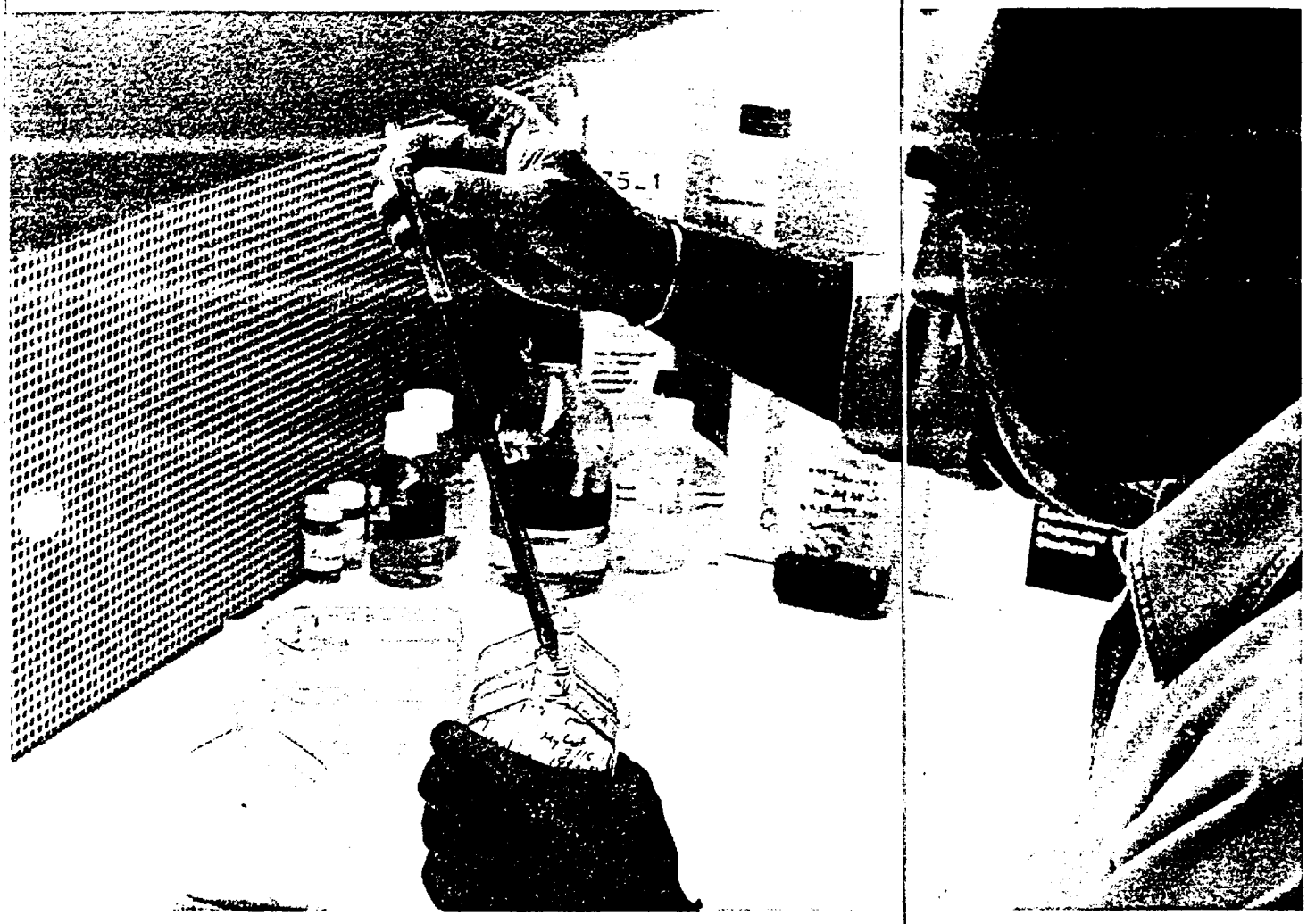
*Yakatan, G. J.; Smith, R. B.; Frome, E. L.; and Doluisio, J. T. 1977. Pharmacokinetics of orally administered hydroflumethiazide in man. J. Clin. Pharmacol. 17:37-47.

Yang, W. K.; Tyndall, R. L.; and Daniel, J. C., Jr. 1976. DNA- and RNA-dependent DNA polymerases: Progressive changes in rabbit endometrium during preimplantation stage of pregnancy. Biol. Reprod. 15:604-613.

*Work done outside ORAU.

1028359

3 Abstracts of Full-Length Publications



This section consists of abstracts of full-length publications. Only published original articles and review articles that contain previously unpublished data are included.

*Armstrong, R. D., and Frome, E. L. 1976. A branch and bound solution of a restricted least-squares problem. Technometrics 18:447-450.

The note proposes an algorithm to solve a least-squares problem when the parameters are restricted to be nonnegative. The algorithm does not use linear programming but utilizes the normal equations to solve a series of unrestricted problems.

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1028360

*Armstrong, R. D., and Frome, E. L. 1976. A comparison of two algorithms for absolute deviation curve fitting. J. Am. Stat. Assoc. 71:328-330.

Linear programming is compared with weighted least-squares approaches to absolute deviation curve fitting. The computational results show that linear programming substantially reduces solution times while requiring only a relatively small amount of additional storage.

Baker, R. C.; Wykle, R. L.; Lockmiller, J. S.; and Snyder, F. 1976. Identification of a soluble protein stimulator of plasmalogen biosynthesis and stearoyl-coenzyme A desaturase. Arch. Biochem. Biophys. 177:299-306.

Stimulation of the desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (GPE), which forms ethanolamine plasmalogens, by a component of the 105,000*g* supernatant has been previously reported. The stimulatory protein has been isolated and identified as catalase. Purified rat liver catalase or commercial bovine liver catalase is as effective in stimulating microsomal 1-alkyl-2-acyl-GPE desaturation as the soluble proteins. The stimulatory effect of these proteins is eliminated by catalase inhibitors. It appears that catalase stimulates the desaturation of 1-alkyl-2-acyl-GPE by preventing inactivation of the enzyme system by H_2O_2 or a decomposition product of H_2O_2. The cytochrome b_5 content and NADH oxidation are depressed in Fischer R-3259 sarcoma microsomes by H_2O_2; this effect is eliminated by catalase. However, since measurable inhibition of 1-alkyl-2-acyl-GPE desaturase by H_2O_2 still occurred in the presence of catalase, the inhibition by H_2O_2 cannot be explained solely on the basis of cytochrome b_5 inactivation. The desaturation of stearoyl-coenzyme A, a reaction analogous in many respects to 1-alkyl-2-acyl-GPE desaturation, was also found to be stimulated by catalase.

Blank, M. L.; Lee, T-c.; Piantadosi, C.; Ishaq, K. S.; and Snyder, F. 1976. Membrane lipid modification and stearoyl-coenzyme A desaturase activity in L-M cells. Arch. Biochem. Biophys. 177:317-322.

The stearoyl-coenzyme A desaturase system of L-M cells, grown as monolayers, was examined in microsomal membranes that contained 8.2% phosphatidylisopropylethanolamine, an unnatural phospholipid analog. Desaturation of both [1-^{14}C]stearic acid by whole cells and [1-^{14}C]stearoyl-coenzyme A by cell-free homogenates, or microsomes, was decreased to about 40% of control levels in cells that had been grown for 24 hr in the presence of 10 mM *N*-isopropylethanolamine. No decrease in microsomal NADH- or NADPH-dependent cytochrome *c* reductase activities or the level of cytochrome b_5 was found in the L-M cells that had been treated for 24 hr with *N*-isopropylethanolamine. Although amino acid transport into L-M cells was not affected by treatment with *N*-isopropylethanolamine, protein synthesis was decreased by about 30%. These results indicate that the decrease in stearoyl-coenzyme A desaturation in the modified membranes is specifically associated with the terminal oxidase activity (cyanide-sensitive factor) of the desaturase enzyme complex.

Blank, M. L.; Rainey, W. T., Jr.; Christie, W. H., Piantadosi, C.; and Snyder, F. 1976. Mass spectral analysis of a homologous series of S- and O-alkylglycerols. Chem. Phys. Lipids. 17:201-206.

Tentative identification of S-alkylglycerols can be accomplished by separating their isopropylidene derivatives using thin-layer chromatography followed by gas-liquid chromatography. However, their absolute identification is only rigorously determined by mass spectral analysis of the individual components separated by gas-liquid chromatography. In this report mass spectra of S-alkylglycerols (C_{10}-C_{18}) are presented and compared with those of the O-alkylglycerols.

All mass spectra exhibit a base peak at *m/e* 101 (characteristic of the isopropylidene moiety). The M^+ peak is very weak in all cases whereas the M^+-15 is intense (5-40% base). No other ions are noted in the high mass region of the O-alkylglycerol derivatives, but characteristic fragments (especially the $R\text{-}S^+$ ions) are found in the spectra of the S-alkylglycerols. High resolution mass spectrometry substantiated the empirical formulas proposed for the high mass ions.

*Charnes, A.; Frome, E. L.; and Yu, P. L. 1976. The equivalence of generalized least squares and maximum likelihood estimation in the exponential family. J. Am. Stat. Assoc. 71:169-172.

The method of iterative weighted least squares can be used to estimate the parameters in a nonlinear regression model. If the dependent variables are observations from a member of the regular exponential family, then under mild conditions, it is shown that the iterative weighted

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1028361

east squares estimates are identical to those obtained using the maximum likelihood principle. An application is provided to illustrate the results.

Frome, E. L., and Armstrong, R. D. 1977. A robust procedure for estimating the trend-cycle component of an economic time series. In Proceedings of Tenth Annual Symposium on the uter Science and Statistics Interface. Gaithersburg, Maryland.

Economic time series are often represented as a composite of trend-cycle, seasonal, and irregular movements. It is proposed that the cubic spline regression method be used to estimate the trend-cycle component and that parameters be estimated by minimizing the sum of the absolute values of the deviations. If there is a seasonal component present, the regression model can be extended using dummy variables. In both cases, least absolute value estimates are obtained using a special purpose linear programming algorithm. An example of the application of the cubic spline smoothing procedure to monthly Texas construction data is discussed.

Fuhr, J. E.; Gengozian, N.; and Overton, N. 1977. In vitro stimulation of primate hemoglobin synthesis by L-thyroxine. Blood 49:407-413.

Bone marrow cells from adult and abortus primates (marmosets) were incubated in vitro to determine their responsiveness to L-thyroxine. ^{3}H-Leucine incorporation into purified globin chains was the parameter assayed to determine responsiveness. Bone marrow from spontaneously aborted animals consistently was stimulated by the presence of physiologic levels of L-thyroxine. Bone marrow cells from adult animals were unaffected by the hormone.

Gengozian, N., and Urso, P. 1976. Status of T- and B-cell cooperation in radiation chimeras: Evidence for a suppressor effect. Transplant. Proc. 8:631-635.

Absolute tolerance may not be in operation in the allogeneic bone marrow chimera, but rather a dynamic state involving interaction not only between the donor and host but also among the donor-lymphoid cells themselves may exist. Whether this observation made in one allogeneic chimera, $CD2F_1 \rightarrow C3BF_1$, will be true for other chimeras (different strain combinations, species) remains to be shown. Thus, the tempo, mode, and *requirement* for the generation of suppressor T cells are factors that may vary for any specific allogeneic bone marrow transplant. Finally, the manner and degree to which the tolerance-inducing mechanism may affect T- and B-cell functions of the chimera with respect to third-party antigens remains to be determined.

Hall, R. L. 1976. Factors affecting uptake of radioactive agents by tumor and other tissues. In Tumor Localization with Radioactive Agents (Proceedings of the International Atomic Energy Agency Advisory Group Meeting, Vienna, Austria, 1974), pp. 29-45.

The subject of factors that affect the uptake of radioactive agents by tumor and other tissues is a broad and complicated one. Factors that may have considerable bearing on the relative affinity of tumor tissue for such agents are, in general, the following: (1) vascularity, blood flow, and interstitial fluid space; (2) uptake of a protein-bound form of the agent; (3) cell proliferation; (4) presence of carrier; (5) capillary and cell permeability; (6) presence of inflammation; and (7) pH. Certain tumor-localizing processes are based on the specific properties of the tissues in question, i.e., radioiodine for functioning thyroid carcinoma, bone seekers for bone lesions, etc. However, there is as yet still no clear indication of the basic process(es) involved in the tumor-localization of agents such as ^{67}Ga and others on which to base projections as to new materials that might be more specific for tumor tissue in general.

Hodges, H. D.; Holloway, S.; and Gibbs, W. D. 1977. Participation of nuclear medicine technologists in radiation accident management. J. Nucl. Technol. 5:46-49.

Even though the probability for a serious radiation accident is extremely small, the reality of such accidents demands serious consideration and preparation. Generally, nuclear medicine technologists are better qualified than any other hospital staff members to deal with such practical problems as how to (1) detect and measure radiation, (2) prevent the spread of radioactive contamination, (3) reduce the radiation dose to the persons involved, and (4) assist the physician and health physicist in caring for radioactivity contaminated accident victims. To be prepared to assume this dormant responsibility, the nuclear medicine technologist needs to test his familiarity with the necessary basic practices, which must be executed immediately after a radiation accident victim is brought to the hospital. The skills needed for the prompt and decisive response of emergency personnel have to be tested and practiced frequently. Nuclear medicine technologists have an unusual advantage here in that many of the skills and instruments required are used daily by the technologist in nuclear medicine laboratory practice. This article discusses how nuclear medicine instruments can be quickly modified for

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1028362

radiation emergencies and how simulated accident drills can test the readiness of the nuclear medicine team.

Johnston, G. S.; Go, M. F.; Benua, R. S.; Larson, S. M.; Andrews, G. A.; and Hübner, K. F. 1977. Gallium-67 citrate imaging in Hodgkin's disease: Final report of cooperative group. J. Nucl. Med. 18:692-698.

A large cooperative study of ^{67}Ga uptake in Hodgkin's disease showed that 88% of untreated patients had a positive uptake in one or more lesions. The percentage of individual lesions seen on scan, however, was significantly lower, indicating that negative findings at any one site do not argue strongly against the possibility of a lesion there. After treatment, the number of visualized lesions decreased sharply, but the exact role of ^{67}Ga in evaluating therapy is still unclear. Of the various histologic types of Hodgkin's disease, there was a high incidence of localization in all except the lymphocyte-predominance type, which showed a slightly lower uptake. No lesions less than 1 cm in diameter were successfully imaged, and the size most easily detected was 4 cm in diameter. As expected, the imaging technique was much less successful for abdominal lesions than for those at other sites because of interfering concentration in bowel and liver. Both radiotherapy and chemotherapy tend to reverse the abnormalities seen on scan. Finding a significant number of unsuspected positive lesions in asymptomatic patients returning for routine follow-up suggests that this is a distinctly valuable use for ^{67}Ga, allowing early therapy for recurrences.

*Machlin, L. J.; Garcia, F.; Kuenzig, W.; Richter, C. B.; Spiegel, H. E.; and Brin, M. 1976. Lack of antiscorbutic activity of ascorbate 2-sulfate in the rhesus monkey. Am. J. Clin. Nutr. 29:825-831.

Oral administration of 10 mg/kg of body weight of ascorbic acid (AA) completely prevented development of scurvy in juvenile rhesus monkeys *(Macaca mulatta)* fed an AA-free liquid diet. The same dose cured scurvy when injected intramuscularly. An equimolar dose of ascorbic acid 2-sulfate (AA-2-S) did not prevent or cure scurvy. Neither AA nor AA-2-S altered serum cholesterol. AA, but not AA-2-S, reduced serum triglyceride. A case of scurvy in an AA-2-S-treated monkey is described in detail.

Nelson, B.; Calvo, W.; Fliedner, T. M.; Herbst, E.; Bruch, C.; Schnappauf, H-P.; and Flad, H-D. 1976. The repopulation of lymph nodes of dogs after 1200 R whole-body X-irradiation and intravenous administration of mononuclear blood leukocytes. Am. J. Pathol. 84:259-282.

Fresh and cryopreserved autologous or allogeneic mononuclear blood cells (MBC) intravenously injected in 1200 R total-body X-irradiated dogs repopulated lymph nodes within 10 days after transfusion. Several parameters of the lymphopoietic regeneration were correlated with the number of cells transfused and with the number of colony-forming units (CFU) contained in the cell suspension when they were cultured in agar. Values within the normal or close to normal range were reached in the mesenteric nodes of most of the animals transfused with 10×10^9 MBC or more. These values were obtained when 5×10^5 CFU or more were transfused. Axillary nodes showed lower values than mesenteric nodes. Axillary nodes were mostly under the normal range but well over those of the irradiated controls. Frozen and thawed MBC seem to be as effective as fresh cells for lymphopoietic restoration. The mesenteric nodes of dogs transfused with allogeneic MBC showed higher cellularity and larger cortical-paracortical areas than those of dogs transfused with approximately the same number of autologous cells. The repopulation of lymph nodes parallels that of the marrow.

Niblack, G. D.; Kateley, J. R.; and Gengozian, N. 1977. T- and B-lymphocyte chimerism in the marmoset. Immunology. 32:257-263.

Marmosets are natural blood chimeras, this condition resulting from the high frequency of fraternal twinning and the consistent development of placental vascular anastomoses between the two embryos. Identification of chimerism by sex-chromosome analysis of cultured blood lymphocytes provided a means of determining the proportion of chimerism among T and B lymphocytes. Peripheral blood lymphocytes were enriched for T or B cells by filtration through a nylon column (yields >95% T cells) or inactivation of T lymphocytes by treatment with a goat antimarmoset thymocyte antiserum in the presence of complement (yields >95% B cells). Mitogenic stimulation of these separated, enriched cell populations yielded metaphase plates that could be scored for percentage male and female cells. Tests on five different blood chimeras showed the T- and B-lymphocyte chimerism to be the same. Stimulation of blood lymphocytes with cells from another species of marmoset in a mixed-lymphocyte culture test revealed the chimeric T-cell response (i.e., host and co-twin cells) to be similar to that obtained with a mitogenic lectin. The demonstration of equivalent T- and P-cell chimerism in these

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1028363

nimals suggests derivation of these cells from a common stem cell pool, and the response of oth T-cell populations to an antigenic stimulus in proportions similar to their percentage himerism suggests complete immunologic tolerance exists in this species for co-twin histo-ompatibility antigens.

..rson, D. A., and Gengozian, N. 1976. Primary in vitro antibody formation by blood eucocytes of a subhuman primate. Cell. Immunol. 27:171-176.

Blood leucocytes from humans and two species of marmosets, *S. fuscicollis* and *S. oedipus oedipus*, were cultured in vitro with sheep red blood cells. Leucocyte cultures from 16 of 20 *S. fuscicollis* marmosets produced significant numbers of plaque-forming cells (PFC) as assayed by the Jerne procedure. Under the same conditions, none of 15 *S. oedipus oedipus* cultures and only 1 of 15 human blood cultures responded. A kinetic study of the response with *S. fuscicollis* leucocytes revealed peak 19S and 7S PFC to appear on day 9 of culture. It is suggested that two factors are acting synergistically in the successful development of antibody-forming cells from leucocytes of *S. fuscicollis:* (1) the innate immune response capabilities of the blood lymphocytes and (2) the natural hemopoietic chimerism that occurs in this species.

Rock, C. O.; Fitzgerald, V.; and Snyder, F. 1977. Activation of alkyldihydroxyacetone phosphate synthase by detergents. Arch. Biochem. Biophys. 181:172-177.

The microsomal enzyme alkyldihydroxyacetone phosphate synthase, which synthesizes alkyldihydroxyacetone phosphate from fatty alcohols and acyldihydroxyacetone phosphate, was examined in the harderian gland and Ehrlich ascites carcinoma. Although the enzyme possessed a higher specific activity in the harderian gland than in the Ehrlich ascites carcinoma, similar results were obtained using the enzyme from both sources. Alkyldihydroxyacetone phosphate synthase was stimulated approximately 50% by the addition of deoxycholate or Triton X-100 to the assay system. The concentration of detergent needed to elicit this response was below the critical micellar concentration of these detergents. The reaction rate as a function of pH was essentially constant in the absence of detergent, but when Triton X-100 was added to the system the synthase showed a distinct pH optimum of 8.0 to 8.5. Unlike cytochrome b_5, a marker for the surface of the microsomal vesicles, the alkyldihydroxyacetone phosphate synthase activity was not removed from microsomal preparations treated with trypsin. Also, alkyldihydroxyacetone phosphate synthase was not solubilized by treatment of microsomes with deoxycholate concentrations below the critical micellar concentration of this detergent. However, enzymatic activity was efficiently removed from microsomes treated in combination with deoxycholate and trypsin. We suggest from these data that the catalytic portion of alkyldihydroxyacetone phosphate synthase is exposed to the lumenal surface of the microsomal vesicles. Since both dihydroxyacetone phosphate and glyceraldehyde 3-phosphate were inactive in the assay system in the presence and absence of detergent, the possibility that acyldihydroxyacetone phosphate functions in the system as a lipophilic molecule that facilitates the transport of a triose phosphate into the microsomal lumen is unlikely.

Rock, C. O.; Baker, R. C.; Fitzgerald, V.; and Snyder, F. 1976. Stimulation of the microsomal alkylglycerol monooxygenase by catalase. Biochim. Biophys. Acta. 450-469-473.

Liver cytosol contains a heat-sensitive nondialyzable soluble factor necessary for maximal activity of the alkylglycerol monooxygenase present in rat liver microsomes. In this report, we demonstrate that the stimulatory component in rat liver supernatant is catalase. Catalase functions to protect the enzyme from inactivation by H_2O_2 in addition to its documented ability to retard the nonenzymatic oxidation of the pterin cofactor. The protective effect of catalase on alkylglycerol monooxygenase and on the aromatic amino acid hydroxylase systems indicates that H_2O_2 sensitivity is a general feature of pterin-dependent hydroxylases.

Rock, C. O.; Fitzgerald, V.; Rainey, W. T., Jr.; and Snyder, F. 1976. Mass spectral identification of 2-(O-acyl)hydroxy fatty acid esters in the white portion of the rabbit harderian gland. Chem. Phys. Lipids. 17:207-212.

A major lipid component of the white portion of the rabbit harderian gland has been shown to be a mixture of 2-(O-acyl)hydroxy fatty acid esters. The fatty acid moieties in this lipid class are exclusively saturated and range in chain length from $C_{14:0}$ to $C_{22:0}$, with $C_{16:0}$ being the major component (65%). The fatty alcohols are also saturated and composed primarily of $C_{20:0}$, $C_{21:0}$, and $C_{22:0}$ chains. The hydroxy fatty acids are composed of $C_{14:0}$, $C_{15:0}$, and $C_{16:0}$, and mass spectroscopy combined with chemical techniques placed the hydroxyl group at the 2-carbon. 2-(O-acyl)Hydroxy fatty acid esters are not found in the pulmonary portion of the rabbit harderian gland nor have they been reported to occur in harderian glands of other species.

1028364

*Sherman, M. I.; Miller, R. A.; and Richter, C. B. 1977. Histologic analyses of experimental tumors from mouse blastocyst-derived cell lines. J. Natl. Cancer Inst. 58:993-1002.

Previous work has shown that established cell lines can be derived from cultured mouse blastocysts. Although these cell lines differed from one another morphologically, immunologically, and biochemically, it has not been easy to determine what embryonic cell type (if any) each cell line represents. This report shows that these blastocyst-derived lines could produce tumors in syngeneic mice at low yields, and it presents the results of histologic analyses of the tumors that were conducted to learn more about the kinds of cells present in each of the lines. Tumors derived from cell line MB2 contained two kinds of cells. Most of the cells were similar to those found in so-called "parietal yolk sac carcinomas," secreted Reichert's membrane-like material, and could be found in subsequent transplant generations. The other cell type resembled trophoblast giant cells morphologically and did not survive transplantation. These results showed that MB2 contained cells that were either parietal endoderm cells or a closely related precursor. The tumor derived from MB4 consisted of small, closely packed cells intermixed with larger cells with foamy cytoplasm and occasional giant cells. Cells in subsequent passages of the tumor were often organized into acinar clusters. Biochemical evidence has suggested that MB4 cells resembled one or both components of visceral yolk sac. Visceral yolk sac sarcomas have not previously been reported; the MB4-derived tumor may prove to be the first. Tumors derived from line MB31 contained both fibroblastic and epithelioid cell types that could not be more definitively identified. Cultures derived from each kind of tumor in each case contained cells morphologically characteristic of those in the originally inoculated population. The results showed that tumors produced from blastocyst-derived cell lines did not resemble the teratocarcinomas formed when blastocysts were implanted into an ectopic site in vivo: They did not contain a wide variety of cell types, nor did they possess pluripotent embryonal carcinoma "stem" cells characteristic of the teratocarcinoma. That each blastocyst-derived line gave rise to a different kind of tumor suggested that each line may have consisted of cells "trapped" at some stage characteristic of the development of a particular embryonic or extraembryonic tissue. However, each tumor did contain at least two distinct cell types; this suggested that cells in the blastocyst-derived lines, though restricted in their developmental potential, may not yet have been completely determined.

Urso, P., and Gengozian, N. 1977. Immune competence of splenic lymphocytes following graft-vs-host disease in mouse allogeneic radiation chimeras. J. Immunol. 118:657-661.

The abnormal immune response of long-term mouse allogeneic chimeras is reflected by qualitative deficiencies in either T or B lymphocytes. The present study was undertaken to determine if a relationship existed between the severity of graft-vs-host disease (GvHD) that these animals had experienced and a functional defect in either the T or B cell population. The in vitro plaque-forming cell (PFC) response of chimera spleen cells to sheep red blood cells (SRBC) was evaluated in the presence of normal T or B lymphocytes 4 to 8 months after marrow transplantation and well beyond the GvHD period. In an analysis of several different allogeneic radiation chimeras, results showed no relationship between the severity of GvHD experienced and the immunologic capacity of either T or B cells. Thus, different chimera combinations showing similar degrees of GvHD were functionally deficient in one or the other of these two cell types or both with no apparent predilection for abnormality in either population. In examining the quantitative in vitro PFC response to SRBC by spleen cells from individual chimeras, we found that the number of PFC formed was related to the severity of GvHD experienced by that animal. A general relationship between severity of GvHD and PFC capacity may also exist between chimeras of different genetic combinations. However, this relationship is not precise since gross exceptions occur. The results, although documenting further the qualitative abnormalities in T and/or B lymphocytes of radiation chimeras, do not reveal the factor or mechanisms by which these cells are made unresponsive. It is suggested that the tolerance-inducing mechanism of these animals, whether it be humoral blocking factors or suppressor cells, is in some way interfering with the collaboration of T and B cells for antibody production.

Voelker, D. R.; Lee, T-c.; and Snyder, F. 1976. Fatty acid biosynthesis and dietary regulation in pulmonary adenomas. Arch. Biochem. Biophys. 176:753-756.

Studies of fatty acid metabolism in mouse pulmonary adenomas demonstrate that the alveolar type II cell tumors synthesize fatty acids at a greater rate than either normal or host lungs. This is reflected in elevated levels of both acetyl-CoA (coenzyme A) carboxylase and fatty acid synthase. Carboxylase activity is 4.4-fold, and synthase activity is 7.9-fold higher in the ade-

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1028365

nomas than normal tissue. Both enzymes from adenomas respond to dietary manipulation in a manner that is qualitatively similar to normal tissue, indicating that the adenomas maintain metabolic control over fatty acid biosynthesis and, therefore, constitute a useful model for normal type II cells. These data suggest that alveolar type II cells have a greater capacity for fatty acid biosynthesis than other cell types of normal lung.

Washburn, L. C.; Coffey, J. L.; Watson, E. E.; Sun, T. T.; and Hayes, R. L. 1976. Radiation dosimetry of some ^{11}C-labeled amino acid radiopharmaceuticals. In Radiopharmaceutical Dosimetry Symposium. Oak Ridge, Tennessee, 1976. Proceedings of a Conference (R. J. Cloutier, J. L. Coffey, W. S. Snyder, and E. E. Watson, eds.). United States Department of Health, Education, and Welfare, Washington, D. C. HEW Publication (FDA)76-8044. pp. 441-451.

The radionuclide ^{11}C ($t_{1/2}$ = 20.4 min) has considerable potential in nuclear medicine as a label for radiopharmaceuticals that have rapid localizing properties. The annihilation radiation associated with the decay of ^{11}C makes possible not only external detection but also the use of tomographic techniques. Several ^{11}C-labeled natural and unnatural amino acids have shown potential as agents for visualizing tumor tissue and the pancreas. [^{11}C]Carboxyl-labeled 1-aminocyclopentanecarboxylic acid has shown promise as a tumor-localizing agent while [^{11}C]carboxyl-labeled DL-valine and DL-tryptophan are potential pancreas-visualizing agents. Studies have been made of the early (15 min-1 hr) tissue distribution of the analogous ^{14}C-labeled compounds in a wide range of animal models, and radiation dosimetry extrapolations have been made to the human for the ^{11}C-labeled agents. In general, ^{11}C doses of these agents up to a level of 100 mCi appear to be acceptable from the standpoint of radiation exposure.

Wykle, R. L., and Kraemer, W. F. 1977. Glycerol kinase activity in adenoma alveolar type II cells. FEBS Lett. 78:83-85.

Glycerol kinase activity was demonstrated in adenoma alveolar type II cells by measuring the synthesis of [^{14}C]glycerophosphate from [U-^{14}C]glycerol. The [^{14}C]glycerophosphate was identified by thin-layer chromatography and paper chromatography. The formation of [^{14}C]glycerophosphate was ATP-dependent with an apparent K_m for glycerol (at 20 mM ATP) of 1.3 μM. The glycerol kinase was localized in the soluble fraction where the activity was 190 pmol/min/mg protein. Although several studies have demonstrated lung tissue can incorporate glycerol into the glycerol backbone of lipids, this is the first direct demonstration of the presence of glycerol kinase in lung-derived cells. The adenomas consist almost exclusively of type II cells that synthesize pulmonary surfactant. The presence of glycerol kinase in the cells explains how [^{14}C]glycerol was incorporated into surfactant in earlier studies and indicates glycerol can be utilized directly by the lung for lipid synthesis without prior phosphorylation by other tissues.

Wykle, R. L.; Malone, B.; and Snyder, F. 1977. Biosynthesis of dipalmitoyl-*sn*-glycero-3-phosphocholine by adenoma alveolar type II cells. Arch. Biochem. Biophys. 181:249-256.

Adenomas induced in lungs of BALB/c mice by urethane treatment consist almost exclusively of pulmonary type II cells and, like normal type II cells, produce dipalmitoylphosphatidylcholine, the major lipid component of lung surfactant [Snyder, C.; Malone, B.; Nettesheim, P.; and Snyder, F. (1973) Cancer Res. 33:2437-2443]. In the present investigation, these adenomas were used for studying the biosynthesis of dipalmitoylphosphatidylcholine in the intact cells. Whole adenomas were incubated in Krebs-Ringer phosphate buffer or Medium 199 with [1-^{14}C]palmitate, [1-^{14}C]acetate, [U-^{14}C]glycerol, or [1,3-^{14}C,2-^{3}H]glycerol. The synthesis of [^{14}C]dipalmitoylphosphatidylcholine was subsequently determined after its isolation by cryochromatography. The [1-^{14}C]acetate was converted to [^{14}C]palmitate, which was incorporated into disaturated phosphatidylcholine in the same manner as exogenous [1-^{14}C]palmitate. After a 1-hr incubation of either [1-^{14}C]palmitate or [1-^{14}C]acetate, approximately 60% of the [^{14}C]phosphatidylcholine was disaturated while 75% to 80% of the label in the disaturated species was in the acyl group at the 2-position. The same results were obtained when minced lung was used instead of adenomas, except the incorporation rate was higher in the adenomas. It was concluded from these studies that palmitate, whether synthesized de novo or provided exogenously, enters a common pool before being used by the type II cells for the synthesis of dipalmitoylphosphatidylcholine. When [1,3-^{14}C,2-^{3}H]glycerol was used to label phosphatidylcholine in the system, all molecular species, based on the degree of unsaturation, retained the same ^{3}H/^{14}C ratio. This indicated that the dihydroxyacetone phosphate pathway is of no more significance for the synthesis of disaturated phosphatidylcholine than for the other molecular species. The study demonstrates the usefulness of the adenoma system as a model for studying surfactant biosynthesis in pulmonary type II cells and

1028366

indicates that the major pathway by which palmitate enters dipalmitoylphosphatidylcholine is by the deacylation and reacylation of phosphatidylcholine.

*Yakatan, G. J.; Smith, R. B.; Frome, E. L.; and Doluisio, J. T. 1977. Pharmacokinetics of orally administered hydroflumethiazide in man. J. Clin. Pharmacol. 17:37-47.

Hydroflumethiazide plasma and urine levels were measured fluorometrically following the oral administration of 100-mg doses (2 x 50 mg tablets) to each of 12 healthy, adult male subjects. The plasma level data as a function of time were fit to a one-compartment open model using a nonlinear regression digital computer program. The means of the individual apparent absorption and disposition rate constants were 0.770 ± 0.46 hr^{-1} ($t_{1/2}$ = 0.9 hr) and 0.358 ± 0.17 hr^{-1} ($t_{1/2}$ = 1.94 hr), respectively, with a 0.49 ± 0.26 hr lag time in absorption. The same parameter estimates obtained by fitting the mean plasma concentration-time curve were 0.461 ± 0.15 hr^{-1}, 0.357 ± 0.13 hr^{-1}, and 0.39 ± 0.09 hr for the apparent absorption and disposition rate constants and the lag time, respectively. The closeness of the parameter estimates and the lack of intravenous data preclude unequivocal assignment of the rate constants to the absorption or disposition processes. Nevertheless, the disposition of hydroflumethiazide is clearly a relatively rapid process. The means of the individual peak plasma levels and times to peak were 0.301 ± 0.094 $\mu g\ ml^{-1}$ and 2.12 ± 0.59 hr, respectively. The mean area under the plasma level time curve was 1.95 ± 0.78 $\mu g \cdot hr/ml^{-1}$ and 46.7 ± 20.0% of the administered dose was recovered in the 24-hr urine. The mean renal clearance of hydroflumethiazide was 453.4 ± 245.6 ml/min.

Yang, W. K.; Tyndall, R. L.; and Daniel, J. C., Jr. 1976. DNA- and RNA-dependent DNA polymerases: Progressive changes in rabbit endometrium during preimplantation stage of pregnancy. Biol. Reprod. 15:604-613.

DNA polymerase activities were studied in rabbit endometria at estrus and through the first 7 days of pregnancy. Extracts of total endometrial homogenates by high salt gave two peaks of polymerase activity by sucrose gradient centrifugation; the 6-8S polymerase showed considerable increase in specific activity that reached a maximum at 5-7 days postcoitum, whereas the 3-4S polymerase showed only a slight increase after 2 days of pregnancy. A 5-6S RNA-dependent DNA polymerase activity, extracted by combined use of hypertonic salt solution and nonionic detergent from particulate subcellular functions, appeared in the endometrium after coitus, reached a maximum at day 3-4, and declined thereafter. This RNA-dependent DNA polymerase activity, isolated by phosphocellulose chromatography at distinct regions of salt gradient, showed preference for template-primers such as $(rA)_n \cdot (dT)_9$ and $(rC)_n \cdot (dG)_6$ but did not utilize 70S RNA of RNA tumor viruses. Attempts to demonstrate oncorna virus expression by other means have been unsuccessful with endometrium of 3-day pregnant rabbits. Interrelation between the changes of these DNA polymerase activities and the DNA replication in the endometrial cells is discussed.

*Work done outside ORAU.

1028367

4 Collaborative Projects

ject	Other Institution;[1] Staff	ORAU Division Staff
dioprotective drug, 1, for possible use radiotherapy	ETCRC; A. Girardi UTMRC; F. Comas WRAIR; M. Heiffer ORNL; R. Ullrich U Pennsylvania; D. Biery U New Mexico; J. Yuhas	G. Andrews, K. Hübner
rification of gallium- and lium-binding proteins	Fordham U; D. Lawless	D. Brown
bcellular distribution platinum complexes	ORNL; J. Hoeschele, N. Johnson	D. Brown
diation sensitivity ytogenetic) of the rmoset	ORNL; J. Brewen	N. Gengozian
tal hemoglobin synthesis	UTMRC; J. Fuhr	N. Gengozian
ntigenic cross-reactivity tween human and marmo- zone pellucidae, a poten- l target for immunocon- ception	U Tennessee; C. Shrivers	N. Gengozian
oduction and assessment of rious radionuclides as aging agents	ORNL; K. Poggenburg	R. Hayes
lmonary disease detection	ETCDH; J. Winebrenner	K. Hübner
nited States Transuranium gistry	HEHF; P. Fuqua	C. Lushbaugh
adiotherapy overdose study	U Cincinnati; E. Saenger	C. Lushbaugh
nthesis of lipid analogs ir precursors	U North Carolina; C. Piantadosi, K. Ishaq	F. Snyder
fect of NO_2 and other xicants on the lung sur- ctant system	ORNL; P. Nettesheim, W. Dalbey	F. Snyder
pid carrier transfer proteins alveolar type II cells	Western Carolina U; R. Lumb	F. Snyder
pid metabolism in trans- rmed and modified cells	ORNL; A. Hsie	F. Snyder
ole of ether lipids in growth, ll differentiation, and oplasia	ORNL; C. Heckman, P. Nettesheim	F. Snyder
ass spectrometry of nique lipids	ORNL; W. Rainey, Jr.	F. Snyder
uclear magnetic resonance udies of unique lipids	ORNL; B. Benjamin	F. Snyder
imethylnitrosamine and its lationship to lipid etabolism	ORNL; N. Clapp, J. Daugherty	F. Snyder
hemical characterization of antihypertensive lipid olated from the kidney edulla	U Tennessee, Memphis; E. Muirhead, B. Leach, L. Byers	F. Snyder
lectron microscopic studies f microfilaments and icrotubules in cell cultures	ORNL; A. Hsie	D. Swartzendruber

[1] Abbreviations
ETCDH—East Tennessee Chest Disease Hospital
ETCRC—East Tennessee Cancer Research Center
HEHF—Hanford Environmental-Health Foundation
ORNL—Oak Ridge National Laboratory
UTMRC—University of Tennessee Memorial Research Center
WRAIR—Walter Reed Army Institute of Research

1028368

5 Grant-Funded Research

Grantor	Title	Principal Investigator
American Cancer Society	Metabolism, regulation, and function of ether-linked glycerolipids in cancer cells	F. Snyder
National Cancer Institute	Concentration of radionuclides by maglignant neoplasms	R. Hayes
National Cancer Institute	Ether lipids in cancer-enzyme mechanisms and inhibitors	F. Snyder
National Institutes of Health	General research support	C. Lushbaugh
National Cancer Institute	Lysophospholipase D in tumors and other tissues	R. Wykle
National Cancer Institute	ORAU-ORNL study of carbon-11 in nuclear medicine	R. Hayes
National Cancer Institute	An interdisciplinary training program in cancer	D. Swartzendruber
National Heart, Lung, and Blood Institute	Surfactant metabolism: alveolar cells and lung disease	F. Snyder
National Institute of Child Health Development	Evaluation of induced cytogenetic lesions in oocytes	G. Littlefield
National Heart, Lung, and Blood Institute	"Autoimmune" thrombocytopenia	N. Gengozian
Damon Runyon–Walter Winchell Cancer Fund	Modification of cancer cell membranes with unnatural lipid analogs (post-doctoral fellowship–Dr. Myles C. Cabot)	F. Snyder, sponsor



1028369

RADIOPHARMACEUTICALS

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Burkhalter, Dianne
Byrd, Billy
Carden, Blanche
Carlton, Elbert
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‖ Kuniyasu, Yoshio
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Washburn, Lee
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McCaskill, Sallie
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Organization and Staff

Research and Training

1028370

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Lushbaugh, C. C.
Ryan, Billie
Snyder, Fred

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Foster, Elizabeth
Hodge, James
Miller, Willie
Vines, Charlie
Young, David

LIBRARY

Garber, Marion
Gramly, Virginia

ELECTRONICS

Barclay, Thomas
Pope, William

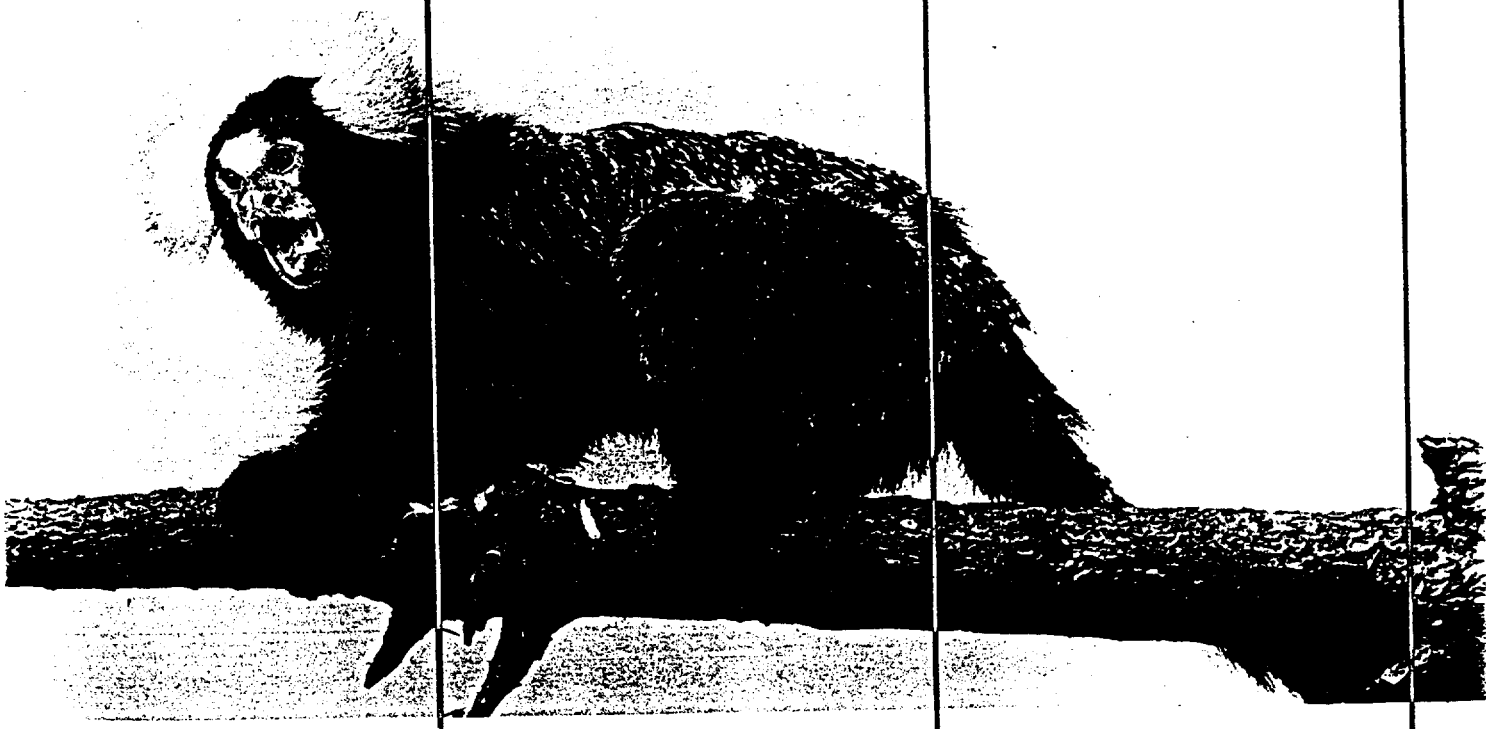
ANIMAL FACILITIES

Barnett, Willie
Caldwell, Isaiah
Carson, Robert
Davis, Suzette
Graham, James
Rice, Cecil
Ruffner, Mack
Suttles, John
Webster, Alfred
Wilson, Claude

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#Humason, Gretchen
Huxford, Virginia
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§ Palisano, John
§ Porcella, Russell
Richter, Conrad
Selle, Eleanor
Swartzendruber, Donald
Tankersley, William

* Student trainee
† Master's graduate student
‡ Predoctoral graduate student
§ Postdoctoral fellow
‖ Guest scientist
\# Consultant
Group leaders' names are in italics



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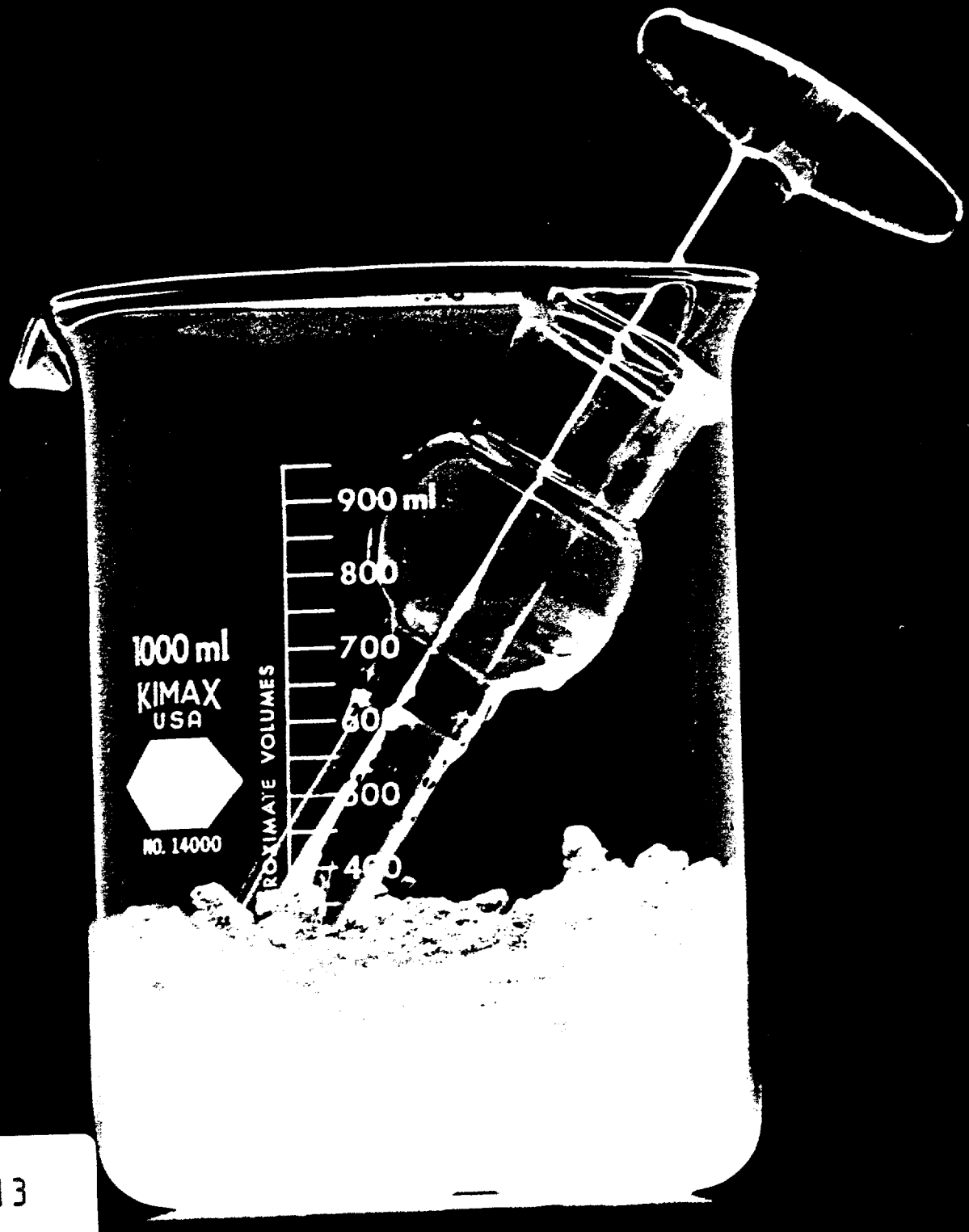
1976-77

1028372

Research Report 1977–1978

Oak Ridge Associated Universities

Medical and Health Sciences Division



1028373

Oak Ridge Associated Universities is a private, not-for-profit association of 45 colleges and universities. Established in 1946, it was one of the first university-based, science-related, corporate management groups. It conducts programs of research, education, information, and training for a variety of private and governmental organizations. ORAU is noted for its cooperative programs and for its contributions to the development of science and human resources in the South.

The Medical and Health Sciences Division conducts biomedical research and training in studies of respiratory and gastrointestinal diseases and cancers related to environmental toxicants and radiation exposure. Immunobiology, lipid biochemistry, radiopharmaceutical chemistry, nuclear medicine, experimental pathology, cytogenetics, cell biology, and epidemiology are the main research disciplines; predoctoral and postdoctoral training and refresher courses are provided in these areas. Besides newly refurbished laboratory units, the Division has two unique facilities: REAC/TS, the Radiation Emergency Assistance Center/Training Site, maintained for the U.S. Department of Energy as a demonstration and training unit for medical preparedness for radiation accidents; and the Marmoset Research Center, which is a source of primates for research at ORAU and elsewhere in the United States.

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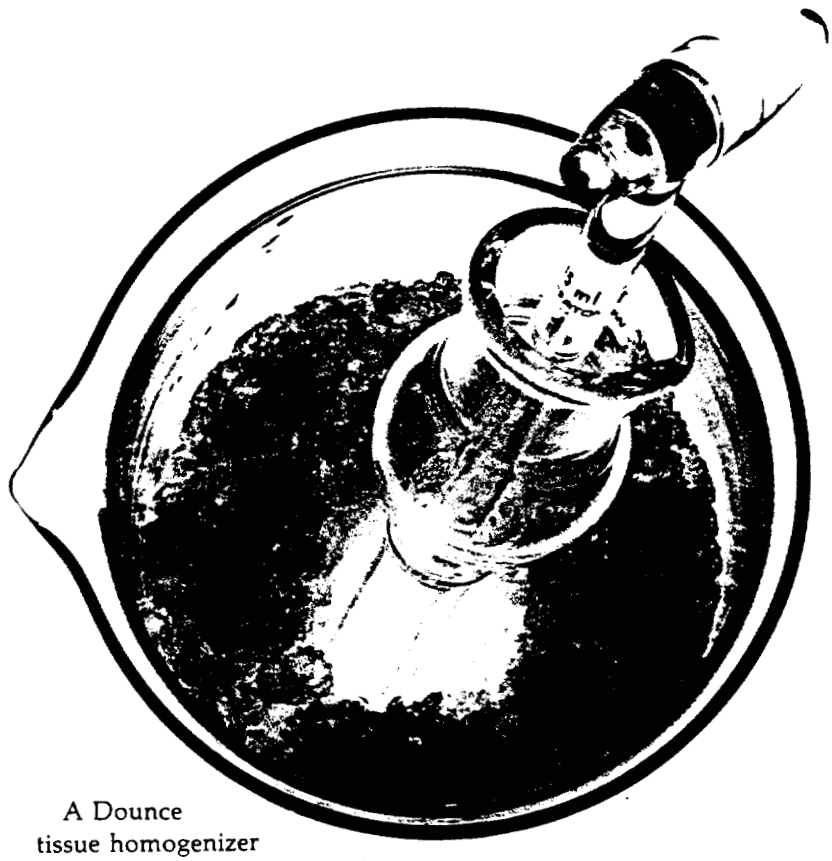
Available from the National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia 22161. Please direct all price inquiries to NTIS.

1028374

Research Report 1977-1978

Oak Ridge Associated Universities

Medical and Health Sciences Division



A Dounce tissue homogenizer (above and cover) used in the Medical and Health Sciences Division to disrupt cells for cellular fractionation.

ORAU-148

October 1978
Oak Ridge, Tennessee

Work for this report was performed under Contract Number EY-76-C-05-0033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

1028375

Preface

The emission computerized axial tomograph—ECAT— provides cross-sectional pictures of the distribution of radioactive tracers in the body, often permitting critical examination not possible with conventional scanners.



The Medical and Health Sciences Division conducts major research programs in the following areas: (1) human radiation effects that involve experimental pathology and cytogenetics, (2) epidemiologic studies of nuclear and nonnuclear workers to identify and lessen occupational dangers related to energy production, (3) biochemical investigations designed to detect, elucidate, and quantitate alterations in the pulmonary surfactant system and in membrane properties that precede the development of respiratory disease and cancer after exposures to toxicants from fossil fuels, (4) immunological and cellular approaches to repairing cell surveillance systems injured by exposure to fuel toxicants, with the aim of achieving protection against occupational cancers and respiratory disease, and (5) development of physiologically appropriate radiopharmaceutical agents to detect the metabolic alterations that lead to various environmental cancers and chronic lung diseases. The scientific research groups responsible for developing and carrying out these programs are Radiation Emergency Assistance Center/Training Site (REAC/TS), which includes cytogenetics; Immunobiology; Biological Chemistry; Radiopharmaceutical Development; Nuclear Medicine; and Epidemiology. These groups also participate in various biomedical training programs, primarily at the predoctoral and postdoctoral levels.

The Radiation Emergency Assistance Center/Training Site occupies a portion of the city hospital. This group provides a unique service that consists of a medical preparedness team and facility for the management of human injuries resulting from accidental exposure to radiation; special consultant services and short-term training courses are also provided on these subjects. The REAC/TS program assumes a major responsibility for the U.S. Department of Energy (DOE) in maintaining a national Radiation Accident and Radiation Overexposure Registry. This program also collaborates with other governmental facilities in developing health and mortality studies for DOE.

Biomedical research programs at the Medical and Health Sciences Division are directed toward meeting the broadened role of DOE responsibilities for environmental and biomedical problems related to developing new energy sources. Cellular lesions caused by biohazardous components in fossil fuels are still poorly understood and even less is known about the basic mechanisms involved. Knowledge of the latter is prerequisite in designing methods for the prevention and therapy of diseases produced by long-term exposure to fossil fuel toxicants.

Our biomedical investigations utilize human subjects, animals, cell and organ cultures, and enzymes as biological systems. The problems of greatest concern in this work encompass acute and chronic diseases induced by chemical toxicants (irritant gases and hydrocarbon carcinogens) of lungs, liver, skin, and the gastrointestinal tract. The disciplines of immunology, biochemistry, radiopharmaceutical development, epidemiology, nuclear medicine, cytogenetics, and cell biology provide the combination of talents and technology for solving these problems.

The cytogenetics program, which has been principally concerned with radia-

1028376



tion dosimetry in the REAC/TS program, is expected to become increasingly independent as methods are developed to detect and quantify chemical rather than radiation damage. Our new tissue culture facilities, just completed, are now under the direction of a cell biologist well versed in procedures of cell and organ cultures. The facilities' purpose is to provide specific cellular systems as models for exploring basic biochemical and toxicant mechanisms at the cellular level. Unique research facilities in the Division include the Marmoset Research Center, an emission computerized transaxial tomograph (one of two in the nation), and in-house computer hardware, including the essential biostatistical and programming expertise. Radiopharmaceutical Development and Biological Chemistry have recently established an interdepartmental lung tomography task force utilizing a molecular approach in visualizing lung functions in health and disease.

Accomplishments of our research programs during the past year are described in the abstracts section of this report. Reprints of all publications are available upon request.

C. C. Lushbaugh, Chairman

Fred Snyder, Assistant Chairman

October 1978



1028377

Table of Contents

Scope of Programs 7
- Biological Chemistry 7
- Epidemiology 8
- Immunobiology 8
- Radiopharmaceutical Development 9
- Radiation Emergency Assistance Center/Training Site 11

Publications 13

Abstracts of Full-Length Publications 19

Training Programs 29

Collaborative Research 31

Grant-Funded Research 33

Organization and Staff 35

1028378

Scope of Programs

A high-pressure liquid chromatograph separates components of tumorous and nontumorous tissue in the Division's Radiopharmaceutical Development program.



1028379

Biological Chemistry

Research in biological chemistry currently centers on (1) the lung surfactant system and alterations induced by toxicant gases and carcinogens, (2) the role of ether-linked lipids and related membrane-bound enzymes in cancer and how they are topographically distributed and regulated, and (3) the modification of membrane properties by lipid analogs and how such changes are involved in cell division and cell-cycle events. All three areas contribute significantly to the training of predoctoral students and postdoctoral fellows.

A major goal of the biochemical research effort for the U.S. Department of Energy is to provide an assessment, at the molecular level, of the biohazards encountered with exposure to environmental toxicants derived from processing and utilizing coal and other fossil fuels as energy sources. Toxic cellular effects depend upon their initial interaction with cell membrane constituents that either prevent or permit their cellular penetration. Thus, by-products of fossil fuel processing and usage can produce hazardous biological effects because of their interaction with the lipid and protein moieties of membranes, thereby altering both intracellular metabolism and protective barrier responses. Long-range studies are centered on the effects of NO_2, SO_2, ozone, hydrocarbons, and their mixtures on the lung surfactant system, on lipid metabolism in respiratory airway tissues, and on the properties of integrated enzyme systems in lung and liver membranes. Also, key enzymes involved in the metabolism of ether lipids, characteristically associated with cancer cells, are being evaluated as indicators of preneoplastic changes induced by toxicants. The approach to this problem is designed to detect initial biochemical events at the subcellular level that eventually lead to respiratory disease and/or cancer, the ultimate risks of toxicant exposures.

Basic experiments are being conducted with normal lung and alveolar type II cells to determine the mode and mechanism of the biosynthesis, transport, storage, secretion, catabolism, and regulation of surfactant lipids and the various properties of the membranes responsible for their metabolism and storage. These results provide the baseline of comparison for toxicant-induced alterations being investigated.

We are also actively studying the use of various analogs of lipid precursors to modify the composition and properties of cell membranes. For example, results from these experiments have shown that *N*-isopropylethanolamine, an analog of choline, produces highly specific alterations in enzymatic properties of microsomal and plasma membranes. Moreover, the analog project is supplying important chemical compounds with specific functional groupings for probing enzymatic mechanisms; furthermore, these studies are also yielding functional ligands used in the preparation of affinity chromatography columns for the purification of enzymes.

An important extension of the Biological Chemistry program has been the addition of cell culture expertise for the Division. The completion of the facility in the fall of 1978 will accelerate existing projects requiring cultured cells. A major goal of the new cell culture



studies is to use alveolar type II cells as a model system for investigating the pulmonary surfactant system and the effect of toxicants upon it.

Epidemiology

The Epidemiology Group consists of three study sections, each working on a different aspect of the question of health and mortality of workers in atomic- and al-related energy production. In the omic workers study section, the epidemiologists are concerned mainly with determining any causal relationship between occupational radiation exposure and subsequent mortality. The chemical workers study section is primarily concerned with the health effects of chemical (especially metal) toxicants, as both primary and confounding (with respect to radiation exposure) occupational stresses. The statistical and computer methodology section is composed of biostatisticians and computer scientists; they are concerned with improving statistical methods for epidemiologic analysis and developing modern data management and data analysis systems.

In the nuclear industry, the Epidemiology Group is continually evaluating the mortality experience of workers in the Oak Ridge operations, which include workers at the National Lead Company and the Mound Laboratory in Ohio. To facilitate these studies and to aid other DOE epidemiologic studies (such as those of omic workers at Hanford, ashington; Los Alamos, New Mexico; and Rocky Flats, Colorado), this group has developed the DOE/Death Certificate Retrieval Center to obtain information on the vital status of specific workers through the cooperation of the Social Security Administration and the vital statistics departments in all 50 states.

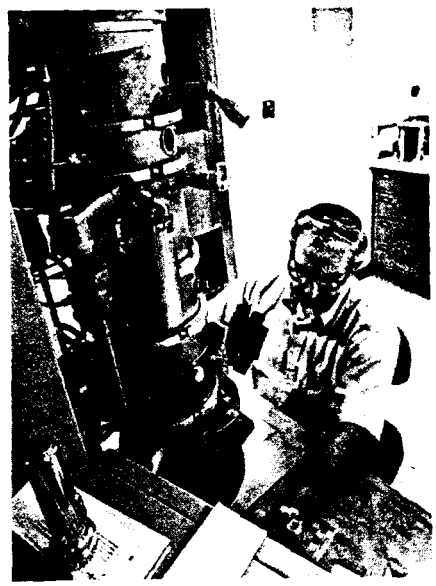
This year a study of the mortality experience of workers exposed to metallic nickel in the Oak Ridge Gaseous Diffusion Plant was completed. Two separate subsets of the data on the Oak Ridge workers (those of the Tennessee Eastman Corporation, 1944–50, and all welders of the Union Carbide Corporation–Nuclear Division) are being prepared for epidemiologic analysis. Another study, in the early stages of data acquisition, is designed to help DOE determine whether the present limit of occupation radiation exposure (5 rem/annum) external whole-body dose is causally related to untoward morbidity or mortality. In parallel with all of these specific tasks, the Epidemiology Group is establishing a computerized data base containing cause-, race-, sex-, and age-specific death rates based on U.S. vital statistics since 1938; the group is also developing extensive retrospective editing and error correction procedures for a data base that has been developed from employment records that extend back to the early 1940s. Both of these data bases will be valuable resources for epidemiologic research on potential occupational risks encountered by workers in atomic- and coal-related energy production.

Immunobiology

The immunobiology program is concerned with the effects of environmental



1028380



pollutants on immunological processes at the organismal and cellular levels. A primary feature of the model systems used—the mouse and the marmoset—is the phenomenon of blood chimerism, a physiologic state that occurs naturally in the marmoset and is induced in the mouse by irradiation and bone marrow transplantation. The immunologic tolerance that mediates the chimeric status provides an extremely subtle and sensitive system on which the effects of chemical pollutants may ultimately be measured.

Our major focus in the mouse has been concerned with the effects of benzo(*a*)pyrene on the immune system of young adults that had been exposed to this agent during fetal life. Dependent upon the dose and age of the fetus (gestational state) at exposure, marked suppression and even enhancement of the immune system are observed. The degree of alteration also depends upon the age of the offspring when antigenic challenge is given, with return to a near normal status at approximately 20 weeks of age. Similar effects are noted when the fetus is exposed to low levels of irradiation. The combination of both insults has yielded marked suppression of the immune system when measured in early neonatal life. Whether exposure of the fetus to chemicals and other toxicants that are not lethal in themselves, but cause abnormalities in the immune system, will also lead to malignancies in later life is a major question under study.

Two species of marmosets, *Saguinus oedipus oedipus* and *Saguinus fuscicollis*, the former showing a high incidence of spontaneous colon cancer, have previously been demonstrated to have varying immune potential with respect to a variety of antigens. Extensive in vitro studies utilizing peripheral blood lymphocytes of these animals have shown comparable capabilities of T and B cells in in vitro antibody formation. Collaborative studies in vitro, however, have suggested a possible dysfunction in those steps necessary to trigger the B cells of the cancer-susceptible species. The relevance of these observations to the sensitivity of either marmoset species to any colon cancer-inducing agent is not yet known. The latter studies were facilitated by the development of specific isoimmune reagents for the two marmoset species. Thus, the ability to selectively kill B or T cells of either species has permitted species "mixing" experiments to determine the relative proportion of different cell types responding to different antigens. Similar reagents are being developed within one species of marmoset so that we may manipulate cells involved in the tolerant state induced by the blood chimerism.



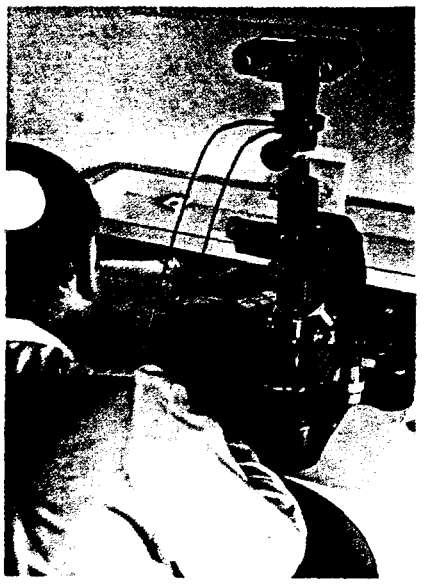
Studies on induced autoimmune thrombocytopenia in the marmoset have shown that the disease appears to be causally related to the deposition of the immunoglobulin IgG on the host's platelets following platelet isoimmunization. Several parallels appear to exist between our experimental model and the disease states manifested in humans, idiopathic thrombocytopenic purpura and posttransfusion purpura. Current studies are aimed at determining the role of soluble antigen-antibody complexes in the syndrome and methods of alleviating the disease once it has been induced.

Radiopharmaceutical Development

The object of the preclinical and nuclear medicine programs is to develop new radiopharmaceuticals for diagnostic use in man. Once a potential agent is identified, it is synthesized in a radiolabeled form and tested in animals for efficacy and safety. Radiopharmaceuticals that have shown sufficient potential are then tested in humans after approval by the U.S. Food and Drug Administration. The mechanisms involved in the localization of radiopharmaceuticals by disease processes are also studied. Collaborative research projects involve Oak Ridge National Laboratory and a number of medical institutions affiliated with ORAU.

During the past year, particular

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emphasis has been placed on the use of positron tomography for the detection of disease states in humans, especially diseases in the lung and pancreas. When positron-emitting radiopharmaceuticals decay in the body, they produce annihilation photons, which can be detected externally by coincidence counting. By viewing these coincident emissions from various angles, it is possible to use computer techniques to reconstruct three-dimensional, cross-sectional images of the tissue distribution of such radiopharmaceuticals.

Using a recently acquired instrument—the Emission Computerized Axial Tomograph (ECAT)—the Division has developed methods for detecting disease processes by taking advantage of physiological alterations in such lesions. The images obtained by positron emission tomography indicate differences in metabolic functions rather than differences in tissue density, which is the basis for transmission X-ray computerized tomography. The ultimate objective of these studies, after having first established methods for detecting functional aberrations, is to quantitate the degree of such differences, a procedure for which the positron tomographic technique should be uniquely suited.

Among the positron-emitting radiopharmaceuticals under investigation at the Division are ^{11}C-carboxyl-labeled 1-aminocyclopentanecarboxylic acid (ACPC), ACBC (the cyclobutane analog of ACPC), DL-valine, and DL-tryptophan. The Division has developed a general technique for producing and purifying multimillicurie levels of ^{11}C-carboxyl-labeled amino acids. [^{11}C]ACBC was identified as a potentially better tumor localizing agent than [^{11}C]ACPC in an animal study of the effect of structural alterations on the tumor specificity of alicylic α-amino acids. As predicted by this preclinical study, [^{11}C]ACBC has been found to be a better tumor-localizing agent in humans than is [^{11}C]ACPC. Also in agreement with animal test results is the fact that [^{11}C]DL-tryptophan has been found to be superior to [^{11}C] DL-valine for the visualization of normal human pancreas. [^{11}C]DL-Valine and [^{11}C]DL-tryptophan are both, however, far superior to [^{75}Se]L-selenomethionine, the agent currently in general use for pancreatic imaging in nuclear medical diagnostic studies. Other positron-emitting radiopharmaceuticals under investigation are ^{68}Ga (available from a 287-day generator), ^{18}F, and ^{64}Cu (the latter two available from reactors).

During the past year, basic studies have utilized a computer code for stimulating accelerator targetry. Experiments at the Oak Ridge National Laboratory 86-inch cyclotron and analysis of reported measurements from the literature have led to design specifications for a dedicated accelerator facility to produce ^{11}C, ^{13}N, ^{15}O, and ^{18}F for use at positron tomography research centers. In this concept, enriched stable isotope targetry, automated radiochemistry equipment, and a positron tomograph would be used to provide a completely integrated system for diagnostic and physiological studies. If the targetry can be developed to permit efficient recovery of the activities in useful chemical forms, a low-energy (8 Mev) proton cyclotron could be used to produce the above radionuclides at the curie level. When fully developed this system would permit a drastic reduction in the size and complexity of accelerators needed for producing positron radiopharmaceuticals.

As the result of continuing study of



1028382



the mechanisms involved in the uptake of tumor-localizing agents by tumor tissue, a ^{67}Ga-binding macromolecular component has been purified and partially characterized. It is an acidic glycoprotein with a molecular weight of approximately 50,000 daltons. Based on animal studies, this material appears to be considerably elevated in tumor tissue. In other animal studies we have obtained strong evidence indicating that normal cells take up ^{67}Ga by endocytosis, whereas tumor cells absorb it through increased permeability of their cell membranes.

Radiation Emergency Assistance Center/Training Site

The REAC/TS program—with its service and training as well as research-oriented projects—has expanded. The facility and staff were involved at various levels in 26 radiation incidents and three major accidents. The facility was also used for teaching, training, and demonstrations. This past year the whole-body counter was calibrated for different radionuclides, and isoresponse curves were established. The "hot" liquid waste system was tested and is now ready to handle large amounts of radioactive fluids accumulating through decontamination procedures. The REAC/TS treatment and decontamination area equipment and procedures are fully coordinated with the emergency response team and surgical staff of the Oak Ridge Hospital of the United Methodist Church.

REAC/TS responsibility and obligation for medical backup through DOE Radiation Assistance Programs for private nuclear industries, DOE and non-DOE contractors, ORAU-affiliated universities, and the DOE naval reactor programs were increased by the addition of another nuclear power plant. The obligations are documented by agreements now with a total of 24 operations, not including the Union Carbide Corporation–Nuclear Division plants located in Oak Ridge. During this reporting period, REAC/TS rendered assistance and services in 26 radiation incidents involving 27 persons.

This past year, 138 persons were trained at the REAC/TS facility. Forty-four physicians, 22 health physicists, and 72 nurses and emergency medical technicians were trained in 5-day or 2-day courses. One of the emergency medical technician courses was reviewed by members of the Nuclear Regulatory Commission–Department of Health, Education, and Welfare (Civil Defense Task Force) for potential use in a nationally designed training program. The courses continue to be approved for continuing education credits in the American Medical Association and in the Tennessee Nurses' Association. Contact with previous course participants has been maintained by a REAC/TS newsletter. Between 500 and 600 representatives of DOE and non-DOE plants and institutions have toured the REAC/TS facility.

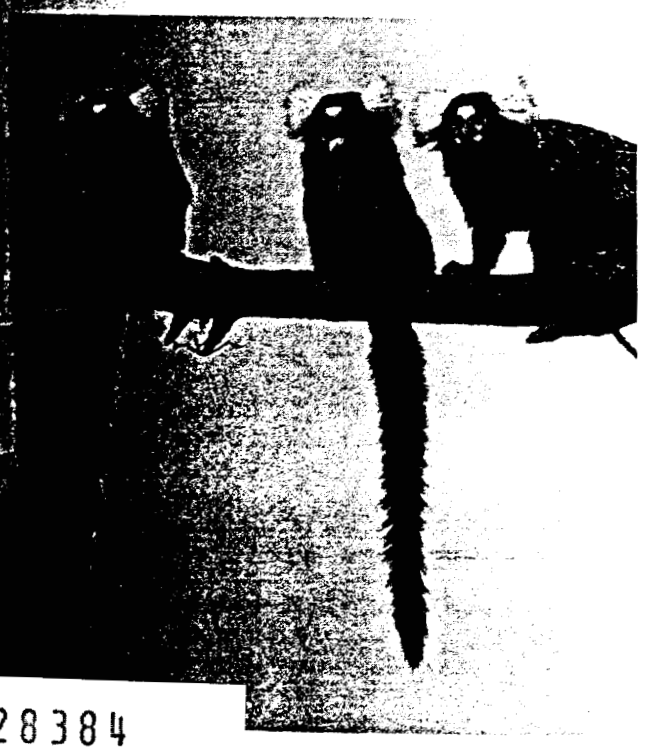
Research activities progressed in radiation biology. The REAC/TS cytogenetic program expanded its activities in routine dosimetry and in standardizing cytogenetic effects from alpha-emitting actinides. In addition, the program continued to develop new cytogenetic approaches to the definition and recognition of genetic risks from human exposure to environmental mutagens. Studies concerning serious high-dose radiation injury of the extremities have resulted in a more sensitive method using ultrasound technology for the early detection of radiation vasculitis.

The REAC/TS Radiation Accident Registries and the Radiation Accident Survivor Follow-Up Program were expanded to include study of all persons who received more than 4.99 rem total-body irradiation in any given calendar year. The roster of these persons (~2000) is being established and the mechanics of annual medical follow-up are being developed.

REAC/TS maintains an active part in chelation therapy with calcium diethylenetriaminepentaacetic acid (DTPA) and zinc-DTPA. Permission has been obtained to use these forms of DTPA in patients. This year medicinal grade zinc-DTPA (1 g/ampule) was distributed to 40 coinvestigators in DOE and non-DOE nuclear facilities. Investigations into the potentially toxic, genetic, and cytogenetic adverse effects, as well as chemical manipulation of DTPA for more effective chelation, are in progress.

1028383

The Marmoset Research Center houses a colony of small New World primates whose biological makeup has proved to be an invaluable tool in research on immunology and colon cancer.



1028384

Publications

All journal articles, abstracts (papers presented at meetings, regardless of publication), review articles, books, chapters, and doctoral dissertations are listed here. All material currently in press is included and is so indicated. Abstracts of full-length publications are found in the next section.

Andrews, G. A.; Hübner, K. F.; and Greenlaw, R. H.
Gallium-67 citrate imaging in malignant lymphoma: final report of cooperative group.
J. Nucl. Med. 19:1013-19. 1978.

Armstrong, R. D., and Frome, E. L.
A remark on algorithm As108.
Appl. Stat. (in press). 1978.

Armstrong, R. D., and Frome, E. L.
A special purpose linear programming alogrithm for obtaining least absolute value estimators in a linear model with dummy variables.
Commun. Stat. B6:383-98. 1977. (Work performed at the University of Texas, Austin.)

Blank, M. L.; Dalbey, W.; Nettesheim, P.; Price, J.; Creasia, D.; and Snyder, F.
Sequential changes in phospholipid composition and synthesis in lungs exposed to nitrogen dioxide.
Am. Rev. Respir. Dis. 117:273-80. 1978.

Cabot, M. C., and Gatt, S.
The hydrolysis of triacylglycerol and diacylglycerol by a rat brain microsomal lipase with an acidic pH optimum.
Biochim. Biophys. Acta (in press). 1978. (Work performed at Hebrew University, Jerusalem.)

Cabot, M. C., and Gatt, S.
Lipases of rat brain microsomes.
Adv. Exp. Med. Biol. 101:101-11. 1978. (Work performed at Hebrew University, Jerusalem.)

Cabot, M. C., and Snyder, F.
The assimilation of unnatural aliphatic moieties into complex lipids of neoplastic cells.
In *Proceedings of the XII International Cancer Congress* (in press), Buenos Aires, Argentina. 1978.

Cabot, M. C., and Snyder, F.
The incorporation of a cyclopentenyl fatty acid into cellular lipids of L-M cells: effects on growth and fatty acid composition.
Presented at the 12th Annual Regional Lipid Conference, Helen, Georgia. 1977.

Cabot, M. C., and Snyder, F.
The manipulation of fatty acid composition in L-M cell monolayers supplemented with cyclopentenyl fatty acids.
Arch. Biochem. Biophys. (in press). 1978.

Chaskes, S., and Tyndall, R. L.
Pigment production by *Cryptococcus neoformans* and other *Cryptococcus* species from aminophenols and diaminobenzenes.
J. Clin. Microbiol. 7:146-52. 1978.

Chepenik, K. P.; Blank, M.; Malone, B.; Snyder, F.; and Waite, B. M.
Some aspects of lipid metabolism during mammalian embryogenesis.
Presented at the 12th Annual Regional Lipid Conference, Helen, Georgia. 1977.

Chepenik, K. P.; Malone, B.; Blank, M.; Borst, D. E.; and Snyder, F.
Lipid metabolism during abnormal limb morphogenesis.
Teratology 17:38A (abstract). 1978.

Dosado, E. A.; Hsie, A. W.; and Snyder, F.
Modification of phospholipid metabolism in dibutyryl-cAMP-mediated morphological conversion of CHO cells.
Biochem. Biophys. Res. Commun. 78:1087-92. 1977.

DuFrain, R. J.; Littlefield, L. G.; and Joiner, E. E.
Cytogenetic dosimetry for ^{241}Am alpha particle irradiation of G_0 stage human lymphocytes.
Radiat. Res. 74:523 (abstract). 1978.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
Chemically induced cytogenetic damage in germinal and somatic cells of the female rabbit.
Mutat. Res. 53:103 (abstract). 1978.

Durum, S. K., and Gengozian, N.
The comparative radiosensitivity of T and B lymphocytes.
Int. J. Radiat. Biol. 34:1-15. 1978.

Frome, E. L., and Armstrong, R. D.
A robust procedure for estimating the trend-cycle component of an economic time series.
In *Computer Science and Statistics: 10th Annual Symposium on the Interface, 1977*, pp. 271-75. U.S. National Bureau of Standards Special Publications 503. 1978. (Work performed at the University of Texas, Austin.)

Frome, E. L., and DuFrain, R. D.
The analysis of dose response curves for radiation-induced chromosome aberrations.
Biometrics (in press) (abstract). 1978.

Gengozian, N.
Immunology and blood chimerism of the marmoset.
Primates Med. 10:173-83. 1978.

Gengozian, N., and McLaughlin, C. L.
Actively induced platelet-bound IgG associated with thrombocytopenia in the marmoset.
Blood 51:1197-1210. 1978.

Genogozian, N.; Batson, J. S.; and Smith, T. A.
Breeding of marmosets in a colony environment.
Primates Med. 10:71-78. 1978.

Gengozian, N.; Kateley, J. R.; and Nickerson, D. A.
Marmoset species variation in the humoral antibody response: in vivo and in vitro studies.
Immunology (in press). 1978.

Gengozian, N., and Deinhardt, F., eds.
Marmosets in Experimental Medicine. Primates in Medicine, vol. 10.
(E. I. Goldsmith and J. Moor-Jankowski, series editors). Basel, Switzerland: S. Karger. 1978.

Gibbs, W. D.; Holloway, S.; and Hodges, H. D.
An approach to quantitation of images obtained by positron emission tomography.
J. Nucl. Med. Technol. 6:104 (abstract). 1978.

Hayes, R. L.
Chemistry and radiochemistry of metal ion nuclides commonly employed in radiopharmaceuticals.
In *The Chemistry of Radiopharmaceuticals*, eds. N. D. Heindel, H. D. Burns, T. Honda, and L. W. Brady, pp. 155-68. New York: Masson Publishing Company. 1978.

Hayes, R. L.
The medical use of gallium radionuclides: a brief history with some comments.
Semin. Nucl. Med. 8:183-91. 1978.

Hayes, R. L.; Byrd, B. L.; and Szymendera, J.
Effect of food intake on the tissue distribution of gallium-67.
In *Proceedings of the Second International Congress of the World Federation of Nuclear Medicine and Biology*, p. 112 (abstract). Washington, D.C. 1978.

Hayes, R. L.; Carlton, J. E.; and Kuniyasu, Y.
A ^{68}Ga albumin preparation for positron tomography of the liver.
In *Proceedings of the 18th Annual Meeting and Continuing Education Lectures, 1977*; A Review of Nuclear Medicine, Part 2, [Section] III, p. 5. Abstracts of Scientific Sessions, Southeastern Chapter, Society of Nuclear Medicine (abstract). 1977.

Hayes, R. L.; Rafter, J. J.; and Butler, T. A.
Copper-64 as a possible positron tomographic agent for detection of cancer.
In *Proceedings of the 18th Annual Meeting and Continuing Education Lectures, 1977*; A Review of Nuclear Medicine, Part 2, [Section] III, p. 5. Abstracts of Scientific Sessions, Southeastern Chapter, Society of Nuclear Medicine (abstract). 1977.

Hayes, R. L.; Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Anon, J. B.; Butler, T. A.; and Callahan, A. P.
Synthesis and purification of [^{11}C]carboxyl-labeled amino acids.
Int. J. Appl. Radiat. Isot. 29:186-87. 1978.

1028385

Highfill, R. R.
The use of cyclotron-produced radioisotopes in nuclear medicine.
Presented at the 8th International Conference on Cyclotrons and Their Applications, Bloomington, Indiana. 1978.

Highfill, R. R., and Gibbs, W. D.
Positron tomographic imaging of the abdomen.
In *Proceedings of the 18th Annual Meeting and Continuing Education Lectures, 1977;* A Review of Nuclear Medicine, Part 2, [Section] III, p. 6. Abstracts of Scientific Sessions, Southeastern Chapter, Society of Nuclear Medicine (abstract). 1977.

Highfill, R. R., and Wieland, B. W.
A choice of targetry to significantly reduce the size of medical cyclotrons for positron tomography.
In *Proceedings of the Second International Congress of the World Federation of Nuclear Medicine and Biology,* p. 17 (abstract). Washington, D.C. 1978.

Hodges, H.; Gibbs, W. D.; and Holloway, S.
Letters to the Editor.
J. Nucl. Med. Technol. 6:96. 1978.

Hodges, H.; Holloway, S.; and Gibbs, W. D.
Reducing CO_2 inhalation in clinical pulmonary investigations.
J. Nucl. Med. Technol. 5:211-12. 1977.

Hübner, K. F.; Andrews, G. A.; Gibbs, W. D.; Holloway, S.; Hayes, R. L.; and Washburn, L. C.
Initial diagnostic results with ^{11}C-labeled amino acids and the emission positron tomograph.
In *Proceedings of the Second World Federation of Nuclear Medicine and Biology,* p. 13 (abstract). Washington, D.C. 1978.

Hübner, K. F.; Andrews, G. A.; Hayes, R. L.; Poggenburg, J. K., Jr.; and Solomon, A.
The use of rare-earth radionuclides and other bone seekers in the evaluation of bone lesions in patients with multiple myeloma or solitary plasmacytoma.
Radiology 126:171-76. 1977.

Hübner, K. F.; Andrews, G. A.; Lushbaugh, C. C.; and Tompkins, E.
A follow-up study program for persons irradiated in radiation accidents.
In *Handling of Radiation Accidents 1977, Proceedings of a Symposium,* pp. 57-70. Vienna, Austria: International Atomic Energy Agency. 1977.

Hübner, K. F.; Andrews, G. A.; Washburn, L.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.; Butler, T. A.; and Winebrenner, J. D.
Tumor location with 1-aminocyclopentane [^{11}C]carboxylic acid: preliminary clinical trials with single-photon detection.
J. Nucl. Med. 18:1215-21. 1977.

Hübner, K. F.; Hayes, R. L.; Washburn, L. C.; Gibbs, W. D.; Byrd, B. L.; and Butler, T. A.
Scanning of the human pancreas with DL-valine-1-^{11}C and DL-tryptophan-1-^{11}C.
J. Nucl. Med. 19:686 (abstract). 1978.

Kateley, J. R.; Nickerson, D. A.; and Gengozian, N.
Mitogenic response of marmoset lymphocytes: cytokinetics and identification of responsive cells.
Immunology 33:653-62. 1978.

Kuniyasu, Y.; Hayes, R. L.; and Carlton, J. E.
A ^{68}Ga albumin preparation for positron tomography of the liver.
In *Proceedings of the Second International Congress of World Federation of Nuclear Medicine and Biology,* p. 90 (abstract). Washington, D.C. 1978.

Lambremont, E. N.; Blank, M. L.; Lee, T-c.; and Snyder, F.
Regulation of glycerolipid synthesis in L-M fibroblasts by *N*-isopropylethanolamine, a choline analog.
J. Am. Oil Chem. Soc. 55:254A (abstract 179). 1978.

Lambremont, E. N.; Lee, T-c.; and Blank, M.
Effect of a choline analog, *N*-isopropylethanolamine, on lipid metabolism of L-M cells.
Presented at the 12th Annual Regional Lipid Conference, Helen, Georgia. 1977.

Lambremont, E. N.; Lee, T-c.; Blank, M. L.; and Snyder, F.
$\Delta 5$ Desaturation of fatty acids in L-M cells.
Biochem. Biophys. Res. Commun. 80:813-18. 1978.

Lawless, D.; Brown, D. H.; Hübner, K. F.; Colyer, S. P.; Carlton, J. E.; and Hayes, R. L.
Isolation and partial characterization of a ^{67}Ga-binding glycoprotein from a rat hepatoma.
Cancer Res. (in press). 1978.

Lee, T-c.; Baker, R. C.; Stephens, N.; and Snyder. F.
Evidence for participation of cytochrome b_5 in microsomal $\Delta 6$ desaturation of fatty acids.
Biochim. Biophys. Acta 489:25-31. 1977.

Lee, T-c.; Blank, M. L.; and Fitzgerald, V.
Oxidation of a fatty alcohol to a fatty acid by rat liver microsomes.
Fed. Proc. 37:1704 (abstract 2398). 1978.

Lee, T-c.; Blank, M. L.; and Snyder, F.
Effect of a choline inhibitor (*N*-isopropylethanolamine) on cellular metabolism of L-M cells.
Biochim. Biophys. Acta 529:351-58. 1978.

Lincoln, T. A., and Lushbaugh, C. C.
Organization, planning, and training.
In *Early Medical Treatment of Radiation Injury (Including Sodium Burns),* ed. J. C. Nenot. Geneva, Switzerland: International Atomic Energy Agency (in press). 1976.

Littlefield, L. G.; DuFrain, R. J.; and Colyer, S. P.
Sister chromatid exchanges in human lymphocytes exposed to ^{60}Co gamma radiation during G_0.
Radiat. Res. 74:522-23 (abstract). 1978.

Littlefield, L. G., and Joiner, E. E.
Cytogenetic follow-up studies in six radiation accident victims (16 and 17 years postexposure).
In *Proceedings of a Symposium on Late Biological Effects of Ionizing Radiation, 1978.* Vienna, Austria: International Atomic Energy Agency (in press). 1978.

1028386

Lushbaugh, C. C.; Hübner, K. F.; and Ricks, R. C.
Medical aspects of nuclear radiation emergencies.
Emergency 10(9):32-35. 1978.

Lushbaugh, C. C.; Humason, G. L.; Swartzendruber, D. C.; Richter, C. B.; and Gengozian, N.
Spontaneous colonic adenocarcinoma in marmosets.
Primates Med. 10:119-34. 1978.

Moore, C.; Blank, M. L.; Lee, T-c.; Benjamin, B.; Piantadosi, C.; and Snyder, F.
Membrane lipid modifications: biosynthesis and identification of phosphatidyl-*N*-methyl-*N*-isopropylethanolamine in rat liver microsomes.
Chem. Phys. Lipids 21:175-78. 1978.

Moore, C.; Lee, T-c.; Blank, M.; Stephens, N.; and Snyder, F.
Enzyme activities in lipid-modified membranes of rat liver.
Presented at the 12th Annual Regional Lipid Conference, Helen, Georgia. 1977.

Moore, C.; Lee, T-c.; Stephens, N.; and Snyder, F.
Enzyme activities in lipid-modified membranes of rat livers.
Biochim. Biophys. Acta (in press). 1978.

Moore, C., and Snyder, F.
Properties of acyl-CoA reductase from the mouse preputial gland.
Presented at the 13th Annual Regional Lipid Conference, Long Beach, North Carolina. 1978.

Nelson, B. M.; Andrews, G. A.; and Watson, E. E.
Histopathologic studies of the liver following intravenous colloidal ^{198}Au therapy.
Radiology 127:239-47. 1978.

Nickerson, D. A.
Primary in vitro antibody formation by marmoset peripheral blood leucocytes.
Ph.D. dissertation, University of Tennessee. 1978.

Nickerson, D. A., and Gengozian, N.
In vitro immune responses of marmoset peripheral blood T and B lymphocytes: species distinction.
Fed. Proc. 37:1487 (abstract 1205). 1978.

Parker, J. C.; Whiteman, M. D.; and Richter, C. B.
Susceptibility of inbred and outbred mouse strains to Sendai virus and prevalence of infection in laboratory rodents.
Infect. Immun. 19:123-30. 1978.

Polednak, A. P.
Age changes in differential leukocyte count in female adults.
Hum. Biol. (in press). 1978. (Work performed at Argonne National Laboratory, Chicago.)

Polednak, A. P.
Bone cancer among female radium dial workers. Latency periods and incidence rates by time after exposure.
J. Natl. Cancer Inst. 60:77-82. 1978. (Work performed at Argonne National Laboratory, Chicago.)

Polednak, A. P.
Long-term effects of radium exposure in female dial workers: differential white blood cell count.
Environ. Res. 15:252-61. 1978. (Work performed at Argonne National Laboratory, Chicago.)

Polednak, A. P., ed.
The Longevity of Athletes, (in press).
Springfield, Illinois: Charles C. Thomas, Inc. (Work performed at Argonne National Laboratory, Chicago.)

Polednak, A. P.; Stehney, A. F.; and Rowland, R. E.
Mortality among women first employed before 1930 in the U.S. radium dial-painting industry. A group ascertained from employment lists.
Am. J. Epidemiol. 10:179-95. 1978. (Work performed at Argonne National Laboratory, Chicago.)

Porcella, R. A.; Swartzendruber, D. C.; and Godbold, J. H., Jr.
A morphometric study quantifying the mucus content of proliferating colonic epithelial cells.
J. Cell Biol. (in press) (abstract). 1978.

Richter, C. B.; Humason, G. L.; and Godbold, J. H., Jr.
Endemic *Pneumocystis carinii* in a marmoset colony.
J. Comp. Pathol. 88:171-80. 1978.

Richter, C. B.; Tankersley, W.; and Webb, A.
Chronic recurrent colitis: A wasting syndrome in marmosets and tamarins.
Presented at the Lab Animal Science Meeting, New York, New York. (Abstract). 1978.

Rock, C. O.; Fitzgerald, V.; and Snyder, F.
Coupling of the biosynthesis of fatty acids and fatty alcohols.
Arch. Biochem. Biophys. 186:77-83. 1978.

Rock, C. O.; Fitzgerald, V.; and Snyder, F.
Properties of dihydroxyacetone phosphate acyltransferase in the harderian gland.
J. Biol. Chem. 252:6363-66. 1977.

Rock, C. O., and Snyder, F.
Asymmetric localization of alkyldihydroxyacetone-P synthase and acyldihydroxyacetone-P acyltransferase in microsomal vesicles.
Adv. Exp. Med. Biol. 101:379-85. 1978.

Sauerbrunn, B. J. L.; Andrews, G. A.; and Hübner, K. F.
Gallium-67 citrate imaging in tumors of the genitourinary tract: report of cooperative study.
J. Nucl. Med. 19:470-75. 1978.

Schremmer, J. M.; Blank, M. L.; and Wykle, R. L.
Studies of arachidonic acid release and prostaglandin synthesis by $HSDM_1C_1$ cells.
Presented at the 12th Annual Regional Lipid Conference, Helen, Georgia. 1977.

Scott, C. C.; Heckman, C. A.; Nettesheim, P.; and Snyder, F.
Lipid turnover in squamous-cell carcinoma cells and primary tracheal epithelial cells.
J. Cell Biol. 75:76a (abstract 7235A). 1977.

Scott, C. C.; Heckman, C. A.; Nettesheim, P.; and Snyder, F.
Metabolism of ether-linked glycerolipids in cultures of normal

1028387

and neoplastic rat respiratory tract epithelium.
Cancer Res. (in press). 1978.

Scott, C.; Heckman, C.; and Snyder, F.
Ether-lipid regulation in cultured cells: relationship to glucose metabolism.
J. Cell Biol. (in press) (abstract). 1978.

Shivers, C. A.; Gengozian, N.; Franklin, S.; and McLaughlin, C. L.
Antigenic cross-reactivity between human and marmoset zona pellucidae, a potential target for immunocontraception.
J. Med. Primatol. (in press). 1978.

Voelker, D. R., and Snyder, F.
Subcellular site and mechanism of synthesis of pulmonary surfactant phosphatidylcholine in alveolar type II cell adenomas.
Fed. Proc. 37:1835 (abstract). 1978.

Washburn, L. C., and Hayes, R. L.
Importance of excess base in the synthesis of sodium phosphorothioate.
In *Inorganic Syntheses*, ed. A. G. MacDiarmid, vol. 17, pp. 193-94. New York: McGraw-Hill Book Co. 1978.

Washburn, L. C.; Sun, T. T.; Anon, J. B.; and Hayes, R. L.
Effect of structural modifications on the tumor specificity of alicyclic α-amino acids.
In *Proceedings of the 18th Annual Meeting and Continuing Education Lectures, 1977;* A Review of Nuclear Medicine, Part 2, [Section] III, p. 9. Abstracts of Scientific Sessions. Southeastern Chapter, Society of Nuclear Medicine (abstract). 1977.

Washburn, L. C.; Sun, T. T.; Anon, J. B.; and Hayes, R. L.
Effect of structure on tumor specificity of alicyclic α-amino acids.
Cancer Res. 38:2271-73. 1978.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and Butler, T. A.
Further preclinical studies of ^{11}C-DL-tryptophan, a potential pancreas-imaging agent.
In *Proceedings of the Second International Congress of the World Federation of Nuclear Medicine and Biology*, p. 41 (abstract). Washington, D.C. 1978.

Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Hayes, R. L.; and Butler, T. A.
[1-^{11}C]DL-Valine, a potential pancreas-imaging agent.
J. Nucl. Med. 19:77-83. 1978.

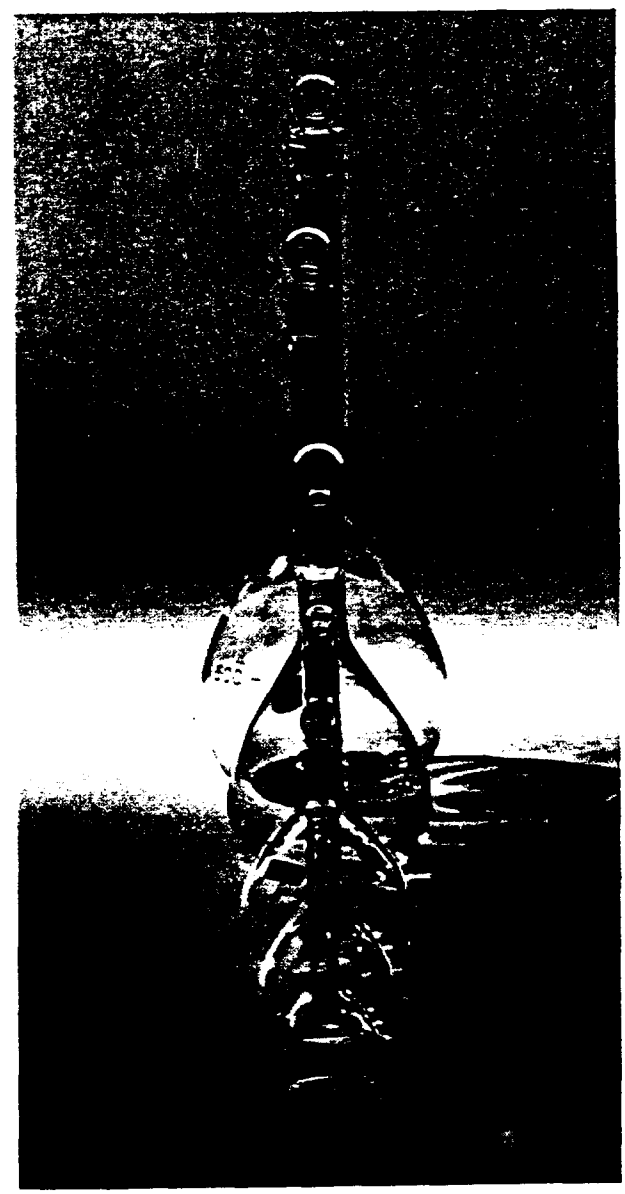
Wieland, B. W.; Highfill, R. R.; Lemon, R. A.; and Howell, R. S.
Applicability of a single-particle fixed-energy accelerator for production of short-lived, positron-emitting nuclides for clinical use.
In *Proceedings of the 18th Annual Meeting and Continuing Education Lectures, 1977;* A Review of Nuclear Medicine, Part 2 [Section] III, p. 10. Abstracts of Scientific Sessions, Southeastern Chapter, Society of Nuclear Medicine (abstract). 1977.

Wieland, B. W., and Howell, R. A.
Computed yields of ^{11}C, ^{13}N, ^{15}O, and ^{18}F for proton bombardment of enriched stable isotope targets.
J. Labelled Compd. Radiopharm. (in press) (abstract). 1978.

Wykle, R. L.; Kraemer, W. F.; and Schremmer, J. M.
Studies of lysophospholipase D of rat liver and other tissues.
Arch. Biochem. Biophys. 184:149-55. 1977.

Wykle, R. L., and Schremmer, J. M.
Biosynthesis of plasmalogens: influence of the composition of the 2-acyl constituent on desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine by tumor microsomes.
Presented at the 13th Annual Regional Lipid Conference, Long Beach, North Carolina. 1978.

Yakatan, G. J., Frome, E. L.; Leonard, R. G.; Shah, A. C.; and Doluisio, J. T.
Bioavailability of acetazolamide tablets.
J. Pharm. Sci. 67:252-55. 1978. (Work performed at the University of Texas, Austin.)



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1028389

REAC/TS provides ultra-modern, multipurpose facilities for handling radiation emergencies. It is designed to cope with any type of radiation-exposure accident that might occur in Oak Ridge or elsewhere.



Abstracts of Full-Length Publications

This section contains abstracts of full-length publications. Only published articles and review articles that contain previously unpublished data are included.

Andrews, G. A.; Hübner, K. F.; and Greenlaw, R. H.
Gallium-67 citrate imaging in malignant lymphoma: final report of cooperative group.
J. Nucl. Med. 19:1013-19. 1978.

In a large cooperative study of gallium-67 uptake in non-Hodgkin's malignant lymphoma, 76% of untreated patients showed positive uptake in one or more lesions. The percentage of known individual lesions seen on scan was significantly lower; thus, negative findings at any one site may have much less significance than positive findings. After treatment, the number of lesions seen decreases sharply, but the role of gallium-67 in evaluating response to therapy is uncertain, especially in view of the fairly large number of lesions undetectable before therapy. Histologic type plays a role in gallium-67 uptake. Large lesions are much more effectively detected than small ones. In spite of numerous false-negative results, gallium-67 scanning is a useful method in evaluating the extent of untreated disease and the presence of lesions post-therapy.

Armstrong, R. D., and Frome, E.
A special purpose linear programming algorithm for obtaining least absolute value estimators in a linear model with dummy variables.
Commun. Stat. B6:383-98. 1977. (Work performed at the University of Texas, Austin.)

Dummy (0,1) variables are frequently used in statistical modeling to represent the effect of certain extraneous factors. This paper presents an algorithm for obtaining least-absolute-value estimators in a linear model with dummy variables. The algorithm employs a compact-basis inverse procedure and incorporates the advanced-basis exchange techniques available in specialized algorithms for the general linear least-absolute-value problem. Computational results with a computer code version of the algorithm are given.

1028390

Blank, M. L.; Dalbey, W.; Nettesheim, P.; Price, J.; Creasia, D.; and Snyder, F.
Sequential changes in phospholipid composition and synthesis in lungs exposed to nitrogen dioxide.
Am. Rev. Respir. Dis. 117:273-80. 1978.

Studies were performed to investigate the time course of biochemical and histologic changes in the lungs of rats after exposure to various concentrations of nitrogen dioxide. Special attention was given to identify lipid classes and the molecular species preferentially affected. Increased incorporation of thymidine, labeled with tritium, into deoxyribonucleic acid was first detected 12 hr after a 5-hr exposure to 40 ppm of nitrogen dioxide and reached a peak after 24 to 36 hr. The first histologic signs of a hyperplastic response in the alveolar ducts and alveoli became recognizable at 24 hr. In contrast, increased incorporation of palmitic acid, labeled with carbon-14, into phosphatidylcholine and the accumulation of this lipid class in the lungs could be detected as early as 6 hr after exposure to nitrogen dioxide. Lung wet weights and lung protein were also increased at this time. These findings suggest that the increase in phosphatidylcholine in the lungs exposed to nitrogen dioxide occurs before commencement of the regenerative hyperplastic response.

Our studies also provide evidence that the increased phospholipid content of the lungs after exposure to nitrogen dioxide is at least in part due to increased biosynthesis. The disaturated species of phosphatidylcholine increased preferentially over unsaturated species. Incorporation of [1-^{14}C]palmitic acid into phosphatidylcholine reached a peak 36 hr after exposure to nitrogen dioxide. The total amount of phosphatidylcholine and phosphatidyglycerol, two components of lung surfactant lipid, were maximal at 48 hr. By 4 days, the incorporation of [1-^{14}C]palmitic acid into phosphatidylcholine returned to near normal values, but the phosphatidylcholine content of the lungs was still significantly increased at this time. On the basis of thin-layer and gas chromatography, no evidence was obtained for oxidation of esterified lipids or appearance of abnormal lipid structures in the lungs exposed to nitrogen dioxide.

Cabot, M. C., and Gatt, S.
Lipases of rat brain microsomes.
Adv. Exp. Med. Biol. 101:101-11. 1978. (Work performed at Hebrew University, Jerusalem.)

This study describes the hydrolysis of acylglycerols by lipases of rat brain microsomes. Two enzymic activities were distinguishable on the basis of substrate specificity, pH optima, and sensitivity to SH reagents: (1) an enzyme that hydrolyzes either monoacylglycerol or a combined hydrolysis of diacylglycerol to monoacylglycerol and glycerol, active at pH 8.0, and (2) an enzyme, active at pH 4.8, that hydrolyzes triacylglycerol and diacylglycerol. Mercuric chloride and N-ethylmaleimide strongly inhibited the monoacylglycerol lipase. At pH 4.8, diacylglycerol was the major product of triacylglycerol hydrolysis. The acidophilic enzyme showed positional specificity for fatty acids located at the primary positions of *sn*-glycerol. When diacylglycerol was the starting substrate, it was hydrolyzed at a rate tenfold greater than triacylglycerol. An alternative method for assaying diacylglycerol lipase is described. The phosphoglyceride fraction of the microsomes was labeled by intracerebral injection of [^{14}C]fatty acids, and the formation of membrane-bound diacylglycerols was promoted by incubating the microsomes with phospholipase C. The action of a membrane-bound enzyme on endogenous substrate was then followed by solely incubating the microsomes at pH 4.8.

Chaskes, S., and Tyndall, R. L.
Pigment production by *Cryptococcus neoformans* and other *Cryptococcus* species from aminophenols and diaminobenzenes.
J. Clin. Microbiol. 7:146-52. 1978.

Cryptococcus neoformans and other *Cryptococcus* species can produce pigment(s) from many aminophenol and diaminobenzene compounds. Pigment production from these compounds is similar to the conversion of diphenols to melanin by *C. neoformans*. Several pigmentation patterns (resulting in the identification or grouping of *Cryptococcus* species) have been observed by using diaminobenzene and aminophenol compounds as substrates. The most common pigmentation pattern observed was pigment production by both *C. neoformans* and *C. terreus*. In contrast to the diphenols, only two aminophenols (4-hydroxymetanilamide and 3-aminotyrosine) were found to be highly specific as substrates. They allowed only *C. neoformans* to produce pigment. When 4-aminosalicylic acid was the substrate, a unique pattern was observed because only *C. terreus*, *C. diffluens*, and *C. albidus* produced pigment. Finally, a pattern was observed in which *C. neoformans* produced large amounts of pigment from aminophenol and diaminobenzene compounds, whereas the other *Cryptococcus* species produced smaller amounts. A simplified scheme with three substrates resulted in the identification of *C. terreus* and *C. neoformans* as well as two groups of other *Cryptococcus* species: group I (*C. albidus* and *C. diffluens*) and group II (*C. laurentii* and *C. luteolus*).

Dosado, E. A.; Hsie, A. W.; and Snyder, F.
Modification of phospholipid metabolism in dibutyryl-cAMP-medicated morphological conversion of CHO cells.
Biochem. Biophys. Res. Commun. 78:1087-92. 1977.

The cellular uptake and incorporation of [1-^{14}C]palmitic acid and ^{32}P into lipids of Chinese hamster ovary cells, clone K_1 (CHO-K_1), have been investigated under conditions where the cells are converted from the compact, epithelial-like shape to the elongated, fibroblast-like morphology by N^6, $O^{2'}$-dibutyryl adenosine 3':5'-phosphate. The primary alteration in lipid metabolism accompanying the morphological conversion to the fibroblast form was an increased incorporation of lipid precursors into all phospholipid classes and a decreased incorporation into the "neutral" lipid fraction. These results reflect the cells' need for phospholipid precursors when the membrane expands to form the fibroblast shape. When the fibroblast-shaped cells were allowed to revert to the epithelial shape, lipid metabolism was similar to that found in untreated cells.

Durum, S. K., and Gengozian, N.
The comparative radiosensitivity of T and B lymphocytes.
Int. J. Radiat. Biol. 34:1-15. 1978.

102839.1

This study deals with the comparative effects of gamma irradiation on T and B lymphocytes in mice. Quantitatively, splenic T cells appeared substantially more radioresistant than B cells. However, in the thymus, mesenteric lymph node, and peripheral blood, both cell types were highly radiosensitive. No repair processes could be detected with respect to lymphocyte killing, since reducing the dose rate or fractionating the dose resulted in no reduction in cell destruction.

Splenic B cells, which remained intact 3 days following 600 R or larger doses, were impaired in the ability to form caps, whereas T cells capped normally. Spleen cells, which were viable 3 days after irradiation, bore latent radiation damage that was manifested as reduced ability to survive and generate plaque-forming cells in vitro. Attempts to separately assess irradiated T and B cell immunocompetence in vitro suggested that at doses of 300 R and below, surviving B cells were more impaired than T cells. After a 600-R dose, neither cell type appeared functional.

Frome, E. L., and Armstrong, R. D.
A robust procedure for estimating the trend-cycle component of an economic time series.
In *Computer Science and Statistics: 10th Annual Symposium on the Interface, 1977*, pp. 271-75. U.S. National Bureau of Standards Special Publications 503. 1978. (Work performed at the University of Texas, Austin.)

Economic time series are often represented as a composite of trend-cycle, seasonal, and irregular movements. We propose that the cubic spline regression method be used to estimate the trend-cycle component and that parameters be estimated by minimizing the sum of the absolute values of the deviations. If a seasonal component is present, the regression model can be extended using dummy variables. In both cases, least absolute value estimates are obtained using a special purpose linear programming algorithm. An example of the application of the cubic spline smoothing procedure to monthly Texas construction data is discussed.

Gengozian, N.
Immunology and blood chimerism of the marmoset.
Primates Med. 10:173-83. 1978.

Marmosets, small South American primates of the *Callitrichidae* family, are unique in that they show a high frequency of fraternal twinning. This twinning is characterized further by the development of placental vascular anatomoses between the fetuses, a condition that leads to hemopoietic chimerism. As demonstrated previously in our laboratory, this blood chimerism permits tissue transplantation between cotwins and to date suggests complete immunological tolerance of the host to antigens expressed by the chimeric elements. In our effort to understand and identify the factors promoting tolerance in this natural blood chimera, we have found it necessary to obtain more information on the chimerism phenomenon itself and basic data on histocompatibility antigens and immunologic responsiveness of this species. This report summarizes our knowledge in these areas and presents information suggesting that blood chimerism may play a role in the immune response capabilities of these animals.

Gengozian, N., and McLaughlin, C. L.
Actively induced platelet-bound IgG associated with thrombocytopenia in the marmoset.
Blood 51:1197-1210. 1978.

Interspecies platelet immunizations among marmosets lead to antibody formation to the donor platelets and a profound thrombocytopenia, which when associated with anemia may result in death of the animal. This actively induced immunologic thrombocytopenia closely resembles two clinical disease entities manifesting autoimmune thrombocytopenia: posttransfusion purpura and idiopathic thrombocytopenic purpura. Although antibody to donor-type platelets could be demonstrated readily, antihost activity was most often nondetectable or, when present, was in very low titer. A consistent finding was the appearance of IgG on the host's platelets shortly after immunization and concomitant with the appearance of antidonor platelet antibody. In 3 of 13 immunized animals, thrombocytopenia did not occur even though antibody was formed and the host's platelets became IgG positive. In those animals that recovered from the induced thrombocytopenia, IgG-positive platelets were found for periods ranging from 30 to greater than 100 days. Splenectomy before and after immunization did not alter the sequential development of antibody formation, appearance of IgG-positive platelets, or thrombocytopenia. Eluates prepared from IgG-positive platelets contained IgG and platelet antigens; the eluted IgG could attach nonspecifically to platelets of host or donor (immunizing) type, in contrast to the species specificity demonstrated for IgG eluted from platelets that had been reacted in vitro with specific antibody. Platelets in a few normal, nonimmunized marmosets had significant amounts of IgG on their surface, comparable to that observed in the immunized animal; interestingly, such IgG-positive platelets were found among imported, but not laboratory-bred, marmosets.

Gengozian, N.; Batson, J. S.; and Smith, T. A.
Breeding of marmosets in a colony environment.
Primates Med. 10:71-78. 1978.

Studies on the marmoset were initiated in our laboratory in 1961 with the specific aim of utilizing this primate in radiation biology. It soon became apparent that as an experimental subject this diminutive primate could offer advantages not observed with other primate species, an impression underscored by successful breeding that first year without any specific efforts made in reproduction. During the past 10 years, our studies have been directed toward understanding the immunologic consequences of the natural blood chimerism that occurs in *Saguinus fuscicollis*. Although specific monies for a breeding program were not available during this time, our research goals necessitated greater attention to breeding, and indeed the concept of nonterminal experimentation was adapted early in our program. The current restrictions on exportation of these animals from the countries of origin place their successful breeding at a high priority if we are to realize the contributions they can make in biomedicine. This report describes pertinent observations that may be of value to those planning to breed or presently engaged in breeding marmosets.

1028392

Hayes, R. L.
Chemistry and radiochemistry of metal ion nuclides commonly employed in radiopharmaceuticals.
In *The Chemistry of Radiopharmaceuticals*, eds. N. D. Heindel, H. D. Burns, T. Honda, and L. W. Brady, pp. 155-68. New York: Masson Publishing Company. 1978.

It certainly behooves those in the nuclear medical field to pay more attention to the basic chemistry involved in the effectiveness of radiopharmaceuticals. In the past, basic chemistry has usually been only an interesting side issue. It did not become important until scientists realized that it did indeed provide insight into the reason for the in vivo behavior of radiopharmaceuticals. Although we are dealing with complex biological systems, many of which are not understood, considerations of the basic chemistry involved can certainly point the way toward developing better agents.

Hayes, R. L.
The medical use of gallium radionuclides: a brief history with some comments.
Semin. Nucl. Med. 8:183-91. 1978.

The use of gallium radionuclides in nuclear medicine dates back to the late 1940s, following the observation in toxicologic studies that gallium localizes to a high degree at sites of osteogenic activity. Initial attempts in the early 1950s to use ^{72}Ga for clinical diagnosis and therapy of malignant bone lesions were unproductive. However, the basic information gained then in the preclinical and clinical investigations was quite instrumental in encouraging the present-day use of gallium radionuclides as effective radiopharmaceutical agents. Although initial clinical trials of ^{72}Ga were unproductive, subsequent studies with ^{66}Ga and ^{67}Ga, together with advances in nuclear medical instrumentation, resulted in the identification of gallium radionuclides as effective tumor- and abscess-localizing agents. A major factor in recognizing the peculiar biologic properties of gallium radionuclides was the existence of a carrier (stable isotope) effect. Also, it appears from basic studies of the mechanism(s) of the uptake of gallium in tumor tissue that the biodistribution of gallium involves many essential biological processes. The future use of gallium radionuclides may, therefore, actually fall more into the field of basic biologic investigations rather than into the field of nuclear medical diagnosis.

Hayes, R. L.; Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Anon, J. B.; Butler, T. A.; and Callahan, A. P.
Synthesis and purification of [^{11}C]carboxyl-labeled amino acids.
Int. J. Appl. Radiat. Isot. 29:186-87. 1978.

A rapid high-temperature modification of the Bücherer-Strecker amino acid synthesis, developed for the production of [^{11}C]carboxyl-labeled ACPC, has been found to be of general use for synthesizing other ^{11}C-labeled neutral amino acids. With certain modifications, we found the technique to be applicable to the synthesis of ^{11}C-labeled DL-tryptophan, a basic amino acid. The method requires 20 min for synthesis and an additional 25 min for purification. Chemical yields of the purified amino acids, based on cyanide, have ranged from 10-70%; ^{11}C activity levels as high as 363 mCi ([^{11}C]DL-valine) have been obtained in the final product.

Hodges, H.; Holloway, S.; and Gibbs, W. D.
Reducing CO_2 inhalation in clinical pulmonary investigation
J. Nucl. Med. Technol. 5:211-12. 1977.

Patient comfort during any scanning procedure alway complements the quality of the scan. Many of our patien undergoing clinical pulmonary investigations were becomin progressively nervous; sometimes we were unable to complet the study. We later determined that this was caused by the rebreathing expired CO_2 gas, which collected in a 12-inch flex ble tube connecting the mouthpiece to the delivery arm of th machine. This problem was alleviated by affixing two one way disposable valves to the mouthpiece to prevent backflo of expired CO_2 gas and at the same time allow fresh room a to be breathed. By eliminating the backflow of CO_2 an making it possible for the patient to breathe easily in ampl room air, we reduced the apprehension previously observed As a result, complete and improved pulmonary function oc curred in patients.

Hübner, K. F.; Andrews, G. A ; Lushbaugh, C. C.; an Tompkins, E.
A follow-up study program for persons irradiated in radiatio accidents.
In *Handling of Radiation Accidents 1977, Proceedings of Symposium*, pp. 57-70. Vienna, Austria: International Atomi Energy Agency. 1977.

Clinical and epidemiologic follow-up studies of survivor of radiation accidents are a major part of the program of th Radiation Emergency Assistance Center/Training Sit (REAC/TS) in Oak Ridge, Tennessee, where training is prc vided for physicians and paramedical staff concerned wit radiation accident victims. Guidelines have been developed fo clinicians and epidemiologists to perform follow-up studie after accidental exposures. The human radiobiological data s obtained are used to improve clinical care and emergenc planning in nuclear energy production. Other aims of thes studies are to provide the best prophylactic and anticipator care for possible late radiation effects and to continuall upgrade radiobiologic risk estimates by epidemiologic in vestigations.

Routine, annual, clinical follow-up studies are recom mended for those survivors who are severely injured and fo others who show clinical or laboratory evidence of the acut radiation syndrome. The extent and protocol for medical ex aminations are described. In U.S. industrial plants where an nual physical examinations are routine, the medical status o serious radiation accident victims is reported to REAC/TS Persons involved in accidents but not seriously exposed i radiation accidents are contacted annually by phone or lette to ascertain their location and health status for epidemiologi data collection. Dosimetric guidelines categorizing the follow up groups are defined. These guidelines are related to total body exposure levels, extent of local exposures, and residua body and lung burdens of transuranic elements. Follow-u studies continue for life. The records are deposited in a cen tralized national radiation accident registry in REAC/T where the information is available to clinicians radiobiologists, and epidemiologists.

1028393

Hübner, K. F.; Andrews, G. A.; Hayes, R. L.; Poggenburg, J. K., Jr.; and Solomon, A.
The use of rare-earth radionuclides and other bone seekers in the evaluation of bone lesions in patients with multiple myeloma or solitary plasmacytoma.
Radiology 125:171-76. 1977.

Twenty-four patients with multiple myeloma and four with solitary plasmacytoma had total-body scans after intravenous injection of [^{67}Ga]citrate alone (17 patients) or combined with other agents (11 patients). The latter agents included [^{99m}Tc]diphosphonate, [^{99m}Tc]polyphosphate, or [^{99m}Tc]sulfur colloid given alone or combined with ^{171}Er, ^{157}Dy, or ^{167}Tm as citrate. In some patients, more than one agent was compared to ^{67}Ga and radiographic bone surveys. In general, localization of the rare-earth "bone seekers" was poor except for ^{157}Dy, which compared well with [^{99m}Tc]polyphosphate and [^{99m}Tc]diphosphonate. In studies of the abdomen and pelvis, ^{157}Dy was also helpful because of its failure to concentrate in the gastrointestinal tract. No toxic or nonspecific effects were noted.

Hübner, K. F.; Andrews, G. A.; Washburn, L.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.; Butler, T. A.; and Winebrenner, J. D.
Tumor location with 1-aminocyclopentane [^{11}C]carboxylic acid: preliminary clinical trials with single-photon detection.
J. Nucl. Med. 18:1215-21. 1977.

High specific activity [^{11}C]carboxyl-labeled 1-aminocyclopentane-carboxylic acid ([^{11}C]ACPC) was tested as a tumor-scanning agent in 38 patients. This artificial amino acid clears the blood to a level of less than 12% within 45 min; thus, imaging is possible within the useful life of ^{11}C. [^{11}C]ACPC can be produced in amounts adequate for clinical scanning. Doses between 12 and 45 mCi were given intravenously, and scans obtained only in the single-photon mode gave clinical information on the sites of tumors. There was no evidence of any toxic effects from [^{11}C]ACPC, and the radiation doses extrapolated from animal data were approximately 0.01 rad/mCi for the whole body and less than 0.06 rad/mCi for the pancreas. In all but 5 of the 38 patients, [^{11}C]ACPC scans were compared with those obtained with [^{67}Ga]citrate. There were 19 positive [^{11}C]ACPC scans and 24 positive ^{67}Ga scans. The results indicate that [^{11}C]ACPC used in conjunction with positron tomography instrumentation is likely to be of diagnostic value for cancer patients.

Kateley, J. R.; Nickerson, D. A.; and Gengozian, N.
Mitogenic response of marmoset lymphocytes: cytokinetics and identification of responsive cells.
Immunology 33:653-62. 1977.

Incorporation of tritiated thymidine ([^{3}H]dThd) into lymphocytes from two marmoset species—New World primates that are haemopoietic chimeras—was studied using peripheral blood lymphocyte (PBL) cultures incubated in vitro for 1 to 5 days with different concentrations of Concanavalin A (Con A), leucoagglutinin, bacterial lipopolysaccharide (LPS) from gram-negative bacteria, rabbit antimarmoset immunoglobulin (anti-Ig) serum, thymosin, or irradiated histoincompatible (xenogeneic) lymphocytes. Only the plant lectins and xenogeneic lymphoid cells stimulated the uptake of [^{3}H]dThd. Lymphocyte enrichment experiments demonstrated that cells responsive to plant lectins and xenoantigens were primarily thymus-derived (T) lymphocytes. Bacterial LPS, however, enhanced the mitogenic response of PBL to Con A when LPS and plant lectin were combined at culture initiation. Thymosin caused either enhancement or suppression of the response to Con A, depending on its time of addition relative to lectin stimulation. Addition of thymosin to lymphocyte cultures with Con A or 24 hr later caused a decrease in isotope incorporation, while addition of thymosin 48 hr later caused a 12–100% increase in [^{3}H]dThd uptake. Lymphocyte chimerism did not influence the mitogenic response since single-born, nonchimeric marmosets responded to plant lectins in a manner similar to marmosets with varying proportions of chimeric blood elements. Cytological analysis of stimulated lymphocytes from heterosexual marmosets revealed the percentage chimerism in the polyclonal mitogenic response and the clonal mixed lymphocyte reaction to be similar.

Lambremont, E. N.; Lee, T-c.; Blank, M. L.; and Snyder, F.
Δ5 Desaturation of fatty acids in L-M cells.
Biochem. Biophys. Res. Commun. 80:813-18. 1978.

L-M cells grown in a lipid-free medium containing ^{14}C-labeled 9,12-linoleic acid incorporated most of this acid into glycerolipids as linoleic acid. Only a small amount (3%) was elongated to eicosadienoic acid. No Δ6 desaturation occurred. When the cells were incubated with ^{14}C-labeled 8,11,14-eicosatrienoic acid, 22% of the activity was found in 5,8,11,14-eicosatetraenoic acid. Treatment of the cells for 24 hr with *N*-isopropylethanolamine, a choline analog, depressed this desaturation reaction to about 60% of control values. The identity of the tetraene product was established by two different chromatographic analyses of the fatty acid methyl esters. Location of the double bond at position C-5 was determined by ozonolysis and subsequent reduction of the ozonides to aldesters followed by gas-liquid chromatography. These results prove that L-M cells have a Δ5 desaturase and an elongation enzyme converting 18:2 to 20:2, but lack a Δ6 desaturase.

Lee, T-c.; Blank, M. L.; and Snyder, F.
Effect of a choline inhibitor (*N*-isopropylethanolamine) on cellular metabolism of L-M cells.
Biochim. Biophys. Acta 529:351-58. 1978.

N-Isopropylethanolamine, a choline analog, is incorporated into L-M cell lipids as 1,2-diacyl-*sn*-glycero-3-phosphoisopropylethanolamine de novo and not by base exchange. In addition, the *N*-isopropylethanolamine effectively blocks choline uptake, which is a reversible process. The following specific time-dependent changes in cell metabolism also occur when *N*-isopropylethanolamine is present: (1) a decrease in total content of phosphatidylcholine, (2) inhibition of both the cellular uptake of [^{3}H]choline and its incorporation into phosphatidylcholine, (3) a decrease in the incorporation of [^{3}H]thymidine into DNA as early as 2 hr after initiating the *N*-isopropylethanolamine block, (4) inhibition of the cellular uptake of [^{3}H]uridine and incorporation into RNA 16 to 24 hr after addition of *N*-isopropylethanolamine, and (5) stimulation of the cellular uptake of [^{3}H]leucine and an inhibition of

1028394

its incorporation into protein, which reached a maximum (68% of controls) 8 hr after *N*-isopropylethanolamine treatment.

T-c; Baker, R. C.; Stephens, N.; and Snyder, F.
:nce for participation of cytochrome b_5 in microsomal Δ6 desaturation of fatty acids.
Biochim. Biophys. Acta 489:25-31. 1977.

The Δ6 desaturation of linoleic acid to γ-linolenic acid and oleic acid to 6,9-octadecadienoic acid by rat liver microsomes was investigated. Using a specific antibody prepared against purified rat liver cytochrome b_5, we demonstrated that cytochrome b_5 participated in Δ6 desaturation of both fatty acids. The reaction products were identified as their methyl ester derivatives by argentation thin-layer chromatography, gas-liquid chromatography, and reductive ozonolysis followed by gas-liquid chromatography.

Lushbaugh, C. C.; Hübner, K. F.; and Ricks, R. C.
Medical aspects of nuclear radiation emergencies.
Emergency 10(9):32-35. 1978.

The greatest problem in developing nationwide emergency preparedness for radiation accidents is caused by their infrequent occurrence. The probability that any particular emergency medical technician team will ever be involved in a serious radiation accident is so low that any need for preparation and training for this rare event seems exaggerated. Only a nedical centers in this country have ever faced the prob- ... of caring for radiation accident cases more than once. Once a hospital or ambulance emergency team has taken care of a radiation accident case, that team can be considered as well trained, but it may not ever have another chance to care for a radiation accident case again. Since, however, radiation accidents do occur, even though rarely, emergency preparedness is essential—but largely to allay the common fears of radioactive materials.

Lushbaugh, C. C.; Humason, G. L.; Swartzendruber, D. C.; Richter, C. B.; and Gengozian, N.
Spontaneous colonic adenocarcinoma in marmosets.
Primates Med. 10:119-34. 1978.

We find that colonic adenocarcinoma, which is an extremely rare neoplasm of all animals except man and carcinogen-treated rodents, occurs spontaneously in some marmosets. The cotton-topped *Saguinus oedipus oedipus* is particularly prone to develop it, but we have found it also at necrospy in *Callimico goeldii* (Goeldi's marmoset). Numerous metastases to regional lymph nodes develop. The cancers arise de novo in the mucosa and invade at an early stage the submucosa and lymphatic apparatus and paracolonic lymph nodes. These findings and the continuing occurrence of this er in our colony suggest that the marmoset may be the sought primate model for experimental intestinal carcinogenesis.

Moore, C.; Blank, M. L.; Lee, T-c; Benjamin, B.; Piantadosi,C.; and Snyder, F.
Membrane lipid modifications: biosynthesis and identification of phosphatidyl-*N*-methyl-*N*-isopropylethanolamine in rat liver microsomes.
Chem. Phys. Lipids 21:175-78. 1978.

In previous studies on the modification of polar head groups of membrane phospholipids with the unnatural base analog, *N*-isopropylethanolamine, we reported an unidentified phospholipid in addition to phosphatidyl-*N*-isopropylethanolamine in the various membrane fractions of rat liver. The structure of this phospholipid has now been identified as phosphatidyl-*N*-methyl-*N*-isopropylethanolamine by nuclear magnetic resonance spectroscopy and by chromatographic and enzymic analyses. In addition, we found that when rats were injected intraperitoneally with *N*-methyl-*N*-isopropylethanolamine, 19% of the liver microsomal phospholipid was phosphatidyl-*N*-methyl-*N*-isopropylethanolamine.

Nelson, B. M.; Andrews, G. A.; and Watson, E. E.
Histopathologic studies of the liver following intravenous colloidal ^{198}Au therapy.
Radiology 127:239-47. 1978.

Histologic studies of liver tissue from 27 patients given up to 395 mCi (cumulative) of intravenous colloidal ^{198}Au showed no definite radiation injury and no correlation between hepatic abnormalities and dose. Demonstration of aggregates of colloidal gold in the Kupffer cells was inconsistent, suggesting slow removal or dispersion. Although the liver ordinarily receives the highest radiation dose, the critical organ is the marrow. Results support the recent introduction of ^{198}Au to supplement teletherapy for certain neoplasms diffusely infiltrating the liver. Apparently the beta distribution, which has been shown to be the cause of radiation hepatitis, minimizes endothelial injury in large vessels.

Parker, J. C.; Whiteman, M. D.; and Richter, C. B.
Susceptibility of inbred and outbred mouse strains to Sendai virus and prevalence of infection in laboratory rodents.
Infect. Immun. 19:123-30. 1978.

Sendai virus is one of the more prevalent and serious virus infections of rodents. Infection was found in 66% of the mouse, 63% of the rat, 83% of the hamster, and 44% of the guinea pig colonies examined. Twenty-four inbred and outbred strains of mice were tested for their sensitivity to lethal Sendai virus infection. The 129/J mice tested were approximately 25,000-fold more sensitive than SJL/J mice; however, both mouse strains were similarly permissive in support of viral replication in their lung tissues. Histopathological studies revealed that whereas lesions in both sensitive and resistant mice were qualitatively similar, the lesions in the more sensitive 129/J mice appeared earlier, were much more extensive, and persisted longer than in the resistant SJL/J mice. These results suggest that the observed variance in sensitivity is not the result of a genetic restriction on virus infection and replication, but rather, the variance is the result of a physiological factor(s) possibly related to some aberration or strain difference in the humoral or cell-mediated immune response.

1028395

Polednak, A. P.
Bone cancer among female radium dial workers. Latency periods and incidence rates by time after exposure.
J. Natl. Cancer Inst. 60:77-82. 1978. (Work performed at Ar[illegible] National Laboratory, Chicago.)

Bone cancer cases in 1250 women exposed to radium while working in the luminous watch-dial industry between 1913 and 1929 were analyzed for times of appearance ("latency periods") and incidence rates over time after first exposure. The lowest radium intake dose associated with bone cancer, among 751 women whose intake doses had been determined, was 202.5 μCi. Mean and median bone cancer latency periods tended to decline, but average survival among women without bone cancer also decreased with increasing intake level. Bone cancer incidence rates over time were compared in two intake-dose groups (200–749 and ≥750 μCi) by means of an actuarial method that takes competing risks into account. Incidence rates were consistently higher in the higher intake-dose group versus the lower dose group at each 5-year period after first exposure. The variability in the odds ratios across the time periods was not statistically significant, and the actuarial method provided little evidence for an effect of intake-dose on the pattern of incidence rate over time. With the use of similar methods, no significant variability was evident in the relative odds of bone cancer over time after exposure between one group of women first exposed to radium at less than 18 years of age and another group exposed when 18 or more years old.

Polednak, A. P.
Long-term effects of radium exposure in female dial workers: differential white blood cell count.
E[illegible]. Res. 15:252-61. 1978. (Work performed at Argonne Na[illegible]al Laboratory, Chicago.)

The long-term effects of bone-seeking radionuclides on bone marrow in humans seem worthy of exploration. Leukocyte counts (total white cells, polymorphonuclears, lymphocytes, eosinophils, and monocytes) were analyzed from 393 female radium dial workers who were first employed between 1913 and 1929 and most recently examined between 1954 and 1975. Mean and median counts were compared by initial intake level (μCi of radium per kg of skeleton) within three age-at-last-examination groups (45-54, 55-64, and 65-74 yr). Medium estimated 40-yr average alpha doses to trabecular bone marrow in the highest intake levels compared were 9 rad, 104 rad, and 40 rad, respectively, in the three age groups. Spearman rank correlation coefficients between radium intake and white cell counts were also calculated. There was little evidence for long-term or late effects of radium exposure on white cell counts. In the younger age groups, lymphocyte count tended to increase with increasing radium intake. In the 65 to 74 year bracket, eosinophil count decreased with increasing intake (Spearman $r = -0.14$, $p < 0.05$). Continued follow-up of this population should help clarify the question of possible long-term effect (direct or indirect) of radium exposure.

Polednak, A. P.; Stehney, A. F.; and Rowland, R. E.
Mortality among women first employed before 1930 in the [illegible]idium dial-painting industry. A group ascertained from e[illegible]oyment lists
Am. J. Epidemiol. 107:179-95. 1978. (Work performed at Argonne National Laboratory. Chicago.)

Mortality is described among a cohort of 634 women who worked in the U.S. radium dial-painting industry between 1915 and 1929 and whose names were obtained from employment lists. Based on death rates for U.S. white females, greater than expected numbers of deaths (from death certificates) were observed for all causes (240 observed vs. 188.5 expected), bone cancer (22 vs. 0.3), cancer of other and unspecified sites (18 vs. 2.6), cancer of the colon (10 vs. 5.0), diseases of blood and blood-forming organs (4 vs. 1.0), and external causes (31 vs. 10.1). Six of 22 certificates coded to radiation accident (underlying cause) also mentioned bone cancer. Compared with women first employed from 1915 to 1924, those first employed from 1925 to 1929 had low cause-specific mortality ratios and an overall mortality ratio of 0.83. The number of deaths from leukemia and diseases of blood was significantly greater than expected in the time period before 1945. Among 521 women alive in 1954, 360 had a radium body-burden measurement while living (in 1954 or later). Mortality ratios (all causes) of greater than 1.00 were observed only in the higher intake-dose groups (i.e., ≥50 μCi). Cause-specific mortality was analyzed in two dose groups (<50 and ≥50 μCi). In the lower dose group, the only cause category with a significantly greater number of observed deaths than expected was cancer of the colon (5 vs. 1.72, $p < 0.05$); the mortality ratio for all causes was 0.86. In the higher dose group, the mortality ratio for all causes was 1.91.

Richter, C. B.; Humason, G. L.; and Godbold, J. H., Jr.
Endemic *Pneumocystis carinii* in a marmoset colony.
J. Comp. Pathol. 88:171-79. 1978.

Retrospective examination of lungs from 441 marmoset necropsies conducted over a decade disclosed endemic pneumocystosis. Age distribution and colony residence time analysis suggest predisposition in the young and long-term residents. Only two cases were judged to have been of possible clinical significance on the basis of histopathological evaluation. Special stains, including periodic acid Schiff's and methenamine silver, were necessary to demonstrate many infections as the organisms were frequently widely scattered in small clusters. The marmoset may be a useful model for study of the human disease.

Rock, C. O., and Snyder, F.
Asymmetric localization of alkyldihydroxyacetone-P synthase and acyldihydroxyacetone-P acyltransferase in microsomal vesicles.
Adv. Exp. Med. Biol. 101:379-85. 1978.

This report summarizes recent results on the localization of dihydroxyacetone-P acyltransferase and alkyldihydroxyacetone-P synthase in microsomal vesicles prepared from the pink portion of the rabbit harderian gland. Such glands are located in the orbit of the eye of animals possessing a third eyelid; the glands function to secrete an oily substance that facilitates eyelid movement. The oily secretion consists largely of ether-linked lipids, and the gland is a rich source of the enzymes responsible for their synthesis. Our laboratory has used this gland as a model for studying those pathways responsible for the synthesis of the ether-linked glycerolipids, a group of lipids that is characteristically prominent in most cancer cells.

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Our findings indicate that a catalytically important segment of both alkyldihydroxyacetone-P synthase and dihydroxyacetone-P acyltransferase is located in the lumen of the microsomal vesicles. The effect of detergents on the pH optimum, substrate specificity, and kinetic properties of these enzymes has been discussed in detail elsewhere, and the results consistent with this view. Alkyldihydroxyacetone-P synase, prepared from Ehrlich ascites cells, also behaved like the harderian gland enzyme, indicating that the properties of this particular enzyme may be a common feature of mammalian cells containing ether lipids.

Rock, C. O.; Fitzgerald, V.; and Snyder, F.
Coupling of the biosynthesis of fatty acids and fatty alcohols.
Arch. Biochem. Biophys. 186:77-83. 1978.

A cell-free system for the biosynthesis of fatty alcohols in the pink portion of the rabbit harderian gland is described. The radiolabeled substrates for the fatty acid reductase were generated using soluble fatty acid synthase from the gland in the presence of acetyl-CoA, malonyl-CoA, and NADPH. Harderian gland microsomes, ATP, and Mg^{2+} were absolute requirements for the synthesis of fatty alcohols and if any of these components were deleted from the assay mixture, no alcohols were detected. We were also unable to detect formation of fatty alcohols if acyl-CoAs were substituted for fatty acid synthase with either NADPH or NADH as reducing agents. The reductase was localized in the microsomal fraction and appears to be on the cytosol-membrane interface of the vesicles, as indicated in experiments using detergents and trypsin. The fatty alcohols formed by the system had the same chain length distribution as the fatty acid synthase. The alkyl moieties of the ether lipids in the harderian gland are exclusively saturated and the properties of the alcohol-synthesizing system described in this report can account for the observed exclusion of unsaturated alkyl moieties from the ether lipids of this gland.

Rock, C. O.; Fitzgerald, V.; and Snyder, F.
Properties of dihydroxyacetone phosphate acyltransferase in the harderian gland.
J. Biol. Chem. 252:6363-66. 1977.

We have used a microsomal preparation from the pink portion of the rabbit harderian gland to study the properties of dihydroxyacetone-P acyltransferase. The enzymatic activity in the microsomes is latent and is stimulated approximately twentyfold by adding detergent to the assay system. Both deoxycholate and cholate stimulated the enzyme at concentrations considerably below the critical micellar concentration of these detergents. The acyltransferase was not solubilized by treatment with these detergent concentrations. In addition, we have shown that the acyltransferase is sensitive to proteolytic digestion in the presence of deoxycholate concentrations that render the microsomal vesicles permeable to the protease, but the acyltransferase is insensitive to the protease treatment in the absence of detergent. Collectively, these data indicate that the active site of dihydroxyacetone-P acyltransferase is exposed to the lumenal surface of the microsomal vesicles. This enzyme is distinct from the glycerol-P acyltransferase in that the dihydroxyacetone-P acyltransferase is sensitive to protease digestion only in the presence of deoxycholate, is not inhibited by sulfhydryl reagents, and is not competitively inhibited by *sn*-glycerol-3-P.

Sauerbrunn, B. J. L.; Andrews, G. A.; and Hübner, K. F.
Gallium-67 citrate imaging in tumors of the genitourinary tract: report of cooperative study.
J. Nucl. Med. 19:470-75. 1978.

Whole-body imaging with [^{67}Ga]citrate in 127 tumors of the genitourinary tract has been evaluated by a cooperative group using a uniform protocol. Primary sites of tumor were not detectable by imaging, except for one bladder and one kidney tumor. Proven and apparent metastases yielded positive scans, however, in 51% of prostatic, 50% of bladder, 72% of kidney, and 53% of testicular neoplasms. In bladder and kidney metastases, if bone sites are excluded, detection of soft tissue metastases was 61% and 75%, respectively. In embryonal-cell carcinoma of the testicle, 74% of metastatic foci were detected.

Washburn, L. C.; Sun, T. T.; Anon, J. B.; and Hayes, R. L.
Effect of structure on tumor specificity of alicyclic α-amino acids.
Cancer Res. 38:2271-73. 1978.

The selective affinity of [*carboxyl*-^{11}C]-1-aminocyclopentanecarboxylic acid (ACPC) for tumor tissue has led us to study the tumor-localizing characteristics of a series of alicyclic α-amino acid analogs of ACPC. The tissue distributions of [^{14}C]-1-aminocyclopropanecarboxylic acid, 1-aminocyclobutanecarboxylic acid (ACBC), 1-aminocyclohexanecarboxylic acid, 1-amino-2-methylcyclopentanecarboxylic acid, and 1-amino-3-methylcyclopentanecarboxylic acid were compared with that of ACPC in Buffalo rats bearing Morris 5123C hepatomas. ACPC and ACBC were found to have significantly higher tumor-to-nontumor concentration ratios than the other four amino acids. ACBC generally had higher tumor-to-nontumor ratios than did ACPC, significantly so for muscle, kidney, and testis, and marginally so for blood. These results suggest that [*carboxyl*-^{11}C]ACBC may be a better agent than [*carboxyl*-^{11}C]ACPC for tumor imaging by positron tomography.

Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Hayes, R. L.; and Butler, T. A.
[1-^{11}C]DL-Valine, a potential pancreas-imaging agent.
J. Nucl. Med. 19:77-83. 1978.

There is a great need for a better pancreas-imaging agent. Studies with [1-^{14}C]DL-valine have shown this amino acid to have a high pancreatic specificity in the four animal species examined. The tissue distribution was almost optimal by 30 min after injection, and no carrier effect was observed through a dose of 5 mg/kg. [1-^{11}C]DL-Valine was synthesized in amounts up to 363 mCi using a rapid ($T_{1/2}$ = 20.4 min for ^{11}C), high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis. Purification was by anion-exchange followed by cation-exchange chromatography. [1-^{11}C]DL-Valine was obtained in a 70% chemical yield with a total synthesis and purification time of

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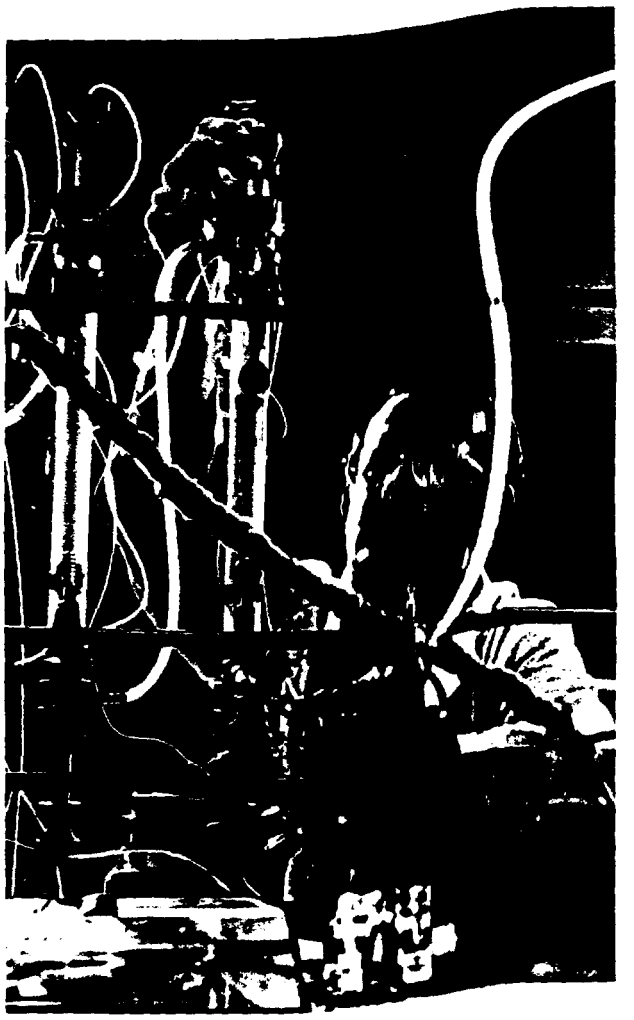
45 min. Animal studies have demonstrated that it is a potentially useful new agent for clinical pancreatic imaging.

Wykle, R. L.; Kraemer, W. F.; and Schremmer, J. M.
Studies of lysophospholipase D of rat liver and other tissues.
Arch. Biochem. Biophys. 184:149-55. 1977.

Lysophospholipase D (EC 3.1.4.39) activity was demonstrated in rat kidneys, intestines, lungs, testes, and liver. The liver enzyme was studied in greatest detail and its labeled products were identified by chemical and chromatographic techniques. This enzyme hydrolyzes 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphoethanolamine and 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphocholine to yield 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphate; the initial product is subsequently dephosphorylated by a phosphohydrolase in microsomes to form 1-[1-^{14}C]hexadecyl-*sn*-glycerol. The possibility that phospholipase C and a phosphotransferase were responsible for the formation of 1-[1-^{14}C]hexadecyl-*sn*-glycero-3-phosphate was ruled out. Neither 1-[1-^{14}C]hexadecyl-2-acyl-*sn*-glycero-3-phosphoethanolamine nor 1-[1-^{14}C]hexadecyl-2-acyl-*sn*-glycero-3-phosphocholine was hydrolyzed. The enzyme requires Mg^{2+}, is inhibited by Ca^{2+}, and is stimulated by high salt concentrations; it is localized in the microsomal fraction and has a pH optimum between 7.0 and 7.6. Inhibition by sulfhydryl reagents and protection by glutathione and dithiothreitol suggest that a sulfhydryl group is required for activity. The enzyme is inhibited by detergents and by organic solvent extraction. It appears to be tightly bound to the microsomes, since repeated freeze-thawing or sonication did not release the activity, and trypsin digestion (either in the presence or in the absence of 0.04% deoxycholate) did not destroy the activity. Lysophospholipase D was previously known to occur only in brain.

Yakatan, G. J.; Frome, E. L.; Leonard, R. G.; Shah, A. C.; and Doluisio, J. T.
Bioavailability of acetazolamide tablets.
J. Pharm. Sci. 67:252-55. 1978. (Work performed at the University of Texas, Austin.)

Plasma acetazolamide levels were measured by an enzymatic assay following single 250-mg oral tablet doses to 20 health volunteers; five different lots of acetazolamide tablets from a single manufacturer were used in a balanced incomplete block design. From the measured plasma levels, estimates of the bioavailability parameters (area under the plasma concentration versus time curve, time to peak plasma concentration, and peak plasma concentration) were obtained by least-squares digital computer fitting. No significant differences among the tablets were observed ($\alpha = 0.05$) for the analysis of variance of the area under the curve or time to peak parameters. Two tablets, however, provided statistically higher peak plasma concentrations than the other three. Thus, lot-to-lot bioinequivalence of acetazolamide tablets was observed. Some in vitro tests employed showed general trends for correlation with the in vivo data. However, considerable refinement of these techniques appears necessary for in vitro prediction of the observed lot-to-lot bioinequivalence.



1028398

Training Programs

The Medical and Health Sciences Division participates in a number of training programs in biomedical research. Graduate students from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, the University of Tennessee (Knoxville and the UT Center for the Health Sciences, Memphis), and the University of North Carolina (Chapel Hill) are currently pursuing research for their Ph.D. degrees in the areas of biological chemistry and immunology. Stipends for the competitive fellowships awarded to these students are obtained from the NCI training grant (CA-05287) at ORNL, University Programs at ORAU (through DOE), and the University of Tennessee (Hilton A. Smith Fellowship). Occasionally research grants are also a source of support.

Postdoctoral fellows also contribute to the various research activities of the Division. Funds for support of postgraduate training have been awarded to fellows from the Damon Runyon-Walter Winchell Cancer Fund (DRG-127-FT) and the NCI Training in Carcinogenesis Research (CA-05296) at ORNL.

Summer research participation sponsored by University Programs at ORAU makes it possible for academic staff and seniors from colleges and universities throughout the United States to partake in the type of laboratory studies described in this report.

The Division also provides training experiences to high school students as secretaries and laboratory aides. These programs are made possible through cooperation with vocational training programs at area high schools.

Short-term courses in radiation management are formally offered to physicians and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program.

Some of the senior staff at the Division conduct special courses and give lectures at the UT-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee (Knoxville and Memphis). These staff members hold joint appointments at the University and are advisors to graduate students pursuing Ph.D. research at ORAU.

The Division's Biochemistry Research Program includes studies of pulmonary lipids and a group of lipids characteristic of most cancerous cells in both animals and man.



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1028400

Collaborative Research

D. Brown
Purification of gallium-binding proteins
Fordham University (D. Lawless)

D. Brown
Subcellular distribution of platinum complexes
Oak Ridge National Laboratory (J. Hoeschele and N. Johnson)

E. L. Frome
Design of a computer-assisted death certificate search system
University of Tennessee, Knoxville (S. Jordan)

E. L. Frome
Least absolute value regression
University of Texas (R. Armstrong)

N. Gengozian
Fetal hemoglobin synthesis
University of Tennessee Memorial Research Center (J. Fuhr)

N. Gengozian
Immunocontraception in the marmoset
University of Tennessee (C. Shivers)

N. Gengozian
Radiation sensitivity (cytogenetic) of the marmoset
Oak Ridge National Laboratory (J. Brewen)

R. L. Hayes
Collaborative studies in radiopharmaceutical development
Oak Ridge National Laboratory (F. F. Knapp)

R. L. Hayes
Synthesis of labeled compounds using organoborane techniques
University of Tennessee (G. Kabalka)

K. F. Hübner
Comparative efficiency of diagnostic methods for detection of pancreatic disease
University of Tennessee Memorial Research Center (I. R. Collmann and E. Buonocore)

K. F. Hübner
Radiation pneumonitis, a model for early detection of pulmonary disease using perfusion and ventilation techniques
University of Tennessee Memorial Research Center (T. Sullivan and F. Comas)

G. Littlefield
Cytogenetic dose response kinetics in G-trisomic lymphocytes exposed to low LET radiation
Medical College of Georgia (D. A. Welter)

G. Littlefield
Cytogenetic follow-up studies in survivors of the LA-O and LA-2 accidents
University of Rochester (L. Hemplemann)

G. Littlefield
Sister chromatid exchange analysis in coke oven workers
National Institute of Occupational Safety and Health (G. Taylor)

C. Lushbaugh
DTPA project
Department of Energy Contractors (40 coinvestigators)

C. Lushbaugh
Radiotherapy overdose study
University of Cincinnati (E. Saenger)

C. Lushbaugh
United States Transuranium Registry
Hanford Environmental-Health Foundation (P. Fuqua)



1028401



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A. Polednak
Epidemiologic studies of thorium workers
Argonne National Laboratory (A. F. Stehney)

C. Richter
Methods of aging of nonhuman primates (tamarins) using osteone analysis
University of Tennessee (F. Smith)

C. Richter
Ophthalmological disease of marmosets
University of Tennessee (N. Buyukmihci)

C. Richter
Saturnine gout in marmosets
University of Tennessee Memorial Research Center (W. Farkas)

C. Richter
Study of the susceptibility variation and vaccination challenge response of mice to Sendai virus infection
Microbiological Associates (J. C. Parker)

F. Snyder
Chemical characterization of an antihypertensive lipid isolated from the kidney medulla
University of Tennessee, Memphis (E. Muirhead, B. Leach, and L. Byers)

F. Snyder
Effect of NO_2 and other toxicants on the lung surfactant system
Oak Ridge National Laboratory (P. Nettesheim and W. Dalbey)

F. Snyder
Mass spectrometry of unique lipids
Oak Ridge National Laboratory (W. Rainey)

F. Snyder
Nuclear magnetic resonance studies of unique lipids
Oak Ridge National Laboratory (B. Benjamin)

F. Snyder
Role of ether lipids in growth, cell differentiation, and neoplasia
Oak Ridge National Laboratory (C. Heckman and P. Nettesheim)

F. Snyder
Synthesis of lipid analogs and their precursors
University of North Carolina (C. Piantadosi and K. Ishaq)

L. Washburn
^{11}C Amino acids for synthesis of labeled antibiotics
University of Kentucky (G. Digenis)



L. Washburn and K. Hübner
WR-2721 as an adjunct to radiother
University of New Mexico (J. Yuh

B. Wieland
Design and testing of ^{18}O (p,n) ^{18}F targets for production of $^{18}F_2$ (
Tandem Van de Graaff-ORNL)
Oak Ridge National Laboratory (P. Miller and F. F. Knapp), Massachus
Institute of Technology (S. M. Senk

B. Wieland
Development of enriched isotope tar systems for production of ^{18}F and ^{13}N
University of California, Los Ange (M. E. Phelps and N. MacDonald)

B. Wieland
Investigation of ^{11}C, ^{13}N, ^{15}O, and for proton bombardment of enrich isotope targets (EN Tandem Van Graaff)
Oak Ridge National Laboratory (P. Miller and J. K. Bair)

B. Wieland and K. Hübner
Development of software for qua titative imaging of gamma emitters a for simulation of accelerator tar systems
Vanderbilt University (P. H. King)

1028403

Grant-Funded Research

N. Gengozian
"Autoimmune" thrombocytopenia
National Heart, Lung, and Blood Institute
$62,500* (1 July 1978 to 30 June 1979)

R. L. Hayes
Concentration of radionuclides by malignant neoplasms
National Cancer Institute
$81,210* (1 May 1978 to 30 April 1979)

G. Littlefield
Evaluation on induced cytogenetic lesions in oocytes
National Institute of Child Health and Human Development
$106,550* (1 June 1978 to 31 May 1979)



C. C. Lushbaugh
Biomedical research support grant
Division of Research Resources (NIH)
$34,979* (1 April 1978 to 31 March 1979)

C. Richter
Marmoset breeding facility
Division of Research Resources (NIH)
$77,705* (1 September 1978 to 31 August 1979)

F. Snyder
Ether lipids in cancer: enzyme mechanisms and inhibitors
National Cancer Institute
$36,788* (1 May 1978 to 30 April 1979)

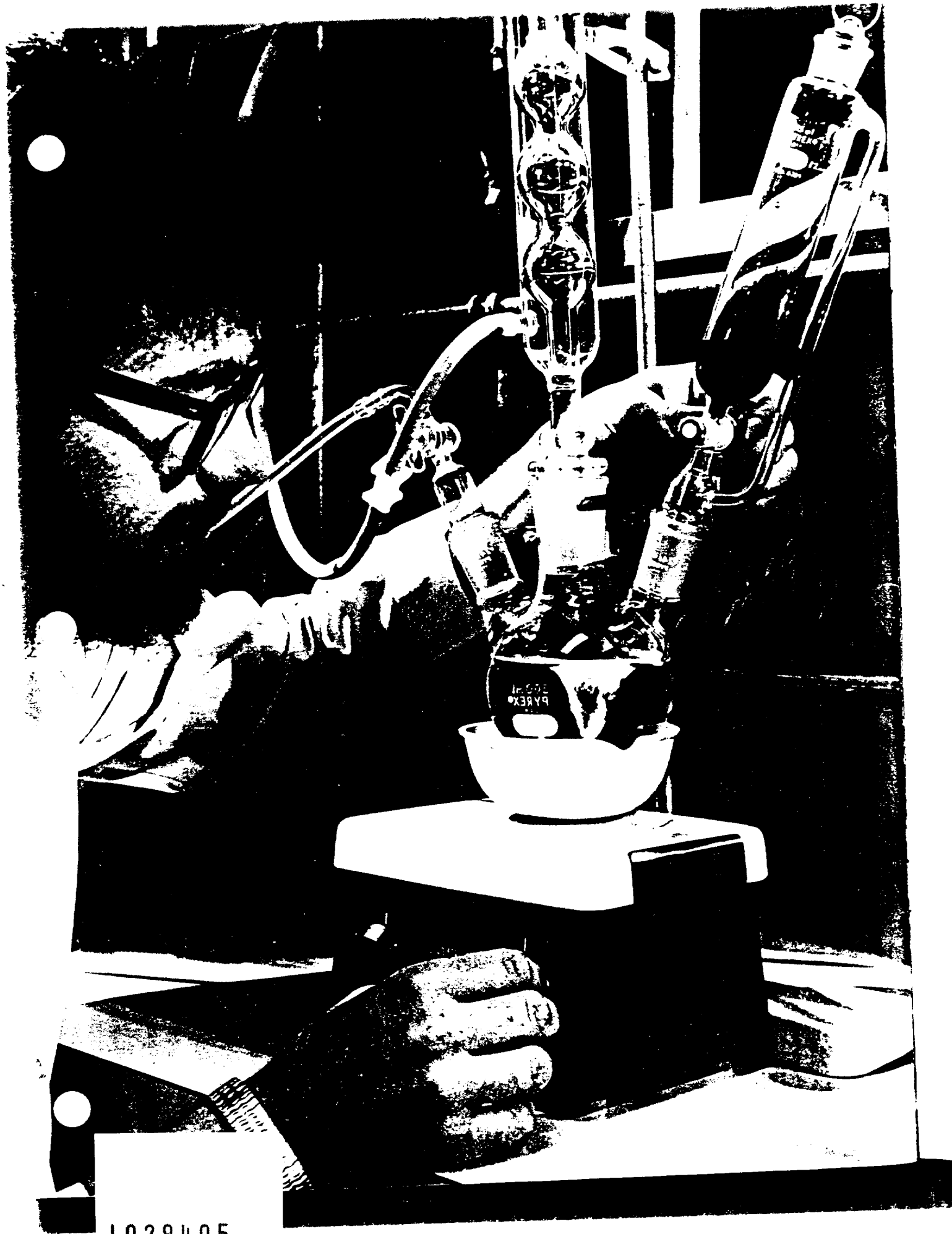
F. Snyder
Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells
American Cancer Society
$53,000** (1 December 1977 to 30 November 1978)

F. Snyder
Surfactant metabolism: alveolar cells and lung disease
National Heart, Lung, and Blood Institute
$37,376* (1 May 1978 to 30 April 1979)

F. Snyder (Sponsor)
Modification of cancer cell membranes with unnatural lipid analogs
Damon Runyon-Walter Winchell Cancer Fund (postdoctoral fellowship to M. C. Cabot)
$15,000** (1 November 1977 to 31 October 1978)

*Direct costs
**Total costs

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1028406

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Marion Garber
Virginia Gramly

[1] Predoctoral student
[2] Postdoctoral fellow
[3] Student trainee
[4] Guest scientist
[5] Master's student
[6] Consultant
Note: Group leaders' names in italics.

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F. E. Dunnam, University of Florida
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Paul Hyde, Louisiana State University Medical Center
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Howard H. Vogel, Jr., University of Tennessee Center for the Health Sciences
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Leonard A. Sagan, Electric Power Research Institute
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'lliam H. Stone, University of Wisconsin, **Chairman**
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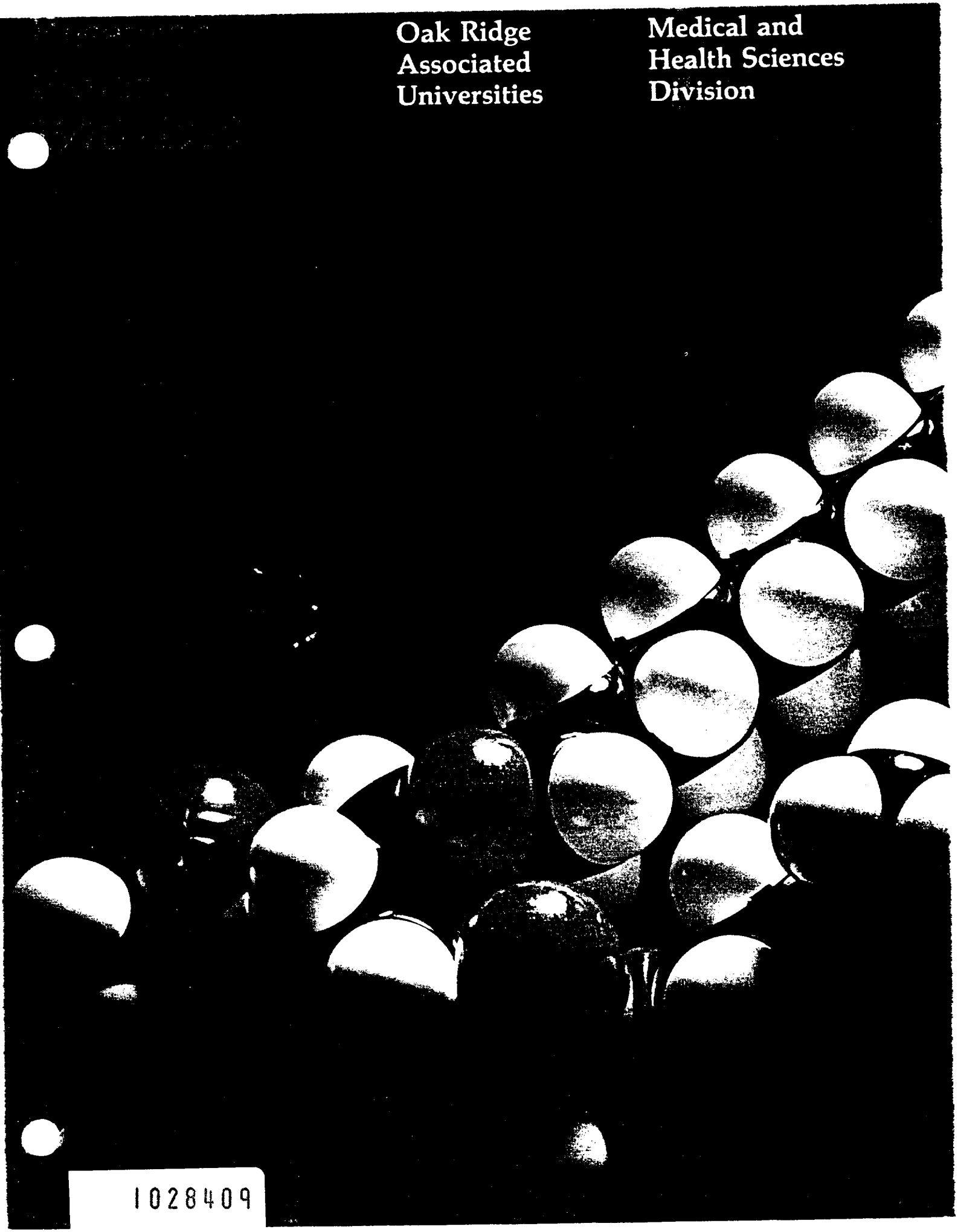
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1978-79

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Oak Ridge
Associated
Universities

Medical and
Health Sciences
Division



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The Medical and Health Sciences Division conducts biomedical research and training in studies of respiratory and gastrointestinal diseases and cancers related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Immunobiology, biological chemistry, cell biology, radiopharmaceutical chemistry, nuclear medicine, experimental pathology, cytogenetics, and epidemiology are the main research disciplines from which pragmatically targeted task groups are structured to enhance efficiency. Predoctoral and postdoctoral training are provided in these areas. Besides newly refurbished laboratory units, the division has two unique facilities: REAC/TS, the Radiation Emergency Assistance Center/Training Site, maintained for the U.S. Department of Energy as a demonstration and training unit for medical preparedness for radiation accidents; and the Marmoset Research Center, which is a source of primates for research in the division and elsewhere in the United States.

Oak Ridge Associated Universities is a private, not-for-profit association of 46 colleges and universities. Established in 1946, it was one of the first university-based, science-related, corporate management groups. It conducts programs of research, education, information, and training for the U.S. Department of Energy and a variety of private and governmental organizations. Oak Ridge Associated Universities is noted for its cooperative programs and for its contributions to the development of science and human resources in the South.

NOTICES

The opinions expressed herein do not necessarily reflect the opinions of the sponsoring institutions of Oak Ridge Associated Universities.

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, nor assumes any legal liability or responsibility for any third party's use or the results of such use of any information, apparatus, product or process disclosed in this report, nor represents that its use by such third party would not infringe privately owned rights.

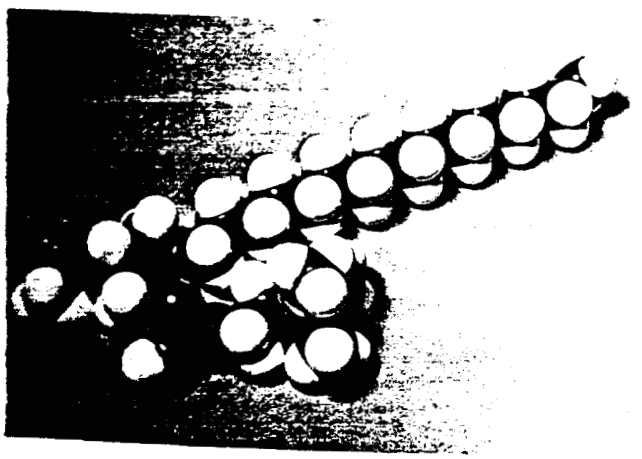
Available from the National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia 22161. Please direct all price inquiries to NTIS.

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Research Report 1978–1979

Oak Ridge Associated Universities

Medical and Health Sciences Division



The molecular model shown on the cover depicts a new type of phospholipid structure (1-alkyl-2-acetoyl-sn-glycero-3-phosphocholine) that possesses profound antihypertensive properties. This unique lipid was discovered at the Medical and Health Sciences Division as part of an ongoing project concerned with ether-linked lipids.

1028411

Table of Contents

Scope of Programs 5
- Biological Chemistry 5
- Epidemiology 6
- Immunobiology 6
- Radiation Emergency Assistance Center/Training Site 7
- Radiopharmaceutical Development and Nuclear Medicine 8

Publications 11

Published Abstracts of Full-Length Publications 17

Training Programs 25

Collaborative Research 26

Grant-Funded Research 29

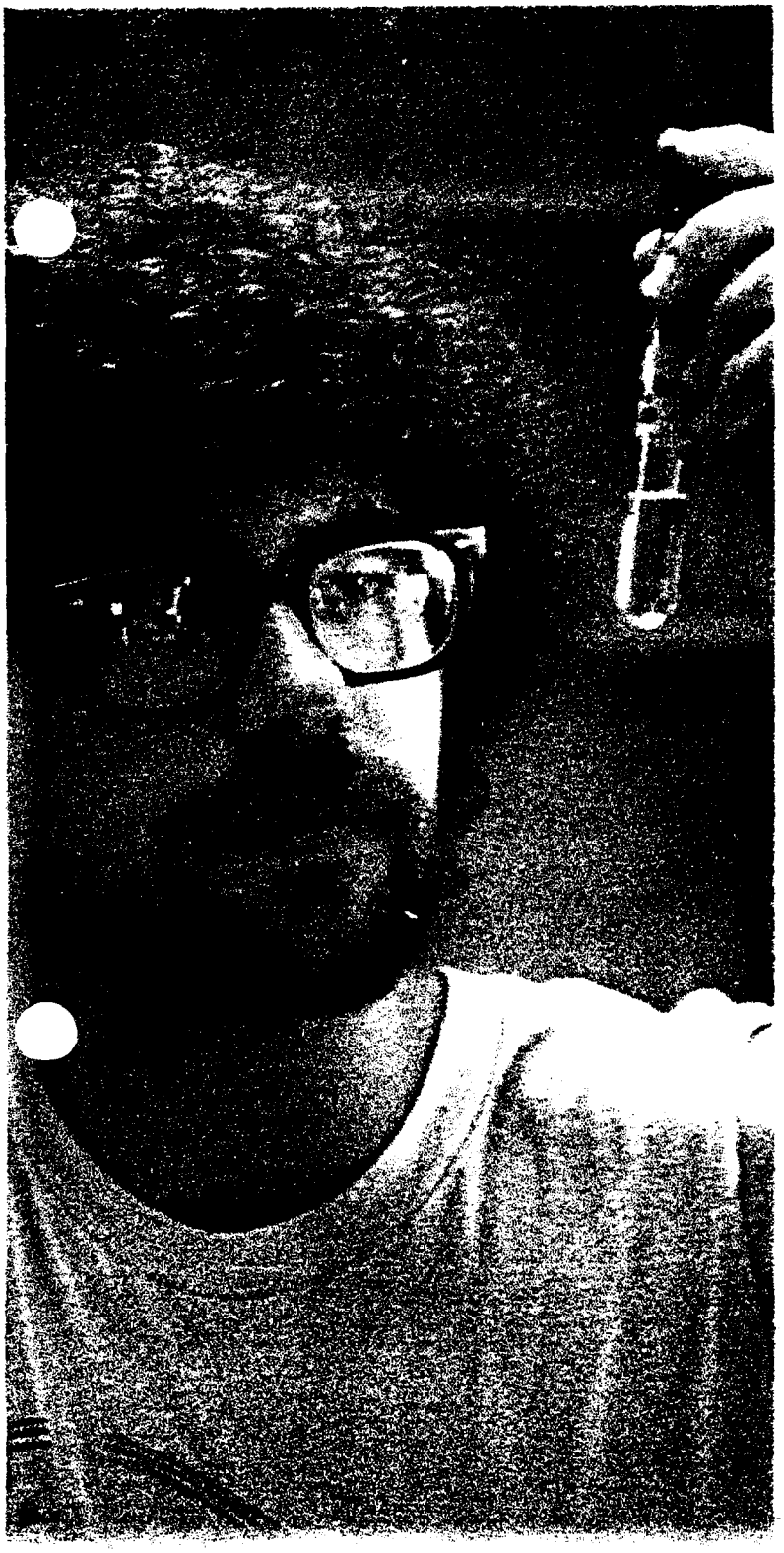
Organization and Staff 31

ORAU-161

October 1979

Oak Ridge, Tennessee 37830

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.



1028412

Preface

Current research at the Medical and Health Sciences Division of Oak Ridge Associated Universities (ORAU) continues to focus on solving occupational biomedical problems associated with the production and use of fuels for energy. This report highlights research accomplishments of the division during the past year and provides a bird's-eye view of other important activities. Biomedical researchers in biological chemistry, immunology, and radiopharmaceutical research and clinicians in nuclear medicine, Radiation Emergency Assistance Center/Training Site (REAC/TS), and epidemiology have been responsible for conducting and supporting research. The scope of the research in these areas is described in the first section of this report. To augment division plans to focus intensively on problems requiring interdisciplinary approaches, the current basic biomedical and clinical research programs are being regrouped under two associate chairmen during FY 1980 as shown in the chart on the next page.

An integral part of our research effort is training. Throughout the year, the Medical and Health Sciences Division provides research training to postdoctoral fellows, graduate students, and visiting scientists. College juniors participate in projects during the summer, and selected high school students are given on-the-job experience as part of a vocational education program. In addition, special courses on the medical management of health problems associated with radiation accidents and nuclear safety practices are taught to medical and paramedical personnel and health physicists as part of the program conducted by REAC/TS.

Major financial support for the division is provided through a contractual arrangement with the U.S. Department of Energy (DOE). However, grants awarded from the National Institutes of Health and the American Cancer Society help support some special projects of individual staff scientists. Despite the continuing competition for research dollars, the total annual budget for the division this past year (FY 1979) was $4.03 million. This amount reflects an increase of 22 percent over the previous year. We believe this is a direct reflection of the scientific strength of the researchers responsible for conducting the programs described in this report.

Solving the complex biomedical problems associated with the safe development of new technologies for alternative energy sources requires an interdisciplinary approach. We believe that such an interplay among scientists within, as well as outside, the division is apparent from our current research efforts. Moreover, during the past year we have established special interdisciplinary task teams to approach specific problems that require a variety of expertise. Such an approach has been initiated for lung tomography with ^{11}C biochemicals and for a project investigating the effectiveness of liposomal encapsulation of diethylenetriaminepentaacetic acid (DTPA) for removing heavy metals from tissues.

The research results described in this report are the summaries of publications in refereed journals or in books. Reprints of the published papers are available upon request to the division. Significant research developments that are in progress and not yet published are mentioned in the first section and can also be gleaned from "in press" papers cited in the publications sections.

C. C. Lushbaugh, Chairman

Fred Snyder, Associate Chairman

October 1979

FY 1980 MEDICAL AND HEALTH SCIENCES DIVISION ORGANIZATION

C. C. Lushbaugh, Chairman

BIOMEDICAL RESEARCH
Fred Snyder, Associate Chairman

PULMONARY FUNCTION
- Pulmonary Biochemistry
- Pulmonary Toxicology
- Pulmonary Cell Biology
- Inhalation Physiology

CANCER GENESIS AND DIAGNOSIS
- Immune Surveillance
- Cancer Biochemistry
- Radiopharmaceutical Design and Testing
- Radiopharmaceutical Synthesis

RESEARCH SUPPORT
- Experimental Pathology and Veterinary Services
- Library
- Electronics

CLINICAL PROGRAMS
C. C. Lushbaugh, Acting Associate Chairman

RADIATION EMERGENCY ASSISTANCE CENTER/TRAINING SITE
- Radiation Accident Management
- Radiation Accident Management Training Program
- REAC/TS Registries and Follow-Up Program
- Radiation Pathology
- Cytogenetic Studies

EPIDEMIOLOGY (ATOMIC WORKERS)
- Health and Mortality Studies
- Biostatistics and Analytic Methodology
- Vital Status Assessment (DCRO)
- Data Base Management and Systems

NUCLEAR MEDICINE
- Emission Computerized Tomography in Pancreatic and Neoplastic Diseases
- Clinical Testing of Radiopharmaceuticals
- Evaluation of New Diagnostic Instruments

1028413

INTERDISCIPLINARY TASK GROUPS
- Lung Tomography
- Marmoset Colon Cancer
- DTPA Project



An electron micrograph of a misplaced endocrine cell from a tamarin with colonic cancer. This cell is found normally in very small numbers in the colonic mucosa. It, or its undifferentiated precursor, has metastasized to a lymph node in this spontaneous disease. Mixtures of endocrine and mucosa cells in these metastases suggests a common origin for these cell types.

1028414

Scope of Programs

Biological Chemistry

Major research programs in biological chemistry are investigating the metabolism and function of lipids and membranes in relation to cancer and pulmonary diseases caused by environmental toxicants.

The cancer-related projects center on the characterization and regulation of several key enzymes involved in the metabolism of alkyl and alk-1-enyl ether-linked lipids, a group of glycerolipids that are closely aligned with neoplastic growth. A major recent accomplishment in this work has been the solubilization of microsomal alkyl synthase, the enzyme that catalyzes the first step in ether bond formation. The use of Triton X-100 in combination with phospholipase A_2 or C has been the key to our successful experiments. With phospholipase C, we obtain a form of alkyl synthase that has a molecular mass of approximately 250,000 to 300,000 daltons. Plans include purifying the alkyl synthase and preparing antibodies against it so that the properties of this unique enzyme can be thoroughly characterized and its mechanism of action clarified.

Properties of other membrane-bound enzymes currently under investigation are (1) acyl-CoA reductase, which forms the long-chain alcohol precursor of the ether chain from acyl-CoAs; (2) fatty alcohol: NAD^+ oxidoreductase, which is responsible for the oxidation of fatty alcohols to fatty acids via an aldehyde intermediate; and (3) alkyl desaturase, which synthesizes plasmalogens. In addition, the topographical location of these enzymes and their products are being determined in microsomal vesicles by using detergents, proteases, and/or phospholipases as probes. Data obtained for the alkyl desaturase, when alkyl phospholipid substrates containing acyl moieties of different chain lengths were tested, indicate that substrates acylated in the microsomal membranes are desaturated at a higher rate than exogenously acylated substrates. The acylation step might be responsible for positioning the substrate in the membrane so it becomes more accessible to the desaturase. We have recently used alkylglycerol supplements (e.g., chimyl alcohol) to increase the level of *O*-alkyl-phospholipids in membranes of Ehrlich ascites and L-M cells. These manipulative systems will be helpful in assessing the cellular role of ether lipids.

An important spin-off of the ether lipid project has been the preparation of an alkyl ether analog of phosphatidylcholine that has potent antihypertensive properties; this is a collaborative project with E. E. Muirhead and L. W. Byers at the University of Tennessee Center for the Health Sciences, Memphis. The antihypertensive lipid (1-alkyl-2-acetoyl-*sn*-glycero-3-phosphocholine) was prepared from choline plasmalogen of fresh beef heart by reducing the alk-1-enyl moiety to an alkyl group and by hydrolyzing the *sn*-2 acyl moiety and replacing it with an acetoyl group. The basis for preparing and testing this compound resulted from experience with antihypertensive lipids derived from the renal medulla and from experience with ether lipid biochemistry.

Recent results obtained from our investigation of the pulmonary surfactant system associated with adenoma alveolar type II cells are the following: (1) Lamellar bodies isolated from the adenoma are similar in phospholipid composition to lamellar bodies isolated from normal lungs of various animal species. (2) Studies of the synthesis of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine demonstrate that when palmitic acid is the substrate, the deacylation-reacylation pathway is the principal mechanism for its synthesis. (3) Pulse-chase experiments with [*methyl*-^{3}H]choline demonstrate that the appearance of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine in the microsomal fraction (derived from the endoplasmic reticulum) precedes its appearance in the lamellar body fraction. This observation indicates that the endoplasmic reticulum is the subcellular site of synthesis for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine via the deacylation-reacylation route. (4) Comparison of the deacylation-reacylation (acyl-CoA:lysophosphatidylcholine acyltransferase) route for surfactant biosynthesis with the lysophosphatidylcholine:lysophosphatidylcholine transacylase route in adenoma type II cells revealed that the total enzymatic activity of the former was about four times greater than that of the latter.

Current and future studies are focusing on the topography of enzymes and products associated with the pulmonary surfactant pathway, the utilization of phosphatidylcholines with defined unsaturated acyl

1028415

groups at the *sn*-2 position as precursors of surfactant, and the development of affinity chromatographic strategies for identifying the existence of subsets of phospholipase A_2 that catalyze the deacylation of the surfactant precursor. The possible role of choline as a regulator of pulmonary surfactant metabolism is being explored by utilizing *N*-isopropylethanolamine as a probe, since our previous studies have shown it to be a competitive inhibitor of choline-linked reactions.

Our toxicology research continues to emphasize the effect of NO_2 on the surfactant system. Studies of the effect of adaptation and modifiers (e.g., vitamin A) on the lung surfactant response to NO_2 are nearing completion. Also, key enzyme activities involved in surfactant lipid metabolism are being investigated in NO_2-exposed rats as part of a Ph.D. thesis research project. Experiments have been initiated to examine whether ozone affects the surfactant system in a manner similar to NO_2; the extent of ozone-induced peroxidation and its subsequent effect on pulmonary membrane properties in rats will also be determined.

Epidemiology

The epidemiology group is concerned mainly with examining associations between occupational radiation exposure and subsequent mortality. The health effects of chemical (especially metal) toxicants are also under investigation as both primary and confounding (with respect to radiation exposure) occupational stresses. In the statistical and computer methodology sections, biostatisticians and computer scientists are developing improved statistical methods for epidemiological analyses and data systems for merging large masses of data into analysis files.

In the area of radiation effects, the epidemiology group is continually evaluating the mortality experience of workers within the Oak Ridge Operations Office area. This area includes workers at facilities run by Union Carbide Corporation, Nuclear Division, in Oak Ridge, Tennessee, and Paducah, Kentucky, and workers at the National Lead Company and the Mound Laboratory, both in Ohio. Editing and correction procedures have been developed for the data bases that extend back to the early 1940s. Preliminary mortality analyses have been completed on two groups of workers at the Oak Ridge Y-12 plant.

Another study, designed to examine possible health effects of occupational exposure to radiation equal to or greater than the current limit (set by the Nuclear Regulatory Commission and DOE) of 5 rem per year, is in the data collection and validation phase. We have constructed a roster of about 2000 DOE (and predecessor organization) employees who have been exposed to 5 or more rem per year. Preliminary analyses of mortality experience for this group will be initiated in late 1979 when death certificate retrieval has been completed.

Other studies in progress include the long-term health effects of exposure to metals and other toxic substances. A completed mortality study of Oak Ridge workers potentially exposed to metallic nickel is being updated to include more follow-up data. Mortality is being examined among welders of the Union Carbide Corporation, Nuclear Division, which contains a subgroup exposed to nickel vapor.

To facilitate these studies and to aid other DOE epidemiological studies, such as those of atomic energy workers at Hanford, Washington; Los Alamos, New Mexico; and Rocky Flats, Colorado, the epidemiology group has developed the DOE Death Certificate Retrieval Center to obtain information on the vital status of specific workers. These data are being accumulated through the cooperation of the Social Security Administration and the vital statistics departments in all 50 states.

Immunobiology

The immunobiology program is concerned with the effects of environmental pollutants on immunologic processes at the organismal and cellular levels. Two animal models are involved – mice and marmosets – each providing biological characteristics conducive to answering the questions pursued. Laboratory mice have diverse genetic strains and varying susceptibilities to chemical oncogenic agents; marmosets have a natural blood chimerism that presents a potentially sensitive system on which the effect of chemical pollutants can be measured.

1028416

Studies in mice have focused on using the fetus as the target for environmental toxicants that might be carcinogenic and show adverse effects on the developing immune system. Inoculation of pregnant mice with benzo(α)pyrene at different gestational ages has led to a marked reduction in the immune response capability of the progeny; most significantly, this immunologic dysfunction has been found to persist up to 78 weeks of age. Paralleling this reduction in immune capacity is an increased incidence of tumors among the experimental animals. Whether there is a direct, causal relationship between the immune status and tumor development is still undetermined. This experimental system is now being extended to examine the cell-mediated immune capacity of mice exposed to the chemical agent during fetal life.

Marmosets are also being used to study immune processes affected by environmental toxicants. A variety of in vitro tests will provide both quantitative and qualitative information. Our inability to obtain a consistent source of splenic tissue for antibody formation, as is commonly done with mice, has necessitated development of a test system that will utilize the most readily available lymphoid tissue – blood lymphocytes. In vitro tests with trinitrophenyl-lipopolysaccharide have demonstrated that marmoset blood lymphocytes are capable of responding with significant numbers of antibody-forming cells. Although the antigen used appears to act through direct stimulation of B lymphocytes, studies with specific immune reagents have shown this response to be controlled by T lymphocytes. These tests appear to be a good model for studying the function of both cell types in animals exposed to the environmental toxicants.

Marmosets have proved to be an excellent model for the study of autoimmune phenomena affecting the platelet-forming system. Induction of an immunologically mediated thrombocytopenia has been obtained consistently by an interspecies immunization of marmosets with platelets. The disease induced closely parallels the clinical situation of posttransfusion purpura, wherein a patient may become severely thrombocytopenic following infusion of genetically incompatible blood platelets. Analysis of the antibodies obtained in our experimental model indicates both antidonor and antihost antibodies are induced by isoimmunization. Preliminary investigations have shown the antihost antibody to have specificities for individual marmosets within the colony; this indicates the existence of a genetic polymorphism comparable to that found in humans.

Radiation Emergency Assistance Center/Training Site

The REAC/TS program includes five major sections: radiation emergency response, teaching and training, the accident registry system, cytogenetics, and experimental radiopathology. The REAC/TS program continues to grow in both its service functions and its research projects. During the past year REAC/TS has rendered assistance and consultation in 4 radiation accidents and 23 radiation incidents. A total of 158 professionals in medicine, health physics, and nursing have been trained in REAC/TS courses. In addition, training in the management of health problems associated with radiation accidents has been provided through lectures, demonstrations, professional exhibits, a newsletter, and preparation of *Response to Radioactive Materials Transport Accidents* for the Department of Transportation.

Major emphasis has been placed on establishing and completing the accident registry system. The REAC/TS registry system has been updated and expanded to 241 entries (each representing a radiation-related event) involving 2870 persons. Of these entries, 95 events (603 persons) qualify for inclusion in the U.S. Radiation Accident Registry (25 rem acute total-body irradiation, 600 rem acute localized irradiation, or one-half maximum permissible body burden for internal irradiation). Medical follow-up information has been obtained for approximately 400 radiation accident survivors including 7 involved in two serious accidents in 1958 and 1971 in Oak Ridge. Twenty DOE sites have identified 1342 persons for inclusion in the Department of Energy Study Registry ($\geqq$5 rem); the names of another 1000 persons from two additional sites are being processed and will be added to this registry shortly. A system of medical surveillance for the approximately 2400 in the DOE Study Registry will be implemented in the near future.

1028417

There are 28 entries (approximately 195 persons) in the Foreign Registry (accurate information about persons involved in foreign radiation accidents is not always available). The remainder of entries in the REAC/TS registry system relate to events brought to the attention of REAC/TS, although they do not meet the criteria for inclusion in any of the registries.

The division continues to manage DOE's program involving diethylenetriaminepentaacetic acid (DTPA) for chelation therapy of internally deposited actinide contaminants. Since the U.S. Food and Drug Administration approved Zn-DTPA as an investigational new drug in late 1978, it has been distributed to 31 coinvestigators. In early 1979, an amendment to the Ca-DTPA regulation was approved by the U.S. Food and Drug Administration to permit its use as an aerosol, which is in addition to the previously approved intravenous and intramuscular routes. Between June 1, 1978, and May 31, 1979, 14 patients were treated with Ca-DPTA and 3 with Zn-DTPA, which brings the total number of patients treated with DTPA to 390. A DTPA Registry has been established to facilitate long-term follow-up of the persons who have received DTPA and to help determine the safety and effectiveness of the therapy.

Research activities also progressed in radiation biology. In addition to responding to requests for biological dosimetry estimates or follow-up studies for 46 persons referred to REAC/TS because of known or suspected radiation exposures, the cytogenetics program conducted several basic research studies aimed at developing improved techniques for assessing the genetic risks of human exposure to environmental mutagens. In vitro dose response curves were developed for chromosome lesion induction in human lymphocytes exposed in vitro to gamma radiation from ^{192}Ir to allow calculation of equivalent whole-body dose effects in persons exposed to this radioisotope. Studies employing sister-chromatid exchange techniques demonstrated that while ionizing radiation does not induce sister-chromatid exchanges in human G_o lymphocytes, in vitro dose response curves can be generated for exposure of human lymphocytes to alkylating chemicals; these curves are useful in assessing dose levels of chemical exposures in vivo.

Progress has been made in the experimental radiopathology work concerned with early recognition of severe, localized radiation injury. Using [^{99m}Tc]-pertechnetate and conventional imaging and quantitation techniques with the gamma camera, radiation damage from necrotizing doses of X rays can now be diagnosed within 24 hours after exposure. This is 2 weeks before clinical manifestations can be seen.

Radiopharmaceutical Development and Nuclear Medicine

The object of the preclinical and clinical nuclear medicine programs is to develop new radiopharmaceuticals and techniques for diagnosing and studying diseases in humans, particularly those diseases resulting from, or aggravated by, environmental pollutants. Potentially useful agents are synthesized in a radiolabeled form and tested in animals for efficacy and safety before clinical testing in humans is approved by the U.S. Food and Drug Administration. Studies of the biochemical mechanisms responsible for the tissue specificity of radiopharmaceuticals are also carried out to aid in developing new classes of radiopharmaceuticals that may be even more effective. Collaborative research projects involve Oak Ridge National Laboratory, Brookhaven National Laboratory, and four medical schools.

When positron-emitting radiopharmaceuticals decay, they emit annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions from various angles with positron emission computerized tomography (ECT), it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of such radiopharmaceuticals. The images are similar to and yield the same type information as those images obtained by autoradiographic techniques, but the positron tomographic technique is not invasive. Using an ECAT$_{TM}$ scanner, the division has continued to develop methods for studying cancer and pancreatic disease by taking advantage of the altered metabolism associated with disease processes. Thus, the images obtained by positron ECT indicate differences in physiological functions rather than differences in tissue density as do conventional X-ray computerized tomographic images.

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Thirty-seven batches of the [^{11}C] carboxyl-labeled amino acids – DL-valine, DL-tryptophan, and 1-aminocyclobutanecarboxylic acid (ACBC) – were prepared last year for use in diagnosing pancreatic disease and detecting solid tumors. We have shown that both [^{11}C] valine and [^{11}C] tryptophan are effective agents for the diagnosis of pancreatic disease, and [^{11}C] ACBC has been shown to be particularly useful in the differential diagnosis of tumors.

With the recent development of improved computer programs for quantitating ^{11}C in tissues, emphasis is now being placed on the amount of [^{11}C] ACBC in tumors before, during, and after therapy to determine whether the quantity of the ^{11}C agent in the tumor can be used to gauge the effectiveness of therapy. Gallium-68, a short-lived positron emitter that is available from a nuclide generator, will undoubtedly find increasing use in positron ECT, since its supply, unlike that of ^{11}C, does not depend on the availability of an accelerator.

Both intravenously administered [^{68}Ga]-citrate and [^{68}Ga] ethylenediaminetetraacetate have been under study in the division's clinical program. Recently, we tested the effectiveness of the regional perfusion of ^{68}Ga-labeled human serum albumin microspheres for visualizing the vascular system of the lung. First results with the microspheres indicate they have considerable promise. In addition, the results from animal studies have revealed that [^{68}Ga]ethylenediaminetetraacetate aerosols can be used for positron ECT ventilation studies in patients.

In a collaborative project with P. H. King of Vanderbilt University, considerable progress has been made in upgrading the division's computer capabilities in nuclear medicine. The ECAT$_{TM}$ scanner software package is being revised to provide improved image reconstruction. An interface between the Pho Gamma V camera and the PDP-11/45 computer of the ECAT$_{TM}$ scanner has been made. As a result, improved quantitative image analysis by both conventional scintiphotography and positron ECT is expected in the near future.

Our two-step, high-temperature, high-pressure technique for synthesizing [^{11}C] carboxyl-labeled amino acids unexpectedly failed for two amino acids – α-aminoisobutyric acid (a potential agent for assessing breakdown in the blood-brain barrier) and 3-aminotetrahydrothiophene-3-carboxylic acid (a potential tumor-imaging agent). Therefore, we developed a single-step Bücherer-Strecker synthesis for synthesizing these amino acids. Enzymatic methods for the rapid resolution of racemic mixtures of ^{11}C-labeled amino acids are now under development in collaboration with the University of Kentucky to provide the naturally occurring optical isomers for metabolic research purposes. D- and L-phenylalanine labeled with ^{11}C have been successfully separated in quantity (~20 mCi each) with this technique.

The computer code designed to simulate accelerator targets was greatly improved and expanded in another collaborative project with P. H. King, Vanderbilt University. This code is being used to design new targets for a small, dedicated cyclotron. It is also being used to evaluate experimental results from existing ^{11}C, ^{13}N, and ^{18}F targets at the Oak Ridge National Laboratory 86-inch cyclotron. The nuclear data library for this code now includes accurate experimental cross sections for the p,n reactions producing ^{11}C, ^{13}N, and ^{18}F. These reactions were confirmed by yield measurements made on the Tandem Van de Graaff generator in collaboration with the Physics Division at Oak Ridge National Laboratory.

An ORAU-initiated collaboration with Brookhaven National Laboratory is anticipated to lead to the development of new cyclotron targets for the production and recovery of ^{11}C, ^{13}N, and ^{18}F in useful chemical forms. This work also involves experiments to define the behavior of high beam current gas targets used to produce ^{11}C, ^{15}O, and ^{18}F and to develop automated systems for radiochemical syntheses.

In our continuing studies of the mechanisms involved in the organ specificity of radiopharmaceuticals, further evidence has been obtained to indicate that ^{67}Ga enters tumor and normal tissues by two different routes. Uptake in tumor tissue appears to involve primarily an unbound or loosely bound form of ^{67}Ga while the uptake in normal soft tissues involves a protein-bound form of ^{67}Ga. Other studies of the uptake of ^{67}Ga by inflammatory lesions suggest that the route involved is similar to that in normal tissue.

1028419



Studies on the [illegible] of ovarian [illegible] fetal tissue at the subsequent immunological consequences after birth is a major [illegible] in the immunobiology program. The molecular model illustrates the structure of benzo(α)pyrene, a potent carcinogen of [illegible]

Benzo(α)pyrene is being administered to a pregnant mouse in a study designed to determine how the exposure of fetuses to a carcinogen at different periods of gestation affects the immune response of the progeny.

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Publications

Full-length journal articles

Armstrong, R. D., and Frome, E. L.
Least-absolute-value estimators for one-way and two-way tables.
Nav. Res. Logistics Quart. 26:79-96; 1979. Work performed at the University of Texas, Austin.

Armstrong, R. D., and Frome, E. L.
A remark on algorithm AS108.
Appl. Stat. 27:378; 1978. Work performed at the University of Texas, Austin.

Armstrong, R. D.; Frome, E. L.; and Kung, D. S.
A revised simplex algorithm for the absolute deviation curve-fitting problem.
Commun. Stat. 8B:175-190; 1979. Work performed at the University of Texas, Austin.

Arneson, R. M.; Geller, A. M.; and Byrne, W. L.
Binding of adenosine 5′-monophosphate to bovine liver fructose 1,6-bisphosphatase.
Enzyme 24:132-136; 1979. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

Blank, M. L., and Snyder, F.
A quantitative chromatographic method for the determination of low levels of nitrilotriacetic and ethylenediaminetetraacetic acids in diethylenetriaminepentaacetic acid.
J. Chromatogr. 170:379-383; 1979.

Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; and Muirhead, E. E.
Antihypertensive activity of an alkyl ether analog of phosphatidylcholine.
Biochem. Biophys. Res. Commun. [1979]. In press.

Brown, A. J., and Snyder, F.
Solubilization of alkyldihydroxyacetone-P synthase from Ehrlich ascites cell microsomal membranes.
Biochem. Biophys. Res. Commun. 90:278-284; 1979.

Buonocore, E., and Hübner, K. F.
Positron-emission computed tomography of the pancreas: preliminary study.
Radiology [1979]. In press.

Buonocore, E.; Hübner, K. F.; and Collmann, I. R.
Differentiation of retroperitoneal tumors using positron emission computerized tomography: a case report.
J. Comput. Asst. Tomogr. [1979]. In press.

Cabot, M. C., and Gatt, S.
The hydrolysis of triacylglycerol and diacylglycerol by a rat brain microsomal lipase with an acidic pH optimum.
Biochim. Biophys. Acta 530:508-512; 1978. Work performed at Hebrew University, Jerusalem, Israel.

Cabot, M. C., and Snyder, F.
The manipulation of fatty acid composition in L-M cell monolayers supplemented with cyclopentenyl fatty acids.
Arch. Biochem. Biophys. 190:838-846; 1978.

Chaskes, S., and Tyndall, R. L.
Pigmentation and autofluorescence of *Cryptococcus* species after growth on tryptophan and anthranilic acid media.
Mycopathologia 64:105-112; 1978.

Chepenik, K. P.; Blank, M.; Snyder, F.; and Waite, B. M.
On the presence of plasmalogens in developing rat embryos.
Int. J. Biochem. [1979]. In press.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
Cyclophosphamide induced SCEs in rabbit lymphocytes.
Environ. Mutagenesis [1979]. In press.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
The effect of washing lymphocytes after in vivo treatment with streptonigrin on the yield of chromosome and chromatid aberrations in blood cultures.
Mutat. Res. [1979]. In press.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Frome, E. L.
Human cytogenetic dosimetry: a dose response relationship for alpha particle radiation from americum-241.
Health Phys. [1979]. In press.

Duffey, D.; Beck, W. L.; Gleason, G. I.; and Wiggins, P. E.
Evaluation of dosimetry film by neutron activation and X-ray fluorescence.
J. Nucl. Sci. Technol. 16:89-94; 1979.

Durum, S. K., and Gengozian, N.
The comparative radiosensitivity of T and B lymphocytes.
Int. J. Radiat. Biol. 34:1-15; 1978.

Fuhr, J. F., and Gengozian, N.
Fetal hemoglobin: in vivo stimulation by D-thyroxin in adult marmosets.
Blood Cells [1979]. In press.

Gengozian, N., and Barton, B. A.
Isoimmunizations among marmosets to identify leucocyte antigens.
J. Med. Primatol. 8:113-121; 1979.

1028421

Gengozian, N.; Brewen, J. G.; and Preston, R. J.
Presumptive evidence for the absence of functional germ cell chimerism in the marmoset.
J. Med. Primat. [1979]. In press.

Gengozian, N.; Kateley, J. R.; and Nickerson, D. A.
Marmoset species variation in the humoral antibody response: in vivo and in vitro studies.
Immunology 35:549-558; 1978.

Germain, G. S., and Arneson, R. M.
Effects of metal ions and selenoamino acids on induction of glutathione peroxidase in mouse neuroblastoma.
Enzyme [1979]. In press. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

Godbold, J. H., Jr., and Tompkins, E. A.
A long-term mortality study of workers occupationally exposed to metallic nickel at the Oak Ridge Gaseous Diffusion Plant.
J. Occup. Med. [1979]. In press.

Hayes, R. L.; Szymendera, J. J.; and Byrd, B. L.
Effect of food intake on the tissue distribution of gallium-67: concise communication.
J. Nucl. Med. 20:938-940; 1979.

Highfill, R. R., and Phelps, M. E.
Positron tomography.
IEEE Trans. Nucl. Sci. NS-26:1632-1634; 1979.

Highfill, R. R., and Wieland, B. W.
The medical applications of short-lived, cyclotron-produced radionuclides.
IEEE Trans. Nucl. Sci. NS-26:2220-2222; 1979.

Hübner, K. F.; Andrews, G. A.; Buonocore, E.; Hayes, R. L.; Washburn, L. C.; Collmann, I. R.; and Gibbs, W. D.
Carbon-11-labeled amino acids for the rectilinear and positron tomographic imaging of the human pancreas.
J. Nucl. Med. 20:507-513; 1979.

Lambremont, E. N., and Wykle, R. L.
Wax synthesis by an enzyme system from the honey bee.
Comp. Biochem. Physiol. 63*B*:131-135; 1979.

Lawless, D.; Brown, D. H.; Hübner, K. F.; Colyer, S. P.; Carlton, J. E.; and Hayes, R. L.
Isolation and partial characterization of a ^{67}Ga-binding glycoprotein from Morris 5123C rat hepatoma.
Cancer Res. 38:4440-4444; 1978.

Lee, T-c.
Characterization of fatty alcohol:NAD^+ oxidoreductase from rat liver.
J. Biol. Chem. 254:2892-2896; 1979.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Comparison of sister-chromatid exchanges in human lymphocytes after G_0 exposure to mitomycin in vivo vs in vitro.
Mutat. Res. [1979]. In press.

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes exposed to ionizing radiation during G_0.
Radiat. Res. 78:514-521; 1979.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. M.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes exposed during G_0 to four classes of DNA-damaging chemicals.
Mutat. Res. 67:259-269; 1979.

McDermott, C. A., and Gengozian, N.
The effect of low-exposure-rate gamma irradiation on T and B lymphocyte function in the mouse.
Int. J. Radiat. Biol. [1979]. In press.

Moore, C.; Lee, T-c.; Stephens, N.; and Snyder, F.
Enzyme activities in lipid-modified membranes of rat livers.
Biochim. Biophys. Acta 531:125-130; 1978.

Polednak, A. P.
Age changes in differential leukocyte count among female adults.
Hum. Biol. 50:301-311; 1978. Work performed at Argonne National Laboratory, Argonne, Illinois.

Polednak, A. P.
Fertility among women exposed to internal and external radiation.
J. Environ. Pathol. Toxicol. [1979]. In press. Work performed at Argonne National Laboratory, Argonne, Illinois.

Polednak, A. P.
Long-term effects of radium exposure in female dial workers: liver function and liver disease.
Environ. Res. 18:454-465; 1979. Work performed at Argonne National Laboratory, Argonne, Illinois.

Polednak, A. P.
Thyroid tumors and thyroid function in women exposed to internal and external radiation.
J. Environ. Pathol. Toxicol. [1979]. In press. Work performed at Argonne National Laboratory, Argonne, Illinois.

Polednak, A. P., and Farnham, J. E.
Osteometry and autoradiography of long bones and skeletons of women exposed to radiation at 13-19 years of age.
Am. J. Phys. Anthropol. [1979]. In press. Work performed at Argonne National Laboratory, Argonne, Illinois.

Polednak, A. P., and Janerich, D. P.
Sickle cell trait, birth characteristics, and complications of pregnancy: a case-control study.
Hum. Biol. [1979]. In press. Work performed at Argonne National Laboratory, Argonne, Illinois.

Porcella, R. A.; Swartzendruber, D. C.; and Godbold, J. H., Jr.
A morphometric study quantifying the mucous content of proliferating colonic epithelial cells.
Am. J. Anat. 155:103-110; 1979.

Richter, C. B., and Buyckmichi, N.
Squamous-cell carcinoma of the epidermis in an aged white-lipped tamarin (*Saguinus fuscicollis leucogenys* Gray).
Vet. Pathol. 16:263-265; 1979.

1028422

Rundo, J.; Polednak, A. P.; Brues, A. M.; Lucas, H. F., Jr.; atten, B. C.; Rowland, R. E.; and Stehney, A. F.
A study of radioactivity and health status of former thorium workers.
Environ. Res. 18:94-100; 1979. Work performed at Argonne National Laboratory, Argonne, Illinois.

Schremmer, J. M.; Blank, M. L.; and Wykle, R. L.
Bradykinin-stimulated release of [^{3}H]arachidonic acid from phospholipids of HSDM$_1$C$_1$ cells: comparison of diacyl phospholipids and plasmalogens as sources of prostaglandin precursors.
Prostaglandins [1979]. In press.

Scott, C. C.; Heckman, C. A.; and Snyder, F.
Regulation of ether lipids and their precursors in relation to glycolysis in cultured neoplastic cells.
Biochim, Biophys, Acta [1979]. In press.

Scott, C. C.; Heckman, C. A.; Nettesheim, P.; and Snyder, F.
Metabolism of ether-linked glycerolipids in cultures of normal and neoplastic rat respiratory tract epithelium.
Cancer Res. 39:207-214; 1979.

Shivers, C. A.; Gengozian, N.; Franklin, S.; and McLaughlin, C. L.
Antigenic cross-reactivity between human and marmoset zonae pellucidae, a potential target for immunocontraception.
J. Med. Primatol. 7:242-248; 1978.

Tankersley, W. G.; Richter, C. B.; and Batson, J. S.
Therapy of filariasis in tamarins.
Lab. Anim. Sci. 29:107-110; 1979.

Tyndall, R. L.; Clapp, N. K.; Davidson, K. A.; Colyer, S. P.; and Burtis, C. A.
Effects of carcinogenic and noncarcinogenic chemicals on plasma esterases in BALB/c mice.
Chem.-Biol. Interact. 23:159-169; 1978.

Urso, P., and Gengozian, N.
Nonspecific suppressor elements in murine allogeneic radiation chimeras.
Cell. Immunol. [1979]. In press.

Voelker, D. R., and Snyder, F.
Subcellular site and mechanism of synthesis of disaturated phosphatidylcholine in alveolar type II cell adenomas.
J. Biol. Chem. 254:8628-8633; 1979.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and Butler, T. A.
DL-[Carboxyl-^{11}C]tryptophan, a potential agent for pancreatic imaging: production and preclinical investigations.
J. Nucl. Med. 20:857-864; 1979.

shburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and tler, T. A.
1-Aminocyclobutane[^{11}C]carboxylic acid, a potential tumor-seeking agent.
J. Nucl. Med. [1979]. In press.

Wieland, B. W.; Highfill, R. R.; and King, P. H.
Proton accelerator targets for the production of ^{11}C, ^{13}N, ^{15}O, and ^{18}F.
IEEE Trans. Nucl. Sci. NS-26:1713-1717; 1979.

Wykle, R. L., and Schremmer, J. M.
Biosynthesis of plasmalogens by the microsomal fraction of Fischer R-3259 sarcoma. Influence of specific 2-acyl chains on the desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine.
Biochemistry 18:3512-3517; 1979.

Wykle, R. L.; Malone, B.; and Snyder, F.
Acyl-CoA reductase specificity and synthesis of wax esters in mouse preputial gland tumors.
J. Lipid Res. [1979]. In press.

Wykle, R. L.; Malone, B.; Blank, M. L.; and Snyder, F.
Biosynthesis of pulmonary surfactant: comparison of 1-palmitoyl-*sn*-glycero-3-phosphocholine and palmitate as precursors of dipalmitoyl-*sn*-glycero-3-phosphocholine in adenoma alveolar type II cells.
Arch. Biochem. Biophys. [1979]. In press.

Chapters, review articles, and other publications

Frome, E. L., and DuFrain, R. J.
Estimation of the amount of exposure to an environmental clastogen using human cytogenetic dosimetry. Davis, H. T.; Prairie, R. R.; and Truett, T., eds.
1979 DOE Statistical Symposium; 1979. Albuquerque, New Mexico, 169-192. US DOE CONF-781108.

Frome, E. L., and Hudson, D. R.
A jackknife procedure for estimating standardized mortality ratios.
Proceedings of the Statistical Computing Section of the American Statistical Association, 1979. Washington, D.C. In press.

Gengozian, N.
The marmoset as a model for twinning, chimerism, and immunologic tolerance. Andrews, E. J.; Ward, B. C.; and Altman, N. H.; eds.
Spontaneous Animal Models of Human Disease; Vol. 1. New York: Academic Press [1979]. In press.

Gengozian, N.; Batson, J. S.; and Smith, T. A.
Breeding of tamarins (*Saguinus* ssp.) in the laboratory. Kleinman, D., ed.
Biology and Conservation of the Callitrichidae, a Symposium; 1975. Washington, D.C. Smithsonian Institution Press; 1977. 207-213;

Hayes, R. L.; Washburn, L. C.; Wieland, B. W.; Sun, T. T.; Turtle, R. R.; and Butler, T. A.
Carboxyl-labeled ^{11}C-1-aminocyclopentanecarboxylic acid, a potential agent for cancer detection.
Year Book Cancer; 1978. 236-237. (Original publication: *J. Nucl. Med.* 17:748-751; 1976.)

Hodges, H. D.
NCRP Report No. 57: Instrumentation and monitoring methods for radiation protection. A review.
NMT Bookshelf.
J. Nucl. Med. Technol. [1979]. In press.

1028423

Hübner, K. F.; Andrews, G. A.; Washburn, L.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.; Butler, T. A.; and Winebrenner, J. D.
Tumor location with 1-aminocyclopentane [^{11}C]carboxylic acid: preliminary clinical trials with single-photon detection. *Year Book Cancer*. In press. (Original publication: *J. Nucl. Med.* 18:1215-1221; 1977.)

Humason, G. L.
Animal Tissue Techniques. 4th ed. San Francisco: W. H. Freeman and Company; 1979.

Littlefield, L. G., and Joiner, E. E.
Cytogenetic follow-up studies in six radiation accident victims (16 and 17 years postexposure).
Late Biological Effects of Ionizing Radiation, Proceedings of a Symposium; Vol. 1; 1978. Vienna, Austria: International Atomic Energy Agency; 1978. 297-308.

Lushbaugh, C. C.
Letter to the editor.
Emergency 11:6-7; 1979.

Lushbaugh C. C., and Washburn, L. C.
FDA IND approval for Zn-DTPA, new clinical agent for decorporation therapy of actinides: letter to the editor.
Health Phys. 36:472; 1979.

Lushbaugh, C. C., and Washburn, L. C.
FDA IND approval for Zn-DTPA, new clinical agent for decorporation therapy for actinides: letter to the editor.
J. Nucl. Med. 20:73; 1979.

Nénot, J. C.; Lushbaugh, C. C.; Lincoln, T. A.; Daw, H. T.; Komarov, E.; Djordjevic, D.; and Eider, W. M.; comp.
Manual on Early Medical Treatment of Possible Radiation Injury with an Appendix on Sodium Burns; Safety Series No. 47. Vienna, Austria: International Atomic Energy Agency; 1978. STI/PUB/506.

Polednak, A. P., ed.
The Longevity of Athletes. Springfield, Illinois: Charles C Thomas; 1979. Work performed at Argonne National Laboratory, Argonne, Illinois.

Richter, C. B.; Lushbaugh, C. C.; and Swartzendruber, D. C.
Cancer of the colon in the nonhuman primate, *Saguinus oedipus oedipus*. Montali, R., and Migaki, G., eds.
Comparative Pathology of Zoo Animals, Proceedings of a Symposium, 1978. Washington, D.C. In press.

Ricks, R. C.
A training course for radiation accident mangement in the health physics/medical environment.
Proceedings of the 13th Annual Mid-Year Symposium of the Health Physics Society, 1979. Honolulu, Hawaii. In press.

Ricks, R. C.; Beck, J.; Berger, J.; and Hübner, K. F.
Response to Radioactive Materials Transport Accidents. Washington, D.C.: Department of Transportation; 1979. DOT/RSPA/MTB-79/8. In press.

Snyder, F.; Lee, T-c.; Blank, M. L.; and Moore, C.
In vivo modification of phospholipid polar head groups and alterations in membrane activities and cellular events. Kates, M., and Kusis, A., eds.
Control of Membrane Fluidity, Proceedings of a Symposium, 1979; Toronto, Canada. Clifton, New Jersey: The Humana Press, Inc. In press.

Voelker, D. R.
Pulmonary surfactant lipid metabolism in the alveolar type II cell adenoma. Oak Ridge, Tennessee: University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences; 1978. Dissertation.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; Butler, T. A.; and Callahan, A. P.
High-level production of [^{11}C]carboxyl-labeled amino acids. *Proceedings of the Second International Symposium on Radiopharmaceuticals*, 1979. Seattle, Washington; 1979. In press.

Abstracts of papers presented at meetings

Blank, M. L.; Snyder, F. L.; Byers, L. W.; Brooks, B.; Smith, K. A.; and Muirhead, E. E.
Similarities in the antihypertensive action of an alkyl ether analog of phosphatidylcholine and a phospholipid derived from the renal medulla.
Clin. Res. [1979]. In press.

Borman, L. S.; Johnson, D. O.; and Snyder, F.
Effects of a choline inhibitor on mammalian cell growth.
In Vitro 15:223a; 1979.

Brown, A. J., and Snyder, F.
Solubilization of alkyldihydroxyacetone-P synthase from Ehrlich ascites cell microsomal membranes.
Soc. Exp. Biol. Med., S.E. Sect. [1979]. In press.

Brown, D. H.; Carlton, J. E.; Rafter, J. J.; and Hayes, R. L.
A large scale extraction procedure for the purification of a ^{67}Ga-binding glycoprotein.
J. Nucl. Med. 20:682; 1979.

Cabot, M. C., and Snyder, F.
The assimilation of unnatural aliphatic moieties into complex lipids of neoplastic cells.
XII Internat. Cancer Congr.: Abstracts, Workshops, Vol. I, 177; 1978. Buenos Aires, Argentina.

Digenis, G. A.; Casey, D. L.; Wesner, D. A.; Washburn, L. C.; and Hayes, R. L.
Preparation of optically active ^{11}C amino acids.
J. Nucl. Med. 20:662; 1979.

DuFrain, R. J.; Littlefield, L. G.; and Joiner, E. E.
Cytogenetics dosimetry for ^{241}Am alpha particle irradiation of G_0 stage human lymphocytes.
Radiat. Res. 74:523; 1978.

DuFrain, R. J.; Littlefield, L. G.; Wilmer, J. L.
Chemically induced cytogenetic damage in germinal and somatic cells of the female rabbit.
Mutat. Res. 53:103; 1978.

1028424

DuFrain, R. J.; Wilmer, J. L.; and Littlefield, L. G.
Analysis of cyclophosphamide-induced sister-chromatid exchange.
Environ. Mutagenesis [1979]. In press.

DuFrain, R. J.; Littlefield, L. G.; Heffernan, K.; Wilmer, J. L.; and Batson, J.
Sister-chromatid in the genus *Saguinus*.
Mamm. Chromosomes Newslett. 20:13; 1979.

Fishman, J. B.; Blank, M. L.; and Snyder, F.
Accumulation of *O*-alkylacyl ethanolamine phospholipid in tumor tissues.
Soc. Exp. Biol. Med., S.E. Sect. [1979]. In press.

Frome, E. L., and DuFrain, R. J.
The analysis of dose-response curves for radiation-induced chromosome aberrations.
Biometrics 34:731; 1978.

Hayes, R. L.; Byrd, B. L.; and Rafter, J. H.
Studies of the mechanisms of ^{67}Ga uptake by tumor and normal tissue.
Proceedings of 19th Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine, 1978; 4.

Hayes, R. L.; Rafter, J. J.; and Byrd, B. L.
Studies of the mechanism of gallium-67 uptake by tumor, abscess, and normal tissues.
J. Nucl. Med. 20:672-673; 1979.

Hoeschele, J. D.; Johnson, N. P.; Rahn, R. O.; and Brown, D. H.
Comparative distribution/binding studies of ^{195m}Pt-labeled dichlorodiammine platinum (II), DDP, in the rat with emphasis on localization in DNA.
Biochimie 60:1054; 1978.

Hübner, K. F.; Buonocore, E.; Gibbs, W. D.; Holloway, S.; and Byrd, B. L.
Differentiation of pancreatic and other retroperitoneal tumors by positron emission computerized tomography (ECT).
J. Nucl. Med. 20:631; 1979.

Huff, V.; Hutton, D. G.; DuFrain, R. J.; and Littlefield, L. G.
Comparison of chromosome lesion frequency in rabbit bone marrow following a single or double injection of cyclophosphamide.
Soc. Exp. Biol. Med., S.E. Sect. [1979]. In press.

Littlefield, L. G.; DuFrain, R. J.; and Colyer, S. P.
Sister-chromatid exchanges in human lymphocytes exposed to ^{60}Co gamma radiation during G_0.
Radiat. Res. 74:522-523; 1978.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. M.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes exposed during G_0 to four classes of DNA damaging chemicals.
Mamm. Chromosomes Newslett. 20:25; 1979.

Moore, C., and Snyder, F.
Partial characterization of acyl-CoA reductase from mouse preputial gland.
Fed. Proc. 38:310; 1979.

Moore, C., and Snyder, F.
Synthesis of long-chain alcohols as catalyzed by the acyl-CoA reductase from mouse preputial glands.
Soc. Exp. Biol. Med. S.E. Sect. [1979]. In press.

Porcella, R. A.; Swartzendruber, D. C.; and Godbold, J. H., Jr.
A morphometric study quantifying the mucus content of proliferating colonic epithelial cells.
J. Cell Biol. 79:20a; 1978.

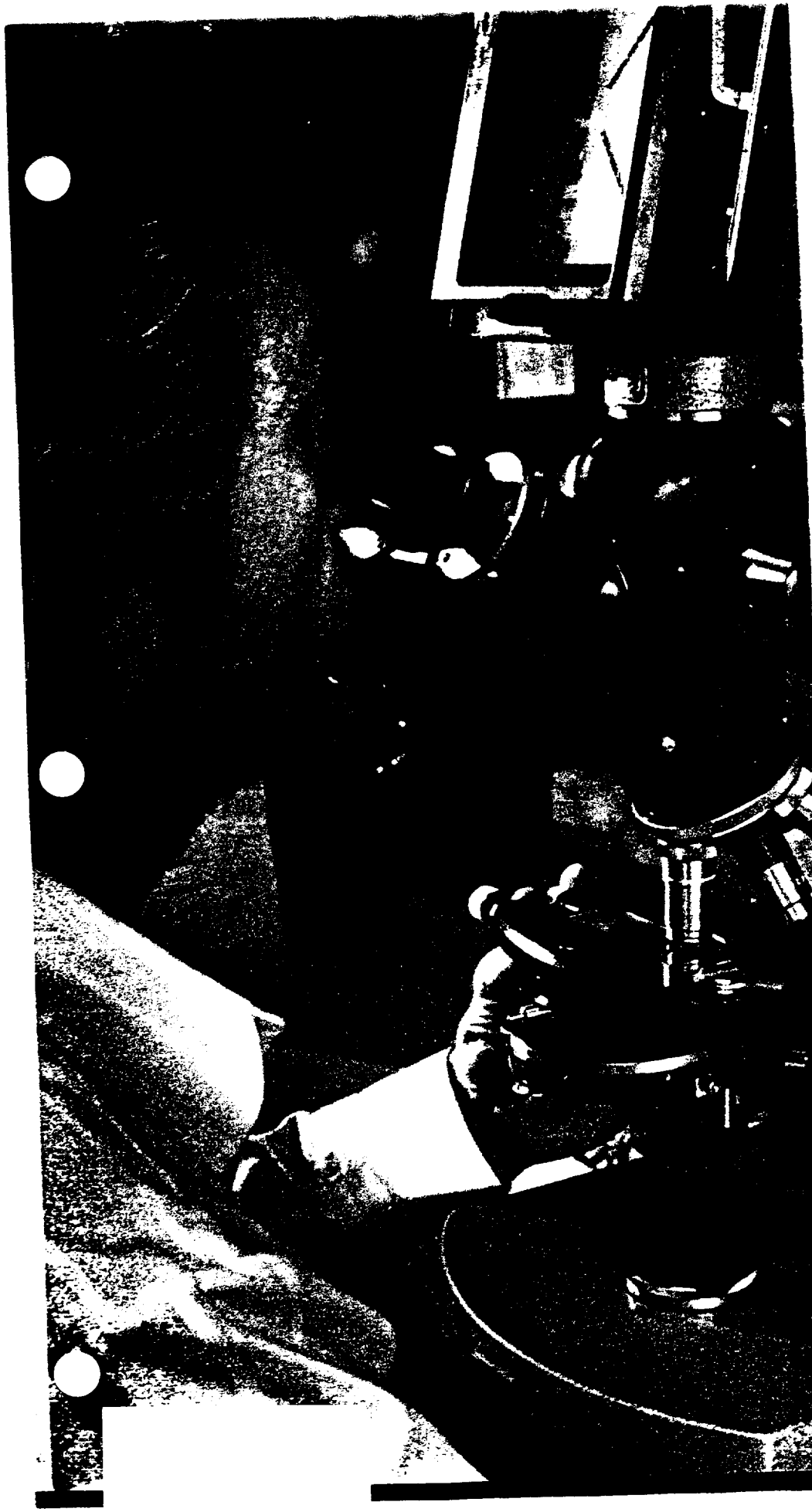
Sayer, A. M.; Littlefield, L. G.; Richter, C. B.; DuFrain, R. J.; and Batson, J. S.
Mutagen-induced chromosome lesions in lymphocytes of *Saguinus oedipus* and *Saguinus fuscicollis*.
Soc. Exp. Biol. Med., S.E. Sect. [1979]. In press.

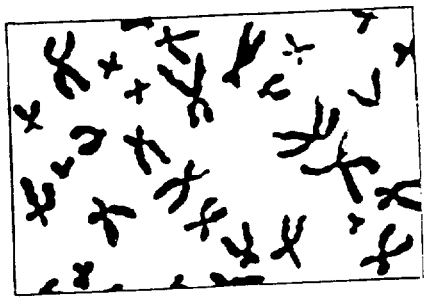
Scott, C.; Heckman, C.; and Snyder, F.
Ether-lipid regulation in cultured cells: relationship to glucose metabolism.
J. Cell Biol. 79:80a; 1978.

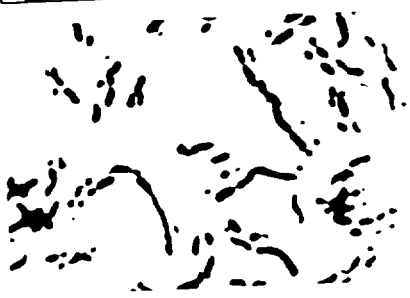
Wieland, B. W., and Howell, R. A.
Computed yields of ^{11}C, ^{13}N, ^{15}O, and ^{18}F for proton bombardment of enriched stable isotope targets.
J. Labelled Compd. Radiopharm. 16:103-105; 1979.

Wykle, R. L., and Schremmer, J. M.
Biosynthesis of plasmalogens: influence of the composition of the 2-acyl constituent on desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine by tumor microsomes.
Soc. Exp. Biol. Med., S.E. Sect. 3:19; 1978.

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(Top) A portion of a human metaphase preparation showing chromosomes from a lymphocyte (about 2000x).

(Bottom) Harlequin-stained chromosomes showing chemically induced sister-chromatid exchanges.

Human cytogenetic dosimetry requires detailed microscopic analysis of cultured lymphocytes from individuals with suspected radiation exposure.

1028426

Published Abstracts of Full-Length Publications

Armstrong, R. D., and Frome, E. L.
Least-absolute-value estimators for one-way and two-way tables.
Nav. Res. Logistics Quart. 26:79-96; 1979. Work performed at the University of Texas, Austin.

This paper concerns the problem of estimating the parameters of one-way and two-way classification models by minimizing the sum of the absolute deviations of the regression function from the observed points. The one-way model reduces to obtaining a set of medians from which optimal parameters can be obtained by simple arithmetic manipulations. The two-way model is transformed into a specially structured linear programming problem, and two algorithms are presented to solve this problem. The occurrence of alternative optimal solutions in both models is discussed, and numerical examples are presented.

Armstrong, R. D.; Frome, E. L.; and Kung, D. S.
Revised simplex algorithm for the absolute deviation curve-fitting problem.
Commun. Stat. 8B:175-190; 1979. Work performed at the University of Texas, Austin.

Least absolute value estimation has long been recognized as an acceptable alternative to least squares. The least absolute value procedure was unpopular until the relatively recent advent of linear programming and the digital computer, primarily due to computational difficulties. In this paper, we present a special-purpose linear programming algorithm that is used to solve the least absolute value regression problem. This algorithm uses the revised simplex method with the principle of LU decomposition in maintaining the current bases. A one-pass partial sort routine is devised to determine the observation to enter the bases at each iteration. All of the original information, including the observation matrix, is preserved by the program during execution. The algorithm is coded in FORTRAN, and comparisons with a similar program are presented.

Arneson, R. M.; Geller, A. M.; and Byrne, W. L.
Binding of adenosine 5′-monophosphate to bovine liver fructose 1,6-bisphosphatase.
Enzyme 24:132-136; 1979. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

Bovine liver fructose 1,6-bisphosphatase bound 4 mol of the allosteric inhibitor AMP per mole of enzyme with half-saturation at 17 μmol/l AMP. The presence of a mixture of positive and negative cooperativity in the binding of AMP to the enzyme was suggested by several procedures for analyzing binding data. In particular, calculating the intrinsic binding constants for AMP yielded the relationships $K_1' < K_2' < K_3' < K_4'$, indicating mixed cooperativity.

Blank, M. L., and Snyder, F.
A quantitative chromatographic method for the determination of low levels of nitrilotriacetic and ethylenediaminetetraacetic acids in diethylenetriaminepentaacetic acid.
J. Chromatogr. 170:379-383; 1979.

A quantitative method for determining low levels (0.05%, w/w) of nitrilotriacetic and ethylenediaminetetraacetic acids in diethylenetriaminepentaacetic acid is described. Palmitic acid is added to the chelator as an internal standard before esterification and methanol containing 2% (v/v) H_2SO_4. The methyl esters of palmitic, nitrilotriacetic, and ethylenediaminetetraacetic acids are first separated from diethylenetriaminepentaacetate by silicic acid column chromatography and are subsequently quantitated by gas-liquid chromatography. The method is both accurate and reproducible with less than 10% relative error. Thin-layer chromatographic separations of the methyl esters and quantitation at the 1% level are also described.

Brown, A. J., and Snyder, F.
Solubilization of alkyldihydroxyacetone-P synthase from Ehrlich ascites cell microsomal membranes.
Biochem. Biophys. Res. Commun. 90:278-284; 1979.

The enzyme, alkyldihydroxyacetone-P synthase, has been solubilized and partially purified from microsomal preparations of Ehrlich ascites cells after treatment with Triton X-100 and phospholipase C, followed by chromatography on Sepharose 4B. When the Triton X-100 was removed after solubilization, the enzyme was still active but eluted in the void volume of the Sepharose 4B column, whereas in the presence of detergent it eluted much later as a single peak of activity, indicating that the solubilized enzyme tends to aggregate unless detergent is present. The lower molecular weight form of alkyldihydroxyacetone-P synthase (in detergent) had an estimated molecular mass of 250,000 to 300,000 daltons.

Cabot, M. C., and Gatt, S.
The hydrolysis of triacylglycerol and diacylglycerol by a rat brain microsomal lipase with an acidic pH optimum.
Biochim. Biophys. Acta 530:508-512; 1978. Work performed at Hebrew University, Jerusalem, Israel.

Lipase activity toward triacylglycerol and diacylglycerol was measured at pH 4.8 using a microsomal preparation from rat brain as the enzyme source. The optimal pH for hydrolysis of triacylglycerol was 4.8, with only minor lipolytic activity in the alkaline pH range. Diacylglycerol was the major product of triacylglycerol hydrolysis, with only little monoacylglycerol being formed. When diacylglycerol was the starting substrate, it was hydrolyzed at a rate 10-fold

1028427

triacylglycerol, and the product was monoacylglycerol. The enzyme showed positional specificity for the fatty acid moieties located at the primary positions of *sn*-glycerol. 1,3-Diacylglycerol was hydrolyzed at greater than twice the rate of the corresponding 1,2(2,3)-isomer.

Cabot, M. C., and Snyder, F.
The manipulation of fatty acid composition in L-M cell monolayers supplemented with cyclopentenyl fatty acids.
Arch. Biochem. Biophys. 190:838-846; 1978.

Mouse L-M fibroblasts, grown in a serum-free medium, were supplemented with fatty acids of 16- and 18-carbon chain lengths that contain a cyclopentene ring in the ω position. These fatty acids, unnatural to mammalian systems, were incorporated into the major lipid classes of L-M fibroblasts. Supplementation with the cyclopentenyl fatty acids caused an accumulation of neutral glycerolipids and a marked inhibition of cell growth. Following the addition of supplement, the cells became more rounded. Of particular interest was the fact that phospholipid fraction isolated from treated cells contained cyclic fatty acids that accounted for as much as 24% of the total phospholipid acyl groups. Unlike the pattern of distribution displayed by endogenous natural monoenes, the majority of the cyclic acid present was esterified in the *sn*-1 position of both phosphatidylcholine and phosphatidylethanolamine. The 18-carbon cyclic fatty acid [chaulmoogric acid, 13-(2-cyclopenten-1-yl)-tridecanoic acid] was incorporated at the expense of the endogenous C-16:0, C-18:0, and C-18:1 fatty acids of the glycerophospholipids. The esterification altered the ratio of saturated to unsaturated acyl groups in the cellular phospholipids. No biochemical modification of chaulmoogric acid was detected. Our results imply that incorporation of unnatural fatty acid analogs, such as chaulmoogric acid, into cellular membranes will alter the functional properties of biological membranes that are dependent on membrane fluidity and structural organization.

Chaskes, S., and Tyndall, R. L.
Pigmentation and autofluorescense of *Cryptococcus* species after growth on tryptophan and anthranilic acid media.
Mycopathologia 64:105-112; 1978.

Cryptococcus neoformans and *Candida albicans* produced a pink pigment from media containing tryptophan. Approximately 30% of the *C. neoformans* strains produced large amounts of the pink (purple after 6 days) pigment in the absence of light, whereas 70% of the *C. neoformans* strains, as well as *C. laurentii*, *C. albidus*, *C. diffluens*, and *C. albicans* also produced the pink pigment with light being required for significant early production (2–6 days). Significant production did occur for *Cryptococcus* but not *Candida* species in the dark after extended incubation (10–25 days). *C. terreus* produced brown pigment from tryptophan, and *C. luteolus* produced a trace of a buff pigment. Most *Candida* species produced either pink or brown pigments, but not both. In contrast, many *Cryptococcus* species producing the pink pigment simultaneously produced brown pigment. *C. terreus*, *C. albidus*, and *C. diffluens* produced brown pigment from anthranilic acid while *C. neoformans*, *C. laurentii*, *C. luteolus*, and the medically important *Candida* species did not produce significant amounts of pigment from anthranilic acid. *Cryptococcus* and *Candida* species were autofluorescent when tryptophan was a major nitrogen source, whereas yeast cell autofluorescence was not observed when anthranilic acid was used. Pigmentation of some *Cryptococcus* species also occurred when *p*-anisidine or *m*-aminobenzoic acid was the substrate.

Duffey, D.; Beck, W. L.; Gleason, G. I.; and Wiggins, P. F.
Evaluation of dosimetry film by neutron activation and X-ray fluorescence.
J. Nucl. Sci. Technol. 16:89-94; 1979.

Photographic film was exposed to γ-rays from ^{226}Ra and ^{60}Co to levels of 5 mR to 5000 R. Measurments of the silver in the developed films by both (a) activation with neutrons from ^{252}Cf and counting ^{110}Ag (half-life 24.4 sec) and (b) X-ray fluorescence correlated with the known exposures. In the lower ranges, conventional optical density measurements were made, and the correlation with the activation and X-ray methods was good.

Durum, S. K., and Gengozian, N.
The comparative radiosensitivity of T and B lymphocytes.
Int. J. Radiat. Biol. 34:1-15; 1978.

This study deals with the comparative effects of gamma irradiation on T and B lymphocytes in mice. Quantitatively, splenic T cells appeared substantially more radioresistant than B cells. However, in the thymus, the mesenteric lymph node, and peripheral blood, both cell types were highly radiosensitive. No repair processes could be detected with respect to lymphocyte killing, since reducing the dose rate or fractionating the dose resulted in no reduction in cell destruction.

Splenic B cells that remained intact 3 days after doses of 600 R or larger were impaired in their ability to form caps, whereas T cells capped normally. Spleen cells that were viable 3 days after irradiation bore latent radiation damage that was manifested as a reduced ability to survive and generate plaque-forming cells in vitro. Attempts to separately assess irradiated T and B cell immunocompetence in vitro suggested that at doses of 300 R and below, surviving B cells were more impaired than T cells. After a 600-R dose, neither cell type appeared to be functional.

Frome, E. L., and DuFrain, R. J.
Estimation of the amount of exposure to an environmental clastogen using human cytogenetic dosimetry. Davis, H. T.; Prairie, R. R.; and Truett, T., eds.
1978 DOE Statistical Symposium; 1979. Albuquerque, New Mexico. 169-192. US DOE CONF-781108.

Cytogenetic analysis of human chromosomes for aberrations is used to assess potential health effects of environmental toxicants. In some situations, a dose-response curve can be derived to quantify the relationship between the amount of exposure to a specific clastogen and the yield of chromosome aberrations. A procedure is described to obtain point and interval estimates of the exposure for an unknown dose. We assume that the dose-response curve is a linear or quadratic polynomial that passes through the origin and that chromosome aberration yields for both the unknown exposure and the dose-response curve follow the Poisson distribution. Two numerical examples that involve accidental exposure to nuclear radiation are presented to illustrate the methods.

1028428

Gengozian, N., and Barton, B. A.
Isoimmunizations among marmosets to identify leucocyte antigens.
J. Med. Primatol. 8:113-121; 1979.

Saguinus fuscicollis ssp., *Saguinus oedipus oedipus*, and *Saguinus nigricollis* marmosets were immunized with white blood cells from *Saguinus fuscicollis illigeri* marmosets. Of the three genetic donor-host combinations attempted, only an interspecies immunization of *S. o. oedipus* marmosets resulted in antibody formation that was detectable by agglutination or cytotoxicity tests. Analysis of one of the antisera with a panel of white blood cells from *S. f. illigeri* marmosets indicated varying specificities, reactions ranging from strong to completely negative. That the serum was detecting genotypic as well as phenotypic markers was suggested by the similarity of reactions of white blood cells from co-twin pairs and in preliminary testing of individual families.

Gengozian, N.; Batson, J. S.; and Smith, T. A.
Breeding of tamarins (*Saguinus* ssp.) in the laboratory. Kleinman, D., ed.
Biology and Conservation of the Callitrichidae, a Symposium; 1975. Washington, D.C.: Smithsonian Institution Press; 1977. 207-213.

Reproductive data from a colony of acclimated and laboratory-bred *Saguinus fuscicollis* ssp. are presented. Information pertaining to the following aspects of breeding has been included: time in residence of imported animals until first pregnancy, a "seasonal" breeding pattern in the laboratory, reproductive performance of imported and laboratory-bred animals and the yearly survival rate of their offspring, interbirth intervals among established breeders, estimation of stages of pregnancy and time of parturition, and twinning frequency. It is concluded that more information on the behavioral characteristics of animals in a captive colony environment relative to that in a more natural setting is required to realize fully the potential already demonstrated in a laboratory whose major research efforts have been directed toward immunology and not reproductive physiology.

Gengozian, N.; Kateley, J. R.; and Nickerson, D. A.
Marmoset species variation in the humoral antibody response: in vivo and in vitro studies.
Immunology 35:549-558; 1978.

A comparison of the in vivo and in vitro antibody response capabilities of two marmoset species, *Saguinus fuscicollis* and *Saguinus oedipus oedipus*, revealed the former to be superior in elaborating humoral antibody. In vivo challenges with *Escherichia coli* lipopolysaccharide and *Salmonella typhi* flagella consistently yielded higher antibody titres in *S. fuscicollis*. Indeed, with lipopolysaccharide antigen, multiple inoculations of *S. o. oedipus* marmosets led ultimately to decrease in antibody formation, in contrast to the anamnestic response of *S. fuscicollis*. This species differential in immune competence was also suggested in the in vitro stimulation of peripheral blood leucocytes and spleen cells with sheep red blood cells. None of 55 *S. o. oedipus* peripheral blood leucocyte cultures and 49 of 89 (55%) *S. fuscicollis* cultures responded to the test antigen. A similar differential in response to sheep red blood cells was noted with the spleen cells of each species. Although this report contrasts the antibody-forming potential of two marmoset species, a comparison of the immunological response profile of marmosets to those of other laboratory animals challenged with similar antigens suggests these primates may be relatively incompetent. The possible relationship between the haemopoietic chimerism of marmosets and a diminished immune competence is discussed.

Hayes, R. L.; Szymendera, J. J.; and Byrd, B. L.
Effect of food intake on the tissue distribution of gallium-67: concise communication.
J. Nucl. Med. 20:938-940; 1979.

Fasting affects the body retention and tissue distribution of ^{67}Ga in experimental animals. In ^{67}Ga experiments, therefore, a difference in food intake between treated and control animals might result in confusing side effects. We have observed this in irradiation studies. We suggest that a fasting regimen should be imposed in any ^{67}Ga animal study where an alteration in food intake might be experienced in the treated group.

Highfill, R. R., and Phelps, M. E.
Positron tomography.
IEEE Trans. Nucl. Sci. NS-26:1632-1634; 1979.

In positron tomographic imaging, a coincidence detection technique is used to record the number of positron annihilations occurring between detectors placed on opposite sides of the body. These data are processed by a reconstructed algorithm to obtain an image of the distribution of positron-emitting radiopharmaceutical in a cross-sectional slice. Quantitative physiological measurements can be made in vivo and noninvasively by applying multicompartmental models.

Highfill, R. R., and Wieland, B. W.
The medical applications of short-lived, cyclotron-produced radionuclides.
IEEE Trans. Nucl. Sci. NS-26:2220-2222; 1979.

There are two classes of cyclotron-produced radioisotopes used in nuclear medicine today: (1) photon-emitting radionuclides that are used with the gamma camera, and (2) positron-emitting radionuclides that are used with the new positron tomographic scanners. The former have achieved widespread clinical application, while the latter are being used to develop an important new approach to the in vivo study of regional physiology.

Hübner, K. F.; Andrews, G. A.; Buonocore, E.; Hayes, R. L.; Washburn, L. C.; Collmann, I. R.; and Gibbs, W. D.
Carbon-11-labeled amino acids for the rectilinear and positron tomographic imaging of the human pancreas.
J. Nucl. Med. 20:507-513; 1979.

Modification of the Bücherer-Strecker amino acid synthesis facilitated the production of DL-[^{11}C] tryptophan and DL-[^{11}C] valine for clinical trials in patients with proven or suspected pancreatic disease. Examples of rectilinear scans and tomographic images of the pancreas are presented in this initial paper. Positron computed tomography was done with

1028429

the ORTEC ECAT$_{TM}$ scanning system. Rapid localization of the ^{11}C-labeled amino acids and fast clearance from the plasma permit almost immediate examination following intravenous injection. Illustrative images include the normal pancreas, pancreatitis, and pancreatic carcinoma. The use of positron tomography with ^{11}C-labeled DL-tryptophan and DL-valine appears to offer a new and promising diagnostic modality for the detection and study of pancreatic diseases.

Lambremont, E. N, and Wykle, R. L.
Wax synthesis by an enzyme system from the honey bee.
Comp. Biochem. Physiol. 63*B*:131-135; 1979.

A cell-free system prepared from the worker honey bee and containing enzymes from active wax glands is described. The system readily incorporated a long-chain fatty alcohol, [1-^{3}H]-tetracosanol (24:0), into wax esters. About 16% of the label is recovered in the wax ester fraction after 1 hr of incubation at 37°C. ATP, CoA, Mg^{2+}, and a pH of 7.1 are required for maximum activity. The fatty acid component of the wax ester is derived from the endogenous lipids of the enzyme preparation. The wax-synthesizing enzyme is not highly specific for the longer chain fatty alcohols, since hexadecanol (16:0) was also incorporated into wax by the system. The system is highly sensitive to detergents. The findings indicate the monoester wax component of beeswax is biosynthesized by the reaction of a long-chain alcohol, such as tetracosanol, with a fatty acyl-CoA.

Lawless, D.; Brown, D. H.; Hübner, K. F.; Colyer, S. P.; Carlton, J. E.; and Hayes, R. L.
Isolation and partial characterization of a ^{67}Ga-binding glycoprotein from Morris 5123C rat hepatoma.
Cancer Res. 38:4440-4444; 1978.

A glycoprotein, particularly high in tumors, has been extracted from Morris 5123C rat hepatomas and purified. The compound constitutes a major binding component for ^{67}Ga in this hepatoma. It has a molecular weight of approximately 45,000. The molecular weight was determined by sodium dodecyl sulfate:polyacrylamide gel electrophoresis and by Sephadex G-200 superfine gel filtration. The steps involved in its extraction and purification include ultrafiltration, gel filtration through Sephadex G-200 superfine, ion-exchange chromatography on diethylaminoethyl Sephadex A-50, and hydroxylapatite chromatography. The homogeneity of the compound was established by gel electrophoresis. The NH_2-terminal residue, the percentage of nitrogen, the nonamino carbohydrate content, and the amino acid composition are reported.

Lee, T-c.
Characterization of fatty alcohol:NAD$^+$ oxidoreductase from rat liver.
J. Biol. Chem. 254:2892-2896; 1979.

Long-chain fatty alcohols are known to be rapidly oxidized to the corresponding fatty acids in normal mammalian tissues in vivo. However, the enzyme system responsible for the reaction has not been well documented. We have demonstrated the conversion of [1-^{14}C]hexadecanol to [1-^{14}C]hexadecanoic acid using a cell-free system from rat liver. The subcellular distribution of this enzyme system parallels that of microsomal marker enzymes. The enzyme shows an absolute requirement for NAD$^+$ (2 to 10 mM) and had a pH optimum of 8.4. Neither NADP$^+$, NADH, nor NADPH can substitute for NAD$^+$ as a cofactor. The reaction does not require oxygen. Albumin (0.5 mg/ml of incubation mixture) increases the rate of reaction. The enzymic activity is not affected by including pyrazole (an inhibitor of alcohol dehydrogenase), sodium azide (an inhibitor of catalase), or AMP, ATP, and quinacrine (analogs or inhibitors of flavin coenzymes) in the incubation mixture. The apparent K_m for hexadecanol is 0.67 μM and the V_{max} is 5.98 nmol/min/mg of protein. The reaction product was identified as the fatty acid by using both thin-layer and gas-liquid chromatography. Fatty aldehyde was also isolated and identified from the reaction mixture. Adding semicarbazide, a trapping agent for aldehydes, to the incubation system increased the amount of fatty aldehyde produced with a concomitant decrease in the formation of fatty acid. This documents that aldehyde is an intermediate in the reaction. This oxidoreductase is found in most tissues but has the highest activity in liver.

Littlefield, L. G., and Joiner, E. E.
Cytogenetic follow-up studies in six radiation accident victims (16 and 17 years postexposure).
Late Biological Effects of Ionizing Radiation, Proceedings of a Symposium; Vol. 1; 1978. Vienna, Austria. International Atomic Energy Agency; 1978. 297-308.

Detailed cytogenetic evaluations were conducted in cultured lymphocytes from six men accidentally exposed to fission neutron and gamma radiation in 1958 (dose range 22.8 to 365 rads). Two-day lymphocyte cultures stimulated with phytohaemagglutinin (PHA) and 7-day cultures stimulated with pokeweed mitogen (PWM) were initiated on all six men for routine microscopic and karyotypic analysis. PHA-stimulated cultures from the two men with the highest exposures were also evaluated using Giemsa-banding procedures. Approximately 9% of the metaphases in the PHA cultures and 11% of the metaphases in the PWM cultures were found to have residual radiation-induced aberrations (symmetrical and asymmetrical exchanges and deletions). In both the PHA and PWM cultures, the highest frequencies of lesions were observed in preparations from three men with the highest radiation exposures. Likewise, in all cultures, most lesions were of the "stable" type (abnormal monocentric chromosomes including translocations, inversions, and deletions without fragments). Among the abnormal metaphases from the various PHA cultures, five possible clones were identified (two or more cells having apparently identical lesions), whereas no stemlines were detected in the PWM cultures. The frequency of stable lesions in 92 banded metaphases from the two men with the highest exposures did not differ significantly from the frequency of lesions detected in 300 metaphases evaluated with routine staining and karyotypic procedures. These data suggest that radiation-damaged lymphocytes responsive to mitogenic stimulation by PHA and PWM may survive for many years following exposure, that the frequency of lymphocytes with persistent aberrations can be roughly correlated with dose, and that in some instances cells bearing "stable" radiation-induced lesions may propagate in vivo. The findings also show that banding procedures, compared with routine microscopic and karyotypic methods, do not significantly improve the rate of detection of symmetrical lesions in lymphocytes from irradiated persons.

1028430

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes exposed to ionizing radiation during G_0.
Radiat. Res. 78:514-521; 1979.

Several authors have reported small, but statistically significant, increases in sister-chromatid exchanges in mammalian cells exposed to low-LET radiation during culture in the presence of [^{3}H]thymidine or bromodeoxyuridine. To determine whether ionizing radiation also induces lesions in the unsubstituted DNA of G_0 lymphocytes that lead to sister-chromatid exchanges in second division metaphases, we exposed human lymphocytes to 150 or 300 R ^{60}Co-γ radiation before culture in the presence of phytohemagglutinin and bromodeoxyuridine. Increased frequencies of sister-chromatid exchanges were not observed in the irradiated cultures, either in second division metaphases having ring and dicentric chromosomes or those that did not have detectable chromosome aberrations. These findings suggest that increased frequencies of sister-chromatid exchanges would not be observed in cultured lymphocytes from persons with in vivo exposures to radiation. The findings also raise questions regarding the efficacy of ionizing radiation in inducing long-lived lesions in native DNA that can subsequently be expressed as sister-chromatid exchanges.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. M.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes exposed during G_0 to four classes of DNA-damaging chemicals.
Mutat. Res. 67:259-269; 1979.

Human lymphocytes were treated before mitogenic stimulation with varying concentrations of six cytostatic drugs representing four classes of DNA-damaging chemicals. Afterwards, the cells were washed to remove residual chemicals and were cultured in the presence of bromodeoxyuridine for analysis of sister-chromatid exchanges. A dose-related increase in sister-chromatid exchanges was observed in cells exposed during G_0 to the alkylating chemicals mitomycin C, chlorambucil, and thiotepa, while significant increases in sister-chromatid exchanges were not noted in cultures exposed to methotrexate, cytarabine, or bleomycin. These findings suggest that not all classes of clastogenic chemicals, which induce sister-chromatid exchanges in proliferative cells substituted with bromodeoxyuridine, are capable of inducing long-lived lesions in the DNA of G_0 lymphocytes that can lead to sister-chromatid exchange formation.

Moore, C.; Lee, T-c.; Stephens, N.; and Snyder, F.
Enzyme activities in lipid-modified membranes of rat livers.
Biochim. Biophys. Acta 531:125-130; 1978.

The unnatural amino alcohol, *N*-isopropylethanolamine, is incorporated into phospholipids of various membrane fractions of rat liver after it is injected intraperitoneally. When this base analog was incorporated into the phospholipids of plasma membranes, the activity of 5′-nucleotidase in cell-free homogenates and in purified plasma membranes decreased by approximately 20 to 50% of that in the control preparations. Arrhenius plots for 5′-nucleotidase in both the control and isopropylethanolamine-treated plasma membranes had break points at the same temperatures. The enzymes from both control and isopropylethanolamine-treated membranes had identical apparent Michaelis constants but different maximum velocities. Removing phospholipids from control and isopropylethanolamine-treated plasma membrane preparations by phospholipase C treatment did not affect their 5′-nucleotidase activities. In addition, incubation of control membranes in the presence of free isopropylethanolamine (1-10 mM) did not alter the 5′-nucleotidase activity. Collectively, these data indicate the lowered 5′-nucleotidase activity in the isopropylethanolamine-treated membranes is due to a decreased amount of the enzyme and cannot be explained by the modification of the phospholipids within the membrane where the enzyme resides. Three other membrane-bound enzyme activities [NADH:cytochrome *c* reductase (EC 1.6.99.3), NADPH:cytochrome *c* reductase (EC 1.6.2.4), and succinic acid dehydrogenase (EC 1.3.99.1)] were not affected by the isopropylethanolamine treatment.

Polednak, A. P.
Age changes in differential leukocyte count among female adults.
Hum. Biol. 50:301-311; 1978. Work performed at Argonne National Laboratory, Argonne, Illinois.

The relationship between age and differential leukocyte count (total white cells, polymorphonuclears, lymphocytes, eosinophils, and monocytes) was assessed in 219 women between 45 and 85 years old who were examined clinically between 1954-1975. Excluded from the study were those women who had died. Cross-sectional data indicated statistically significant correlations (Spearman r) between age and number of lymphocytes ($r = -0.14$, $P < 0.05$), eosinophils ($r = +0.28$, $P = 0.001$), and monocytes ($r = +0.25$, $P = 0.001$), with little change in total white cell count ($r = -0.02$, $P > 0.80$). Longitudinal data on 31 women examined at age 50 to 59 and again at age 65 to 74 tended to confirm these changes. Exclusion of women from the original sample with various chronic diseases and drug allergies had little effect on the Spearman r's. Partial correlation coefficients, controlling for age, were calculated between leukocyte counts and height, weight, and the weight/height ratio. Only the r's between monocyte count and weight ($r = -0.23$, $P < 0.01$) and weight: height ($r = -0.21$, $P < 0.01$) were statistically significant.

Polednak, A. P.
Long-term effects of radium exposure in female dial workers: liver function and liver disease.
Environ. Res. 18:454-465; 1979. Work performed at Argonne National Laboratory, Argonne, Illinois.

The question of the long-term effects of α-emitting radionuclides on the liver is of considerable interest. Liver function test results and data on the prevalence of, and mortality from, diseases of the liver and biliary tract were examined among women who were first employed before 1930 in the U.S. radium watch-dial painting industry and who had a radium body-burden measurement while living (1958–1976). By using both univariate and multivariate statistical analyses, I found little evidence for a relationship between radium intake dose (initial systemic burden in microcuries) and serum levels of albumin, total bilirubin, glutamic oxaloacetic transaminase, or cholesterol in 142 long-term survivors. The mean serum glutamic oxaloacetic transaminase level was significantly higher in the highest intake dose (> 50 μCi) than in the lower

1028431

intake dose groups, suggesting the need for continued clinical follow-up. Among 264 women with a serum alkaline phosphatase determination, a lack of significant association with intake dose was noted. The prevalence of diagnosed diseases of the liver or biliary tract (i.e., cirrhosis, infectious hepatitis, and cholecystitis) was not significantly related to the intake dose level in 683 women, nor were the observed numbers of deaths from cirrhosis or cancer of the liver significantly increased relative to the U.S. white female population. The findings were discussed in terms of estimated absorbed (rad) doses and in relation to the findings of studies on other radiation-exposed human populations.

Porcella, R. W.; Swartzendruber, D. C.; and Godbold, J. H., Jr.
A morphometric study quantifying the mucous content of proliferating colonic epithelial cells.
Am. J. Anat. 155:103-110; 1979.

We report a quantification of the maximum musous accumulation in proliferating rat colonic epithelial cells. The proliferative potential was determined by radioautographic study of 1-hr pulse exposures to titrated thymidine; mucous content was determined by periodic acid-Schiff staining. We examined 55 labeled mucous cells in 0.5- to 1-μm serial sections. The maximum thecal and nuclear profiles of these cells were photographed and their surface areas were determined utilizing a coordinate sensor. The data were expressed as a theca-to-nucleus ratio. The maximum theca-to-nucleus ratio for a labeled mucous cell was 3.0. We performed a similar analysis on 22 unlabeled mucous cells from upper crypt regions and surface epithelium to derive the range of theca-to-nucleus ratios for terminally differentiated mature mucous cells. The range of theca-to-nucleus ratios from these cells was from 4.8 to 16.4. Our study shows that proliferative potential of mucous cells is determined by the interrelationship between mucous accumulation and nuclear size.

Richter, C. B., and Buyckmichi, N.
Squamous-cell carcinoma of the epidermis in an aged white-lipped tamarin (*Saguinus fuscicollis leucogenys* Gray).
Vet. Pathol. 16:263-265; 1979.

Invasive squamous-cell carcinoma of the epidermis ventral to the intertragic notch of the left eye was observed in an aged female white-lipped tamarin. The neoplasm exhibited rapid growth but had not metastasized at the time of her death. Clinically, she exhibited left facial palsy caused by encroachment of the tumor on the left facial nerve. Squamous-cell carcinoma of the head and neck region are common in man and frequently more malignant than those arising elsewhere on the body. This female was a colony resident for 13 years and 8 months, during which time she had 33 offspring.

Rundo, J.; Polednak, A. P.; Brues, A. M.; Lucas, H. F., Jr.; Patten, B. C.; Rowland, R. E.; and Stehney, A. F.
A study of radioactivity and health status of former thorium workers.
Environ. Res. 18: 94–100; 1979. Work performed at Argonne National Laboratory, Argonne, Illinois.

Studies have been started on the late effects of thorium on the health of 4316 persons (80% are males) who worked at a thorium refinery during the period from the mid-1930s to 1973. The population and the study are described briefly, as are the methods and results to date of measurements of radioactivity in vivo and in the exhaled breath. Of 46 men examined so far, all but 3 had readily measurable amounts of thoron in the breath, but there was little correlation between the amount of activity in the breath and the ^{212}Bi content of the chest.

Scott, C. C.; Heckman, C. A.; Nettesheim, P.; and Snyder, F.
Metabolism of ether-linked glycerolipids in cultures of normal and neoplastic rat respiratory tract epithelium.
Cancer Res. 39:207-214; 1979.

To study the mechanism of ether lipid accumulation, which is characteristic of many tumors, we have compared the metabolism of lipids in primary rat tracheal epithelial cells and in a cell line derived from a benzo(α)pyrene-induced tumor. Growth of the primary epithelial cells in vitro resulted in the accumulation of alkyldiacylglycerols at levels (10% of total lipid) comparable to those found in the tumor cell line, B2-1. This class of ether lipids could not be detected in normal rat tracheal epithelium in vivo. A double isotope labeling method using [^{3}H]- and [^{14}C]palmitic acid was used to study metabolic stability of lipid classes in the cell cultures. Primary cells and B2-1 cells labeled with palmitic acid showed the greatest loss of label from triacylglycerols and free fatty acids during incubation in unlabeled media. A slight loss of label from the ester linkages of the alkyldiacylglycerols was observed in the primary epithelial cells, but not the B2-1 cells. No label was lost from the ether linkages of the alkyldiacylglycerols of either the B2-1 or primary cells. With the exception of diradylglycerophosphocholine, phospholipids of primary and B2-1 cells labeled with palmitic acid accumulated label during incubation in unlabeled media. Similar results were observed with the B2-1 cells using labeled glycerol as the lipid precursor. The results demonstrate that alkyldiacylglycerols can be induced in nontumorigenic cells by growth in culture and can thus provide a useful model for studying the regulation of ether lipid metabolism.

Shivers, C. A.; Gengozian, N.; Franklin, S.; and McLaughlin, C. L.
Antigenic cross-reactivity between human and marmoset zonae pellucidae, a potential target for immunocontraception.
J. Med. Primatol. 7:242-248; 1978.

Cross-reactivity between marmoset, chimpanzee, human, and pig zona pellucida antigens was demonstrated by immunofluorescence and zona precipitation. In marmosets, antizona antibody prevented sperm attachment to eggs in vitro, and the antibody could be detected on zonae of ovarian oocytes following passive immunization. Use of the marmoset as an animal model in testing the feasibility of the zona approach to immunocontraception is discussed.

Tankersley, W. G.; Richter, C. B.; and Batson, J. S.
Therapy of filariasis in tamarins.
Lab. Anim. Sci. 29:107-110; 1979.

A regimen of thiaectarsamide sodium (0.22 ml/kg twice daily for 2 days) plus levanmisole phosphate (11 mg/kg/day

1028432

for 10 days) was effective in eliminating unidentified microfilariae from the blood in seven of eight tamarins. No serious adverse effects resulted from the treatment. All of the animals were initially freed of circulating microfilariae after treatment, and five have remained microfilaria-negative for 1 year. Two of the tamarins died of causes unrelated to filariasis and were microfilaria-negative before death. One tamarin remained microfilaria-positive after two courses of this treatment.

Tyndall, R. L.; Clapp, N. K.; Davidson, K. A.; Colyer, S. P.; and Burtis, C. A.
Effects of carcinogenic and noncarcinogenic chemicals on plasma esterases in BALB/c mice.
Chem.-Biol. Interact. 23:159-169; 1978.

Esterase profiles of plasma from female BALB/c mice treated with a variety of carcinogenic and weakly or noncarcinogenic chemicals were analyzed. Mice treated with the potent carcinogens diethylnitrosamine, dinitrosopiperazine, dipropylnitrosamine, dimethylhydrazine, urethane, and dimethyldinitrosopiperazine had similarly altered plasma esterase profiles after 7 days' exposure to the chemicals. The alterations consisted of increased activity in four esterase bands. The increased activity persisted in some of the bands after cessation of carcinogen exposure. Exposure to high concentrations of the weakly or noncarcinogenic compounds nitrosohydroxyproline, nitrosomethoxymethylamine, nitroso-4 methylpiperazine, nitroso-2,6 dimethylpiperidine, and ethyl methanesulfonate caused no obvious plasma esterase alterations. Ingestion of carbon tetrachloride resulted in increased activity in one esterase band with concomitant decrease in a second band. Analysis of serum from test mice for levels of serum glutamic oxaloacetic transaminase, alkaline phosphatase, lactate dehydrogenase-lactate substrate, and *D*-gammaglutamyl transpeptidase did not differentiate between mice treated with selected carcinogens and those treated with noncarcinogens and/or carbon tetrachloride.

Voelker, D. R., and Snyder, F.
Subcellular site and mechanism of synthesis of disaturated phosphatidylcholine in alveolar type II cell adenomas.
J. Biol. Chem. 254:8628-8633; 1979.

The site of synthesis of 1,2-disaturated-(diacyl)-*sn*-glycero-3-phosphocholine (Sat_2PC) in mouse alveolar type II cell adenomas has been studied by conducting pulse-chase experiments. Isolation of microsomal and lamellar body fractions from adenomas after a 20-min pulse with [*methyl*-^{3}H] choline demonstrates that Sat_2PC first appears in the microsomal fraction and, after a short lag, subsequently appears in the lamellar body fraction. The kinetics of labeling of Sat_2PC are consistent with the microsomal membranes functioning as the cellular site of synthesis for this pulmonary surfactant phospholipid. Short-term labeling experiments with [9,10-^{3}H]-palmitate demonstrate that this fatty acid is incorporated into the *sn*-2 position of Sat_2PC at a faster rate than its incorporation into the *sn*-1 position. This finding indicates that the synthesis of Sat_2PC occurs by a deacylation-reacylation mechanism.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and Butler, T. A.
DL-[Carboxyl-^{11}C] tryptophan, a potential agent for pancreatic imaging: production and preclinical investigations.
J. Nucl. Med. 20:857-864; 1979.

In animal studies, DL-[carboxyl-^{14}C] tryptophan showed a high specificity for the pancreas, which suggested the potential of DL-[carboxyl-^{11}C] tryptophan for clinical pancreatic imaging. The blood clearance and tissue uptake of the amino acid were very rapid, and no carrier effect was observed through a dose of 5 mg/kg. None of three transplanted hamster pancreatic adenocarcinomas we studied showed a selective uptake of DL-[carboxyl-^{14}C] tryptophan by the tumor, and none of the three enzymatic regimens investigated gave significant enhancement of the pancreatic specificity. Commercial L-[carboxyl-^{14}C] tryptophan gave slightly better pancreatic specificity than the analogous racemic compound, but without enough improvement to warrant attempts at optical resolution. DL-[Carboxyl-^{11}C] tryptophan was synthesized in amounts up to 325 mCi using a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis. Yields ranged from 30 to 60%, and 40 min were required for synthesis and chromatographic purification. DL-[Carboxyl-^{11}C] tryptophan thus appears to have significant potential as a clinical pancreas-imaging agent, particularly when used in conjunction with positron computerized transaxial tomography.

Wieland, B. W.; Highfill, R. R.; and King, P. H.
Proton accelerator targets for the production of ^{11}C, ^{13}N, ^{15}O, and ^{18}F.
IEEE Trans. Nucl. Sci. NS-26:1713-1717; 1979.

The p,n out reactions on enriched stable isotope targets offer the potential of producing high yields of ^{11}C, ^{13}N, ^{15}O, and ^{18}F at low energies with an economical small proton accelerator. To approach this goal, it will be necessary to develop techniques allowing efficient recovery of activity in chemical forms appropriate for radiopharmaceutical synthesis. Another important consideration will be the design and testing of beam windows allowing targets to be operated at high beam currents (and high pressures, if necessary) without imposing unreasonable energy attenuation of the beam. With this information available, the performance of all the proton and deuteron target systems should be compared. By combining the resulting energy and beam current requirements with radiopharmaceutical production needs, the problems of optimum accelerator specifications can be addressed.

Wykle, R. L., and Schremmer, J. M.
Biosynthesis of plasmalogens by the microsomal fraction of Fischer R-3259 sarcoma. Influence of specific 2-acyl chains on the desaturation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine.
Biochemistry 18:3512-3517; 1979.

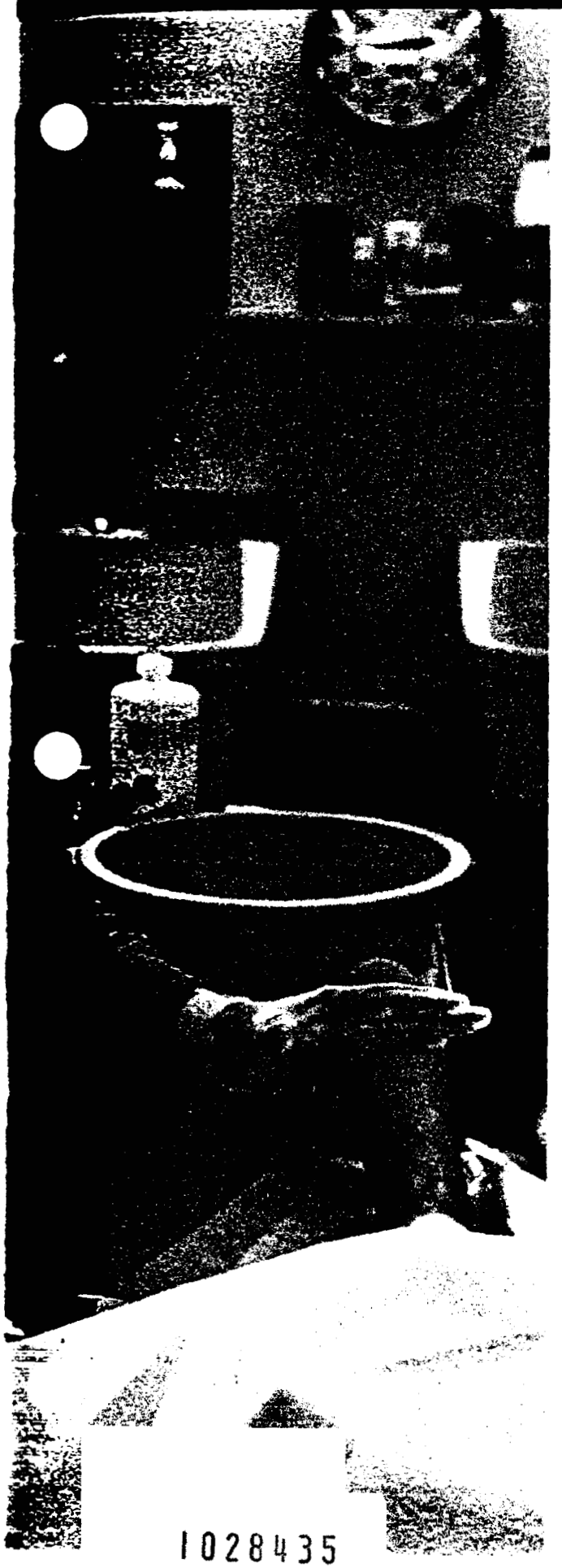
In the Fischer R-3259 sarcoma, ethanolamine plasmalogens (1-alk-1′-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine) are synthesized from 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (1-alkyl-2-acyl-GPE) by a microsomal desaturase that inserts a Δ1 double bond in the alkyl chain. In the present study, a series of 1-[1-^{14}C] hexadecyl-2-acyl-GPE substrates

1028433

containing specific acyl groups ranging from $C_{2:0}$ to $C_{20:4}$ at the 2 position were prepared and tested as substrates for the microsomal Δ1-alkyl desaturase. The microsomal preparations contained an acyl hydrolase that removed the $C_{2:0}$, $C_{4:0}$, and $C_{7:0}$ acyl groups from the 2 position. By inhibiting the hydrolase with diisopropylfluorophosphate, it was possible to test conversion of the unaltered substrates to plasmalogens. The alkyl desaturase exhibited little discrimination among the specific acyl derivatives tested. The highest rate of desaturation was obtained with 1-[1-^{14}C]hexadecyl-2-acyl-GPE synthesized in situ in the microsomes via acylation of 1-[1-^{14}C]-hexadecyl-GPE; this rate was threefold that observed with exogenously acylated substrates. The 1-[1-^{14}C]hexadecyl-2-acyl-GPE synthesized in situ contained highly unsaturated acyl groups. No selectivity of the desaturase for specific acyl chains was detected when the different molecular species of 1-[1-^{14}C]-alkyl-2-acyl-GPE and 1-[1-^{14}C]alk-1′-enyl-2-acyl-GPE were compared. The short-chain substrates, being more hydrophilic, mimicked the chromatographic behavior of 1-alkyl-GPE, yet they did not resemble the lyso compound in its higher conversion to plasmalogens. Thus, despite their similar R_f values, the packing of the short-chain acyl homologues in the membrane may be quite different from that of the lyso compound. Binding of 1-hexadecyl-2-acyl-GPE and 1-hexadecyl-GPE to microsomal membranes was similar. Detergents stimulated the desaturation of exogenously added 1-hexadecyl-2-acyl-GPE, but the level of activity remained at only one-third that obtained by using 1-hexadecyl-GPE as the precursor. The results suggest that the acylation step positions the substrate in the membrane so that it becomes more accessible to the alkyl desaturase.



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1028435

Training Programs

The Medical and Health Sciences Division participates in a number of training programs in biomedical research. Graduate students from the University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences; the University of Tennessee (Knoxville campus and the Center for the Health Sciences in Memphis); and the University of North Carolina (Chapel Hill) are currently pursuing research for their Ph.D. degrees in the areas of biological chemistry and immunology. Stipends for the competitive fellowships awarded to these students are obtained from the National Cancer Institute training grant (CA-05287) at Oak Ridge National Laboratory, from DOE through university programs at ORAU, and from the Hilton A. Smith Fellowship at the University of Tennessee. Occasionally research grants are a source of support.

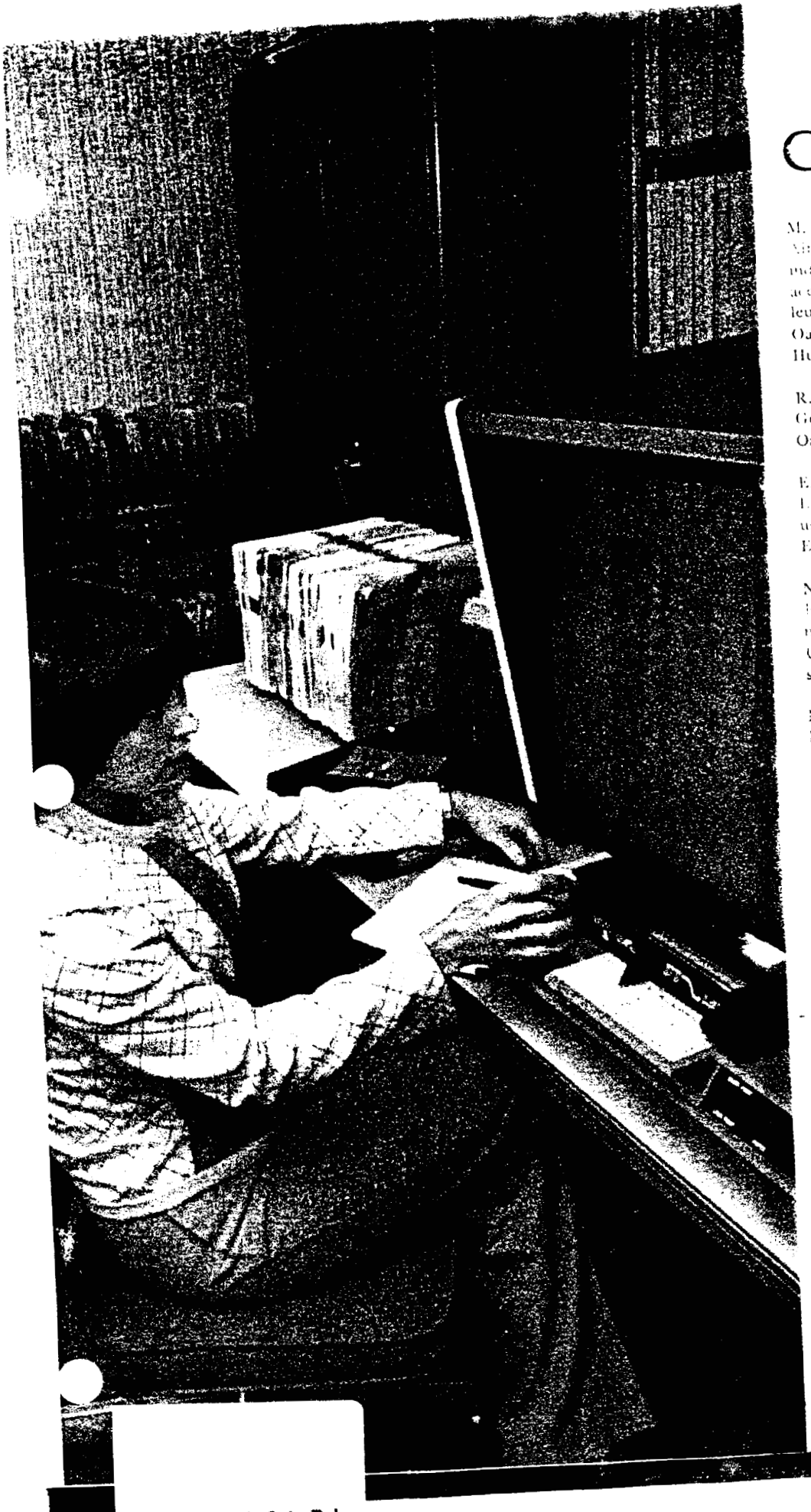
Postdoctoral fellows also contribute to the various research activities of the division. In the past, funds for support of postgraduate training have been awarded to fellows from the Damon Runyon—Walter Winchell Cancer Fund (DRG-127-FT) and the National Cancer Institute Training in Carcinogenesis Research (CA-05296) at Oak Ridge National Laboratory.

Summer research participation sponsored by DOE through university programs at ORAU makes it possible for teachers and undergraduate seniors from colleges and universities throughout the U.S. to partake in the type of laboratory studies described in this report.

The division also provides training experiences to high school students as secretaries and laboratory aides. These opportunities are made possible through cooperation with vocational training programs at area high schools.

Short-term courses in radiation management are offered at regular intervals by REAC/TS to physicians and paramedical personnel. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program.

Some of the division senior staff conduct special courses and lectures at the University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences, and at the University of Tennessee (Knoxville and Memphis). These staff members hold joint appointments at the university and are the advisors to graduate students pursuing Ph.D. research at ORAU.



Collaborative

M. Cabot
Alterations in phospholipid metabolism induced by phorbol-12-myristate 13-acetate in differentiating human myeloid leukemia cells
Oak Ridge National Laboratory, I. Huberman

R. J. DuFrain
Gene mapping the laboratory rabbit
Oak Ridge National Laboratory, P. [illegible]

E. L. Frome
Evaluation of record-linkage methods for use in DOE health and mortality studies
Emory University, B. Blumenstein

N. Gengozian
Fetal hemoglobin synthesis in the marmoset
University of Tennessee Memorial Research Center, J. Fuhr

N. Gengozian
Immunocytotoxicity in the marmoset
University of Tennessee, C. Shivers

N. Gengozian
Radiation sensitivity of cells of the marmoset
Oak Ridge National Laboratory, J. Brown

N. Gengozian
Thrombopoiesis in the marmoset
University of Tennessee Memorial Research Center, T. McDonald

R. Hayes
Collaborative studies in radiopharmaceutical development
Oak Ridge National Laboratory, F. Lamb

R. Hayes
Purification of gallium-binding protein
Fordham University, D. Lawlis

R. Hayes
Synthesis of labeled compounds using organoborane techniques
University of Tennessee, C. Lasche

K. Hübner
Comparative efficiency of [illegible] methods for detecting [illegible] disease

1028436

Research

University of Tennessee Memorial Research Center, E. Buonocore and I. Collmann

K. Hübner
Positron emission tomography in patients with brain tumors
University of North Carolina, C. Partain and J. Scatliff

K. Hübner
Radiation pneumonitis, a model for early detection of pulmonary disease using perfusion and ventilation techniques
University of Tennessee Memorial Research Center, F. Comas and T. Sullivan

G. Littlefield
Cytogenetic dose response kinetics in G-tr[illegible] lymphocytes exposed to low LE[illegible]adiation
Medical College of Georgia, D. Welter

G. Littlefield
Cytogenetic follow-up studies in survivors of the LA-0 and LA-2 accidents
University of Rochester, L. Hempelmann

G. Littlefield
Sister-chromatid exchange analysis in coke oven workers
National Institute of Occupational Safety and Health, G. Taylor

C. Lushbaugh
Diethylenetriaminepentaacetate (DTPA) project
Department of Energy contractors, 40 coinvestigators

C. Lushbaugh
United States Transuranium Registry
Hanford Environmental Health Foundation, B. Breitenstein

A. Polednak
[illegible]ologic studies of thorium workers
Argonne National Laboratory, A. Stehney

C. Richter
Microbiology and pathology of *Klebsiella* infections in marmosets and tamarins
Center for Disease Control, J. Farmer

C. Richter
Pathology of respiratory viruses in mice and the pathology of Sendai virus vaccination in mice
Microbiological Associates, J. Parker

C. Richter
Saturine gout using *Callithrix jacchus* as a model
University of Tennessee Memorial Research Center, W. Farcus

C. Richter
Studies on the skeletal maturation and aging in marmosets and tamarins
University of Tennessee, F. Smith and D. Glassman

F. Snyder
Chemical characterization of an antihypertensive lipid isolated from the kidney medulla
University of Tennessee Center for the Health Sciences, E. Muirhead and L. Byers

F. Snyder
Effect of NO_2 and other toxicants on the lung surfactant system
Oak Ridge National Laboratory, W. Dalbey

F. Snyder
Mass spectrometry of unique lipids
Oak Ridge National Laboratory, W. Rainey

F. Snyder
Nuclear magnetic resonance studies of unique lipids
Oak Ridge National Laboratory, B. Benjamin

F. Snyder
Role of ether lipids in growth, cell differentiation, and neoplasia
Oak Ridge National Laboratory, C. Heckman

F. Snyder
Synthesis of lipid analogs and their precursors
University of North Carolina, C. Piantadosi and K. Ishaq

L. Washburn
Pharmacological studies using ^{11}C-labeled compounds
University of Kentucky, G. Digenis

L. Washburn and K. Hübner
WR-2721 as an adjunct to radiotherapy
University of New Mexico, J. Yuhas

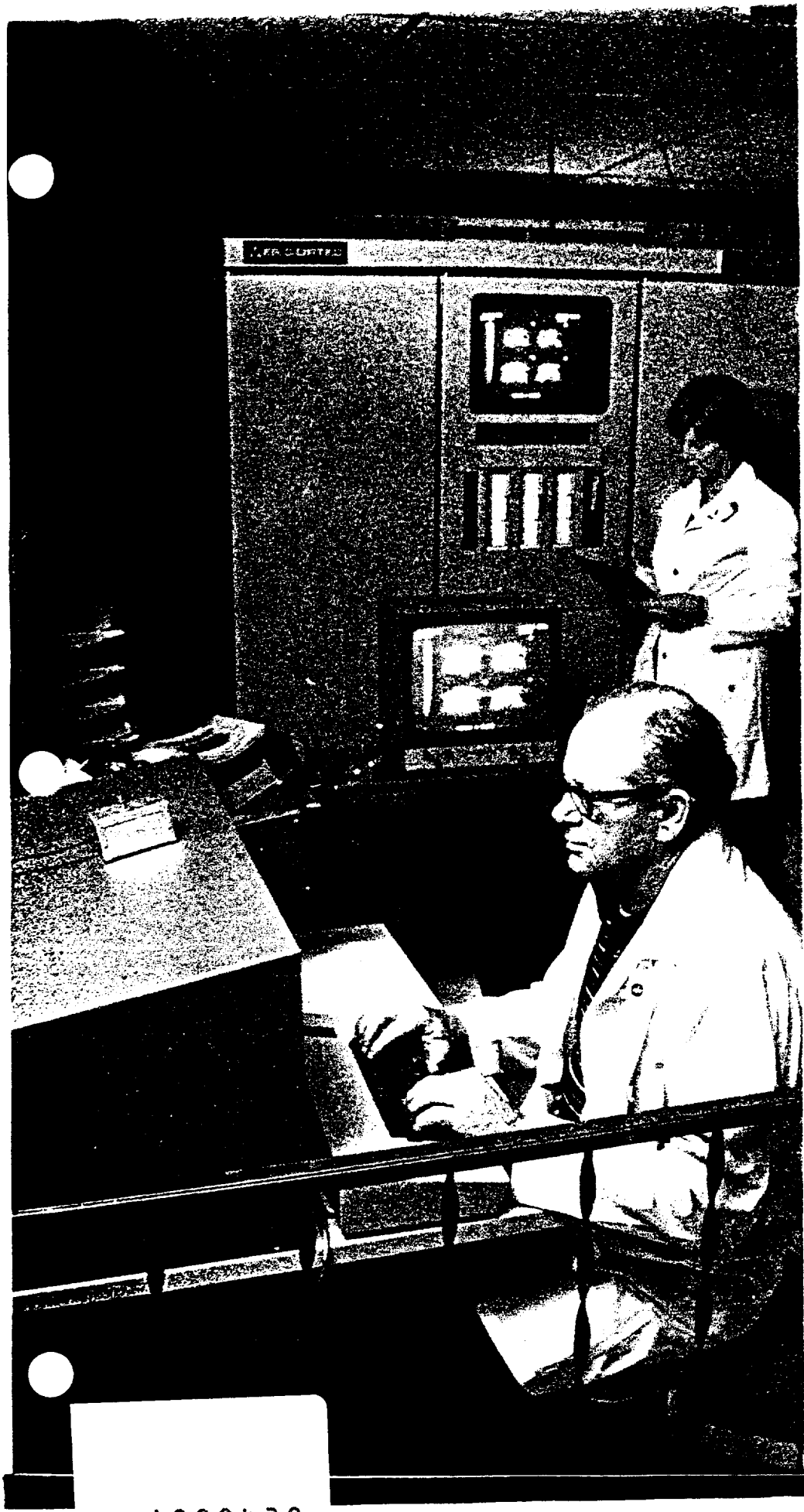
B. Wieland
Design of ^{18}O (p,n) ^{18}F gas targets system for the ORNL EN Tandem Van de Graaff accelerator
Massachusetts Institute of Technology School of Chemical Engineering Practice at Oak Ridge National Laboratory, S. Senkan

B. Wieland
Measurement of the p,n cross sections for production of ^{11}C, ^{13}N, and ^{18}F using the ORNL EN Tandem Van de Graaff accelerator
Oak Ridge National Laboratory, P. Miller and J. Bair

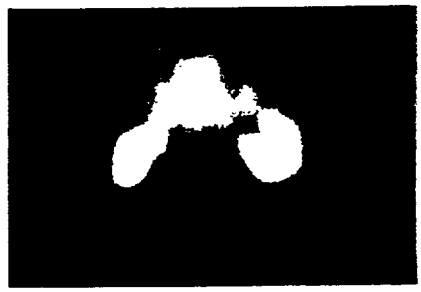
B. Wieland
Research on targetry and automated radiochemistry systems for the production of ^{11}C, ^{13}N, ^{15}O, and ^{18}F
Brookhaven National Laboratory, A. Wolf

B. Wieland and K. Hübner
Development of software for quantitative imaging of gamma emitters and for simulation of accelerator target systems
Vanderbilt University, P. King

1028437







Top: A scan of a normal human pancreas as visualized by the ECAT, in 25 minutes after injection of 22 mCi of 11C-Clorgyline.

Bottom: Tomographic images through the upper abdomen obtained between and 25 minutes after intravenous injection of 5.7 mCi of 11C-Clorgyline in a patient with cancer of the pancreas. The 11C-Clorgyline uptake is localized in the liver and kidneys and in a large mass of normal mass and necrosis in the cancer of the pancreas.

1028438

Grant-Funded Research

R. Arneson
Antioxidants in mouse neuroblastoma.
National Cancer Institute
$18,578* (1 June 1979 to 30 November 1979)

N. Gengozian
"Autoimmune" thrombocytopenia
National Heart, Lung, and Blood Institute
$66,152* (1 July 1979 to 30 June 1980)

N. Gengozian
Development of markers for assessment of damage to the immune system
Environmental Protection Agency
$310,000** (1 October 1978 to 30 September 1979)

N. Gengozian
Immunocontraception in the marmoset
World Health Organization
$12,910* (1 March 1979 to 31 December 1979)

R. L. Hayes
Concentration of radionuclides by malignant neoplasms
National Cancer Institute
$49,676* (1 October 1978 to 30 April 1979)

G. Littlefield
Evaluation on induced cytogenetic lesions in oocytes
National Institute of Child Health and Human Development
$94,523* (1 June 1979 to 31 May 1980)

C. C. Lushbaugh
Biomedical research support grant
National Institutes of Health, Division of Research Resources
$71,710** (1 April 1979 to 31 March 1980)

C. Richter
Marmoset breeding facility
National Institutes of Health, Division of Research Resources
$77,705* (1 September 1978 to 31 August 1979)

R. Ricks
"Responder's Guidebook for Radioactive Materials Transportation Incidents"
Department of Transportation
$18,500** (1 April 1979 to 31 July 1979)

F. Snyder
Ether lipids in cancer: enzyme mechanisms and inhibitors
National Cancer Institute
$39,344* (1 May 1979 to 30 April 1980)

F. Snyder
Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells
American Cancer Society
$53,000** (1 December 1978 to 30 November 1979)

F. Snyder
Surfactant metabolism: alveolar cells and lung disease
National Heart, Lung, and Blood Institute
$32,752* (1 May 1979 to 30 April 1980)

*Direct costs
**Total costs

1028439



Naturally occurring colon carcinoma of the cotton-top tamarin has been transplanted beneath the skin of this athymic nude mouse. The tumor can be seen on the side of the mouse just behind the right forelimb.

A microbiologist holds an athymic nude mouse. Natural immune deficiency renders these mice suitable hosts for transplanted tumors from humans and other animal species.

1028440

Organization and Staff

BIOLOGICAL CHEMISTRY

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Raymond Hayes
Paul King[3]
Heino Luts[3]
John Rafter
Rae Ringinberg[2]
Tan Tan Sun
Lee Washburn
Bruce Wieland

[1] Predoctoral student
[2] Student trainee
[3] Guest scientist
[4] Employment terminated during year
[5] Master's student
[6] Consultant
[7] Fulbright Fellow
[8] Temporary appointment
NOTE: group leaders' names in italics

1028441

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Lois Hodges[8]
Karl Hübner
William Kraemer[4]
C. C. Lushbaugh
Anthony P. Polednak
Robert Ricks
Ann Sipe
Sheryl Wells

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John Crockett
C. C. Lushbaugh
Billie Ryan
Fred Snyder

LIBRARY

Marion Garber
Virginia Gramly

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Willie Barnett[4]
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Robert Carson
James Graham
Cecil Rice
Mack Ruffner
John Suttles
Gregory Weatherspoon
Alfred Webster
Claude Wilson[4]

ELECTRONICS

Maynard Ludwig
William Pope

CUSTODIAL SERVICES

Mark Brewer
Elizabeth Foster
Willie Miller
Sherrill Talley
Gregory Weatherspoon
David Young[8]

[1]Predoctoral student
[2]Student trainee
[3]Guest scientist
[4]Employment terminated during year
[5]Master's student
[6]Consultant
[7]Fulbright Fellow
[8]Temporary appointment
NOTE: group leaders' names in italics

ORAU Council Committee on Medical and Health Sciences – 1979

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Kenneth A. Fry, Texas Woman's University
R. W. Henningson, Clemson University
Paul Hyde, Louisiana State University Medical Center
James U. Lowe, Meharry Medical College
Paul F. Parks, Auburn University, Chairman
John J. Salley, Virginia Commonwealth University
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Advisory Committee for Epidemiology – 1979

Louis Hempelmann, University of Rochester School of Medicine (Consultant)
George B. Hutchison, Harvard University School of Public Health, Chairman
Norton Nelson, New York University Institute of Environmental Medicine
Marvin A. Schneiderman, National Cancer Institute

1028442

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Florida State University
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Tulane University
Tuskegee Institute
Vanderbilt University
Virginia Commonwealth University
University of Virginia
Virginia Polytechnic Institute and State University
West Virginia University
College of William and Mary

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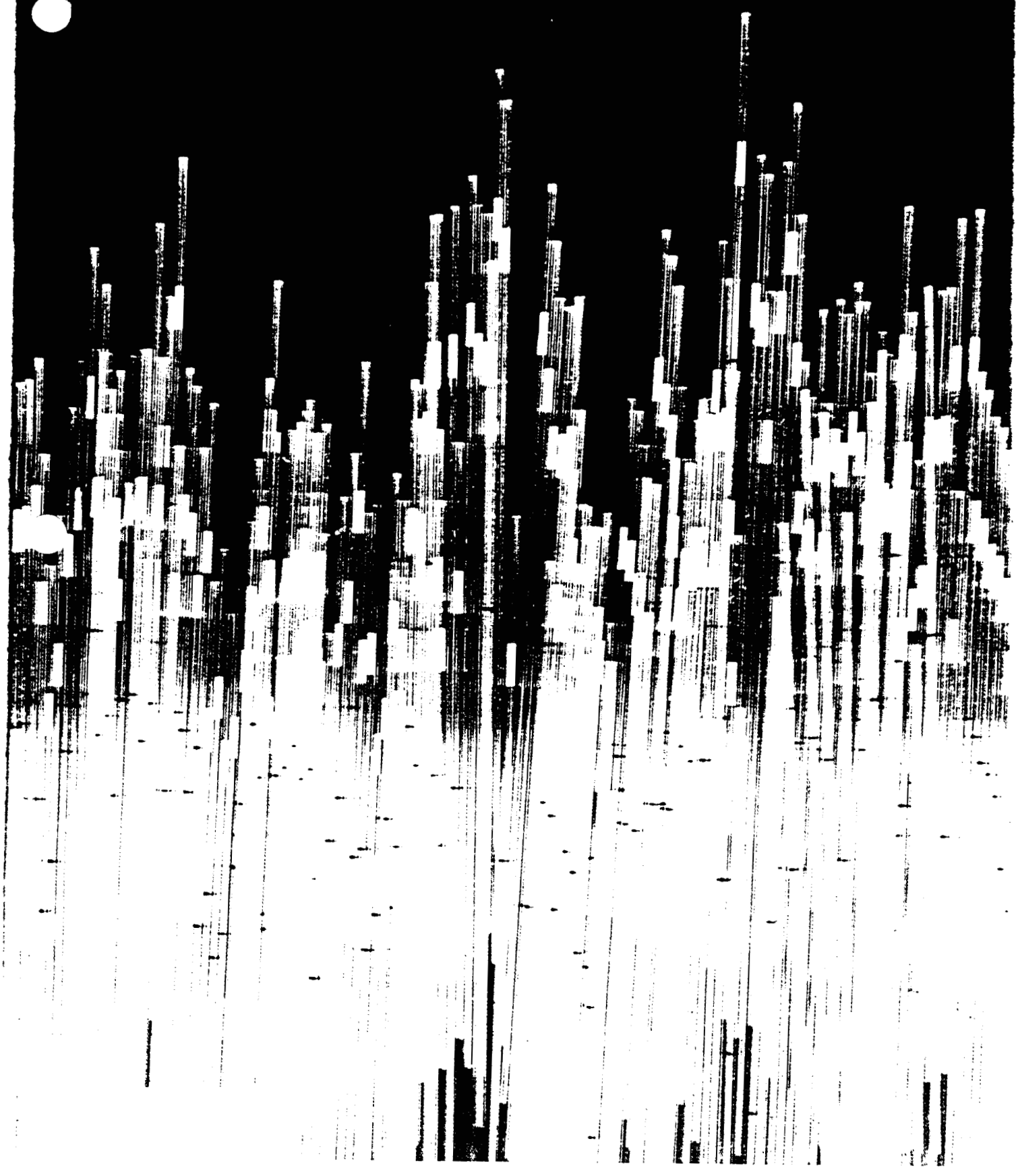
1979-80

1028444

Research
Report
1979-1980

Oak Ridge
Associated
Universities

Medical and
Health Sciences
Division



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The Medical and Health Sciences Division conducts biomedical research and training programs in studies of respiratory and gastrointestinal diseases and cancers related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Immunobiology, biological chemistry, cell biology, radiopharmaceutical chemistry, nuclear medicine, experimental pathology, cytogenetics, and epidemiology are the main research disciplines from which pragmatically targeted task groups are structured to enhance efficiency. Predoctoral and postdoctoral training programs are provided in these areas. Besides newly refurbished laboratory units, the division has two unique facilities: REAC/TS, the Radiation Emergency Assistance Center/Training Site, maintained for the U.S. Department of Energy as a demonstration and training unit for medical preparedness for radiation accidents; and the Marmoset Research Center, which is a source of primates for research in the division and elsewhere in the United States.

Oak Ridge Associated Universities is a private, not-for-profit association of 50 colleges and universities. Established in 1946, it was one of the first university-based, science-related, corporate management groups. It conducts programs of research, education, information, and training for the U.S. Department of Energy and a variety of private and governmental organizations. Oak Ridge Associated Universities is noted for its cooperative programs and for its contributions to the development of science and human resources.

NOTICES

The opinions expressed herein do not necessarily reflect the opinions of the sponsoring institutions of Oak Ridge Associated Universities.

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Available from the National Technical Information Service, U.S. Department of Commerce, Springfield, Virginia 22161. Please direct all price inquiries to NTIS.

Cover:

Biochemical studies of a recently identified biologically active class of ether phospholipids with hypotensive and platelet-activating properties have established the metabolic pathways responsible for the biosynthesis and degradation of this unique lipid.

The regulation of ether-linked lipids that are characteristically high in cancer cells was investigated in animal models and tissue culture systems; the activities of several key enzymes in the metabolic pathway were found to be coordinately controlled.

Title page:

Certain long-chain alkyl amine analogs were shown to have a high affinity and relatively long residence time in the lung; such compounds labeled with carbon-11 show promise as indicators of pulmonary function in studies being pursued by the lung tomography task group.

1028446

Research Report 1979-1980

Oak Ridge Associated Universities

Medical and Health Sciences Division

ORAU-172
October 1980
Oak Ridge, Tennessee 37830

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.



1028447

Table of Contents

Preface 3

Scope of Programs 4
- Cancer and Pulmonary Research Section 4
  - Immune Surveillance of Carcinogens 4
  - Cancer and Pulmonary Biochemistry 5
  - Radiopharmaceutical Design, Synthesis, and Testing 6
  - Pulmonary and Cancer Cell Biology 6
- Interdisciplinary Task Groups 7
  - Marmoset Colon Cancer 7
  - ^{11}C-Lung Tomography 7
  - Benzene Toxicity 8
  - DTPA 8
- Cardiovascular Research Section 8
- Radiation and Nuclear Medicine Section 9
  - Nuclear Medicine 9
  - Radiation Emergency Assistance Center/ Training Site 9
  - Cytogenetics 10
- Occupational Epidemiology 10

Publications 11
- Full-Length Journal Articles 11
- Chapters, Review Articles, and Other Publications 14
- Published Abstracts of Papers Presented at Meetings 16

Published Abstracts of Full-Length Publications 18

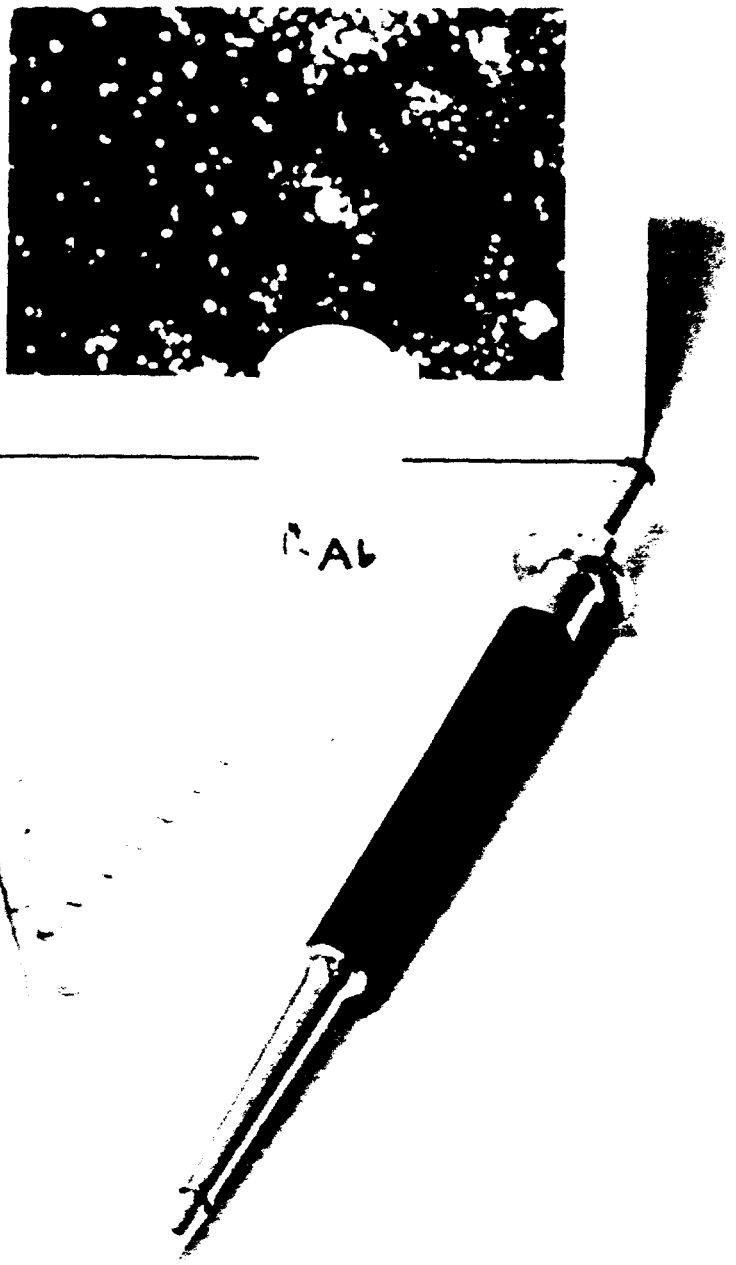
Training Programs 26

Collaborative Research 28

Grant-Funded Research 29

Organization and Staff 30

1028448



In vivo and in vitro studies showed that benzo(α)pyrene can have an adverse effect on T lymphocytes, cells considered to play a major role in immune surveillance with respect to tumor development.

Preface

The Medical and Health Sciences Division conducts research programs relevant to neoplastic, pulmonary, gastrointestinal, and cardiovascular diseases. Basic biological science, nuclear medicine, and epidemiology provide an integrated approach to solving biomedical problems directly related to occupational medicine and environmental health effects. The central theme of this research is focused on both the mechanisms and risk assessments of diseases caused by accidental exposure to chemical toxicants derived from fossil and synthetic fuels or to radiation.

A major reorganizational change made this past year restructured the division into two branches. The environmental and health sciences branch contains a cancer and pulmonary research section, an interdisciplinary task group section, a cardiovascular research section, and a research support section. The radiation and nuclear medicine branch consists of a radiation and nuclear medicine section and an occupational epidemiology section. In addition, special task groups have been created to provide an interdisciplinary team approach in certain research efforts. Charts reflecting these changes and listing affiliated staff are located at the end of this report.

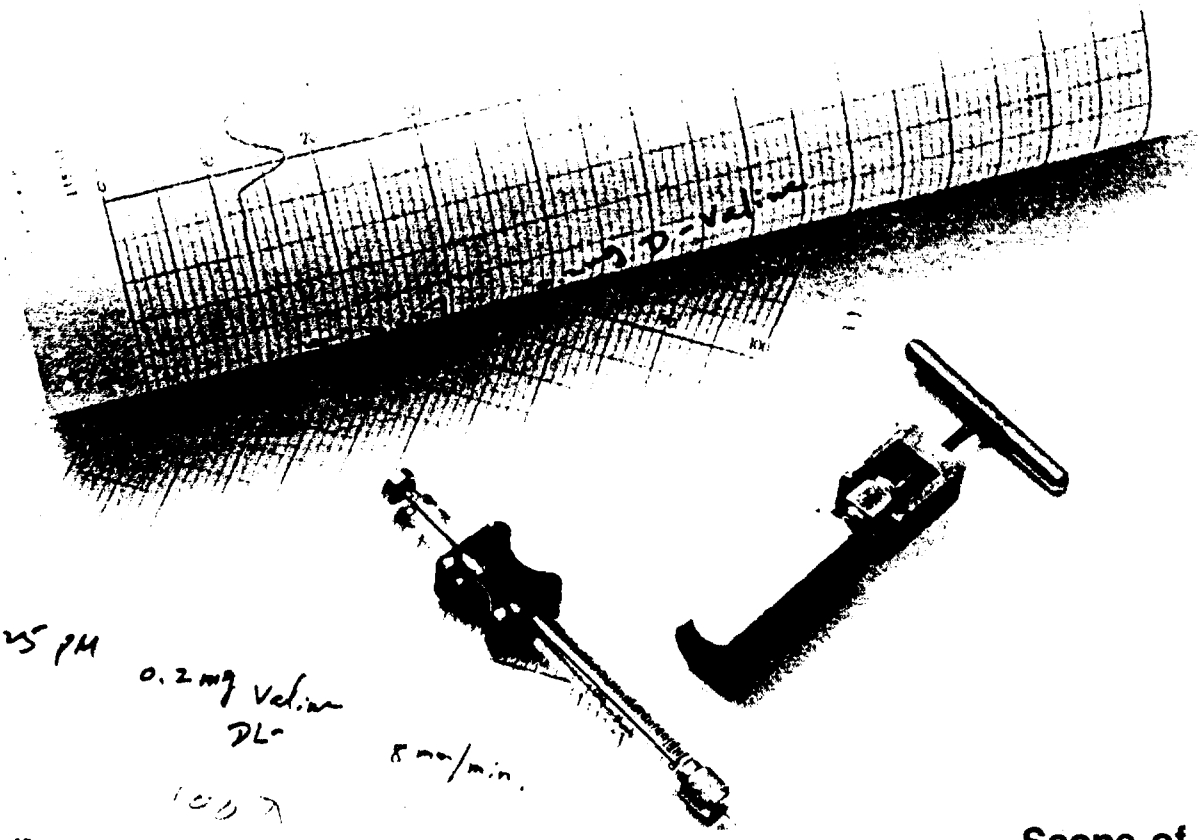
Information included in this booklet summarizes our research results and related activities for the period from October 1, 1979, to September 30, 1980. A more comprehensive description of research results can be obtained from the division by requesting reprints of specific publications.

C. C. Lushbaugh, Chairman

Fred Snyder, Associate Chairman

October 1980

1028449



An important accomplishment in basic radiopharmaceutical studies was the development of a high-pressure liquid chromatographic method for rapid resolution of small quantities of ^{11}C-labeled racemic amino acids (DL-tryptophan, DL-valine, and DL-leucine). A scale-up procedure of this analytical technique is expected to provide clinically useful quantities of these and other amino acids in optically pure form.

Scope of Programs

Cancer and Pulmonary Research Section

Immune Surveillance of Carcinogens

This program studies the effects of environmental pollutants on immunologic processes at the organismal and cellular levels in two animal models—mice and marmosets. The diverse genetic strains of laboratory mice have varying susceptibilities to chemical oncogenic agents, and marmosets have a natural blood chimerism that provides a potentially sensitive system for measuring the effect of chemical pollutants.

1028450

Benzo(α)pyrene, an environmental toxicant, has been studied for its effect on the developing immune system of mice. Treatment of pregnant mice with this carcinogen leads to profound and persisting immunologic incompetence in the offspring. The immune status of mice has been evaluated by assessing humoral antibodies and the ability of T cells to respond to major histocompatibility antigens in mice. The lowered response capability of the T cells may contribute to the increased incidence of tumors found among these animals. Adaptation of an in vitro antibody-forming system to analyze the effects of benzo(α)pyrene at the cellular level has also been accomplished. Primary in vitro antibody formation by normal spleen cells is significantly depressed upon treatment with this chemical at levels that are not toxic to the cells. Application of such an approach to other specific and nonspecific stimulations of immunocompetent cells should lead to a better understanding of the mechanism by which compounds such as benzo(α)pyrene exert their immunosuppressive effects. Preliminary results obtained by using this in vitro approach in lymphocytes isolated from marmosets have indicated a variable but significant effect on cells challenged with plant mitogens and allogeneic histocompatibility antigens.

A new direction of study with environmental toxicants is anticipated in the coming year. Through the support of the U.S. Environmental Protection Agency, studies will begin in inhalation toxicology with benzene as the toxicant. The hemopoietic toxicity of benzene has been recognized for many years, but surprisingly little is known about the effects of this solvent on immune processes. The projected studies will entail low-level, long-term exposures of mice and marmosets to benzene vapors; humoral and cell-mediated immune capabilities will then be monitored as a function of exposure time.

Cancer and Pulmonary Biochemistry

The major thrust of this research is to investigate the structural, functional, and metabolic roles of membrane lipids in cancer and pulmonary diseases—especially as they relate to toxicants found in the environment.

The cancer-related projects are designed to identify the enzymatic control points and factors responsible for the regulation of ether-linked glycerolipids and related membrane lipids that are closely associated with malignancies. Recent results have shown that the level of ether-linked lipids in tissues is regulated in a coordinated manner by increased activity of the biosynthetic enzymes and decreased activity of the enzymes responsible for their metabolism. Use of a fluorescent probe has demonstrated that the presence of an O-alkyl ether linkage can cause significant alterations in the phase transition temperature of lipids in model systems. The same technique will be used to determine whether such physical properties can affect the fluidity of membranes that have a high ether-lipid content in L-M cells. Studies completed in 1980 with L-M cells have shown that elevated levels of cellular ether lipids can affect certain membrane transport systems (e.g., they decrease choline transport).

The properties of two membrane-bound enzymes that are important in the regulation of ether-lipid levels have been further characterized. Alkyl synthase, which catalyzes the first step in ether bond formation, is now partially purified; and the kinetic properties of the soluble form have been found to be similar to those of the "membrane-bound" form. Other results have shown that acyl-CoA reductase, which forms the long-chain alcohol precursor of the ether chain from acyl-CoAs, requires lipid for the expression of its activity and is stimulated by a cytosolic protein. Purification of this soluble protein and elucidation of its physiological role are currently under investigation. The reductase catalyzes a two-step reaction, but the two activities have been impossible to resolve as separate proteins.

Ongoing studies of lipid metabolism in the HL-60 line of tumor promoter-differentiated human leukemia cells have shown that phorbol esters stimulate the incorporation of fatty acids into triacylglycerols and cause a reduction in the activity of stearoyl-CoA desaturase. Enzymatic pathways in glycerolipid metabolism are now being investigated in the leukemic cells that are undergoing differentiation towards "normal" lineage. In a comparative study of the lipid composition in mouse C3H10T1/2 cells and an in vitro oncogenically transformed clone, a 1.2-fold increase in the content of the ether lipids (alkyldiacylglycerols) was found in the "neoplastic cells"; this is consistent with earlier results that have described the characteristically elevated levels of ether lipids in cancerous tissues.

Completed experiments with the pulmonary surfactant system in adenoma alveolar type II cells describe the relative contributions of the lysolecithin:lysolecithin transacylase and acyl-CoA:lysolecithin acyltransferase reactions toward the formation of dipalmitoyl phosphatidylcholine, the major surface-active phospholipid in lung tissue. Both pathways were found to contribute significantly to the in vivo synthesis of pulmonary surfactant; however, the relative importance of each appears to depend on the level and nature of the precursors of the disaturated phosphatidylcholine available in the cells. Topographical studies of the enzyme sites responsible for pulmonary surfactant production indicate that a sizable portion of the active center of palmitoyl-CoA: lysolecithin acyltransferase is embedded in the interior of the endoplasmic reticulum membrane.

Toxicant studies during 1980 centered on the effects of ozone and nitrogen dioxide (NO_2) on pulmonary function. Increases in pulmonary surfactant after NO_2 exposure were found to be a more specific measurement of alveolar damage than the extent of hyperplasia as measured by [^{3}H]thymidine uptake. Moreover, the increased level of disaturated phosphatidylcholine appears to depend primarily on the level (ppm) of NO_2 in the air and not on the duration of NO_2 exposure. For example, repeated 1- or 5-hour exposures of rats to NO_2 given 24 hours apart failed to enhance the

1028451

increase in phosphatidylcholine caused by the initial NO_2 exposure. However, if 1-week periods occurred between the NO_2 exposures, the level of surfactant lipid again increased after a subsequent exposure. Recent experiments with rats treated with ozone (0.2 to 2 ppm) have shown that it causes the same response in pulmonary phosphatidylcholine levels as previously observed with NO_2, except that about 10 times less ozone was required to produce the same effect.

Radiopharmaceutical Design, Synthesis, and Testing

The objectives of the preclinical portion of the nuclear medical program are (1) to identify, prepare, and test new radiopharmaceuticals in animal models in order to evaluate their diagnostic potential in human diseases (particularly those that result from, or are intensified by, environmental pollutants); (2) to produce radiopharmaceuticals for clinical trials; and (3) to determine the biochemical mechanisms responsible for the selective localization of radiopharmaceuticals so that even better agents can be developed.

Emphasis is on the development and production of positron-emitting radiopharmaceuticals. Positron decay produces annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions from various angles with a positron-emission computerized tomograph (ECT), it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of such radiopharmaceuticals. The images and information obtained are similar to those resulting from autoradiographic techniques, except that the positron-ECT technique is noninvasive. The images obtained by positron-ECT can reflect differences in tissue function rather than differences in tissue density as do conventional X-ray images. Thus, they can be used to detect metabolic alterations associated with disease processes.

A major effort in this program is focused on ^{11}C-labeled amino acids. A general method was developed for the rapid organic synthesis and purification of racemic mixtures of these materials. Resolution of such racemates is important because the L-enantiomer occurs naturally; therefore, ^{11}C-labeled L-amino acids are required for truly physiological studies. Preliminary studies indicate that many ^{11}C-labeled amino acid racemates can be resolved rapidly and efficiently by high-performance liquid chromatographic techniques. Enzymatic methods for resolution of the racemates are also being investigated in collaboration with Dr. George A. Digenis of the University of Kentucky. ^{11}C-Labeled L-tryptophan and L-valine have shown potential for differential diagnosis of pancreatic disease; and the metabolism of ^{11}C-labeled L-leucine, L-valine, and L-serine are being investigated in brain studies. Properties of ^{11}C-labeled D-leucine have indicated that it could be a useful agent for tumor imaging, particularly in colon cancer.

Results obtained with [^{11}C]α-aminoisobutyric acid suggest that it has possibilities as an agent for assessing the breakdown of the blood-brain barrier. In addition, preliminary experiments with [^{11}C]α-aminoisobutyric acid indicate it is also a good tumor-imaging agent.

In collaboration with Dr. Digenis, [^{11}C]phenethylamine, an amphetamine analog, was synthesized and its pharmacology studied in the brain. Another collaborative project, with Dr. George Kabalka of the University of Tennessee, involves the use of organoborane techniques for incorporating radionuclides into compounds of nuclear medical interest. So far, a novel method has been developed for radioiodination of several important compounds such as estrogens and fatty acids.

Exploration of the mechanisms involved in the uptake of ^{67}Ga has shown that ^{67}Ga apparently enters inflammatory tissues in a protein-bound form similar to that observed for normal tissues. On the other hand, uptake of ^{67}Ga by tumor tissue appears to involve primarily an unbound form of this nuclide. Comparison of ^{169}Yb ($T_{1/2}$ = 32 days) and ^{67}Ga ($T_{1/2}$ = 3.3 days) for detection of inflammatory lesions suggests that ^{169}Yb may be more valuable for the rapid detection of inflammatory lesions. The utility of ^{169}Yb is particularly attractive as a potential agent for such studies because of its cost-effective shelf life.

Development of targetry systems for a small proton cyclotron to be used to produce ^{11}C, ^{13}N, ^{15}O, and ^{18}F compounds for applications in clinical positron tomography has continued. A study of deuteron and proton penetration in Ne and N_2 target gases has been completed through ORAU-initiated collaborative research with the 60-inch cyclotron at the Brookhaven National Laboratory. The reduction of gas target density was experimentally measured by two methods for varying incident energies, beam currents, collimator sizes, and target lengths. Additional collaborative work at Brookhaven included (1) study of a $^{14}N(p,\alpha)^{11}C$ gas target and design of an associated radiochemistry system for the automated production of $^{11}CO_2$, ^{11}CO, and $H^{11}CN$ of high specific activity; and (2) research on a system for the on-line conversion of $^{13}N_2O$ from a $^{12}C(p,n)^{13}N$ target into $^{13}N_2$ and $^{13}NH_3$ using microwave discharge techniques. In conjunction with the targetry research, a computer model was developed in ORAU-initiated collaborative research with Dr. Paul King of Vanderbilt University to compare effects of temperature and stress on beam window designs for the proposed clinical accelerator.

Pulmonary and Cancer Cell Biology

Cell biology research is focused on solving problems related to growth and differentiation of epithelial cells in vivo and of isolated cell lines in culture. The growth and characterization of alveolar type II cells isolated from urethan-induced lung adenomas in mice require a major effort. Successful cultures of adenoma cells were established this past year. Electron microscopic studies have demonstrated that a fraction of the cells in early passage cultures contained multilamellar inclusions, a characteristic organelle in alveolar type

1028452

II cells. The cultures also incorporated a high proportion of palmitic acid, which is a major fatty acid component of surfactant, into disaturated phosphatidylcholine. Subsequent culturing efforts have produced rapidly growing, homogeneous type II cell lines, with lamellar bodies.

Another significant achievement is that cultures of the transplantable 4047 rat colonic adenocarcinoma have been established. Two cell lines and several clones isolated from each line are presently being maintained. All cultures are tumorigenic in rats although morphologic differences exist between each cell line and its clones. These cells will be used in investigations designed to answer questions about the potential multidirectional differentiation of colonic cancer cells, and attempts are planned to alter their tumorigenecity by differentiation induced in vitro. Information derived from this study could ultimately apply in developing methods potentially useful for the control colorectal cancer in humans.

Electron microscopic autoradiography and cytochemistry have also been used in identifying the maturation stages of colonic cancer cells in tamarins that develop the disease naturally. These studies are based on earlier ultrastructural and histochemical observations that have identified four types of cells in metastases: (1) undifferentiated, (2) cystic, (3) mucous, and (4) argentaffin or endocrine. The fact that endocrine cells are present in metastases proves that they are an integral part of these neoplasms since benign cells do not metastasize. It is also consistent with the notion that endodermal and endocrine cells of the gut may differentiate from a common stem cell.

Interdisciplinary Task Groups

Marmoset Colon Cancer

The objective of this interdisciplinary group is to investigate the usefulness of naturally occurring large bowel neoplasms in the cotton-top tamarin as a model for human colorectal cancer. Tamarins and marmosets are small simian primates belonging to the family Callitrichidae. During the past decade, division pathologists have observed a high incidence (approximately 20 percent) of this disease in adult cotton-tops born in the jungle and in captivity. This observation is unique since man is the only other species known to suffer from a high frequency of natural large bowel cancer. Because colorectal carcinoma ranks as one of man's most common neoplastic diseases, the tamarin surrogate is especially important. Since the cotton-top is no longer available from its natural habitat, extensive efforts have been made at ORAU to propagate the species in captivity. Grant funds from the National Institutes of Health were provided to support this project, now in its second year; and while it is too early to predict the outcome, results so far are promising. Careful pedigrees are maintained on all cotton-tops, and records show that two examples of familial cancer have been observed, one in a father-daughter pair, and one in twins. The fact that several human disease syndromes are characterized by familial patterns of large bowel cancer makes the tamarin model even more interesting. As pedigree data accumulate, efforts will be made to propagate the cancer "trait."

The usefulness of the animal model has been enhanced by the recent ability to diagnose the disease in animals well before death. Presently, approximately 50 percent of new cases are diagnosed antemortem.

There are three basic areas of interest actively under study. First, preneoplastic changes in the large bowel are being characterized. Efforts are made to identify quantitative and qualitative changes in colon mucosa cell populations to determine if specific changes can be interpreted as preneoplastic and used as a diagnostic warning. In addition, specific segments of large bowel are labeled with ^{3}H-methyl thymidine, biopsied, and examined autoradiographically to study the rate of cell population turnover. Patterns of pathology are also studied to determine if recognizable pathological changes may precede neoplastic change.

Secondly, peripheral lymphocytes from cancer-carrying and noncancer-carrying tamarins are assessed for susceptibility to the induction of sister chromatid exchange when these cells are cultured in the presence of a mutagen. Preliminary evidence indicates that patterns of susceptibility may be familial and, furthermore, may be related to cancer susceptibility. Grant funding has been sought to explore this further.

Third, the ultrastructure of cell populations of tamarin colon cancers is being examined. To date, four cell "types" have been identified by this method: undifferentiated (stem cell), cystic, goblet (signet ring or mucous), and argentaffin. The origin, role, and fate of these different cells are under study. Similar mixed populations have also been observed in human colon cancer. In addition to these studies, attempts to culture tamarin cancer cells in vitro are in progress, and studies of antigen-antibody complexes and lipids associated with this form of cancer are planned. Grant funds are presently being sought to investigate the tamarin colorectal carcinoma further.

^{11}C-Lung Tomography

An interdisciplinary lung tomography task group was formed to develop new radiopharmaceuticals that can be applied to positron-emission computerized tomographic studies of pulmonary function. The project seeks to identify compounds that show high uptake by lung tissue and forms that can be conveniently labeled with a positron-emitting radionuclide via organic synthesis. ^{11}C-Labeled straight-chain aliphatic amines are rapidly and selectively taken up by the lung, but they are also actively metabolized, resulting in substantial loss of radioactivity from the parent compound. This short residence time in the lung makes ^{11}C-labeled aliphatic amines, such as [^{11}C]octylamine, unsuitable for quantitative positron tomographic studies of pulmonary function. Therefore, two analogs—[^{14}C]2-methyloctylamine and [^{14}C]2,2-dimethyloctylamine—were synthesized, and their uptake and

1028453

clearance in lungs tested. 2-Methyloctylamine gave about twice the lung specificity at early times after administration as octylamine, and in addition it was metabolized only about half as fast. 2,2-Dimethyloctylamine had less lung uptake than the other two amines, but it showed virtually no metabolic loss of label. [^{14}C]N-Methyloctylamine and [^{14}C]N,N-dimethyloctylamine were also synthesized; these compounds exhibited lung uptake and clearance rates similar to those of octylamine. Although 2-methyloctylamine is superior to the other agents studied, in terms of both uptake and clearance from the lung, its residence time is still too short to permit quantitative tomographic studies of the lung.

Other structural modifications in the amine series are planned in an effort to identify an agent with useful lung uptake and adequate residence time at this site. A systematic in vitro investigation of lung monoamine oxidase specificity toward various amines will also be carried out. This information will be useful in selecting amines that are partially resistant to monoamine oxidase and display longer tissue residence times. Basic amines, such as chlorpromazine and imipramine, have specific high affinity sites on the plasma membrane of the pulmonary endothelium. This high affinity of the endothelium for basic amines could make such agents useful for evaluating endothelial integrity in disease states, since the endothelial cell lining of the pulmonary vasculature is of major importance in nonrespiratory functions of the lungs.

Benzene Toxicity

Benzene is used widely by industry as a precursor in many chemical syntheses, in automotive fuels, and as a solvent in household products (paints, adhesives, cements, insecticides). These numerous uses of the more than 12 billion pounds produced annually dictate the need for a greater understanding of the biologic hazards associated with exposure to benzene. Although the hematotoxic properties of benzene have been known for some time, more recent epidemiologic studies indicate this chemical may be leukemogenic for man. Limited laboratory data have hindered the establishment of a safe exposure criterion for benzene. The difficulties in setting risk levels are also compounded by development of aplastic anemia in some individuals following a single exposure to vapors of this chemical. Finally, while laboratory studies have verified the cytopenic effects, attempts to demonstrate carcinogenic activity have been uniformly unsuccessful.

This task group, which has just been organized, will integrate the development of future goals in this area. Another goal of this group is to obtain background information and to coordinate the detailed planning of a new inhalation facility that is expected to be built in the near future. The research program expected to emerge will use marmosets and mice to investigate the mechanism of action of benzene toxicity at the molecular and cellular levels and in biochemical and immunological terms.

DTPA

The DTPA (diethylenetriaminepentaacetic acid) task group manages for the Department of Energy (DOE) the national use of DTPA chelation drugs for decorporation therapy of plutonium and other actinides. Both Ca-DTPA (the calcium salt of DTPA) and Zn-DTPA (the analogous zinc salt) have been approved for human use by the U.S. Food and Drug Administration (FDA) under investigational new drug (IND) status. The two INDs are managed by the division's DTPA task group and involve approximately 40 industrial physicians, mostly at DOE contractor sites, as co-investigators. A national registry of individuals treated with DTPA is maintained for DOE. The registry was organized to document the safety and efficacy of treatment with DTPA drugs and to aid in facilitating long-term follow-up of the treated individuals.

The program also evaluates new developments in chelation therapy involving both improved protocols for use of Ca- and Zn-DTPA and research on new chelators. When indicated, the appropriate IND amendments or new applications are submitted through DOE to FDA. One such evaluation currently in progress involves liposome-encapsulated Ca- or Zn-DTPA as a potential agent for increased removal of intracellular actinide contamination. Preliminary results suggest that the lipophilic form may be more effective than Ca- or Zn-DTPA, particularly for removing insoluble, colloidal contaminants; however, the splenic enlargement that occurs after administration of liposomes could be a limiting factor in its use.

Cardiovascular Research Section

Ongoing research in this area includes the investigation of thrombocytopenia in the marmoset and a new class of biologically active phospholipids that possesses platelet aggregation and hypotensive properties. Thrombocytopenia can be induced in marmosets by a single interspecies inoculation of platelets. The disease syndrome closely resembles that of post-transfusion purpura of man and has many features in common with idiopathic thrombocytopenic purpura.

Three major findings have been made this year. The first is that interspecies immunizations lead not only to formation of antibody to the donor platelets, but also to the development of antibodies reactive to autologous platelets and to platelets of other members of the host species. Antibodies that selectively react to either autologous or host-type platelets can be found during the course of thrombocytopenia in the immunized marmoset. Serum obtained from these animals has been used to identify potential immunogenetic markers for platelet antigens within one species of marmoset.

Secondly, a mechanism of immunologically mediated destruction of platelets not previously recognized in clinical autoimmune syndromes has also been found. This involves the activation of naive lymphocytes upon contact with

1028454

antibody-coated platelets, leading cytolysis of the target platelets ıtibody-dependent cellular cytotoxicity).

Third, recent presumptive evidence has indicated that immune complexes may also contribute to immune elimination of platelets. These complexes have been detected through the nonspecific Raji cell assay. This study of autoimmmune thrombocytopenia is now in its sixth year of grant support from the National Institutes of Health.

Research on membrane lipids at the division led to the discovery of a unique phospholipid with potent physiologic properties. The active glycerolipid contains an ether-linked moiety, phosphate, choline, and a short-chain acid (acetate) group. It exhibits profound antihypertensive effects after intravenous or oral administration to rats. The same phospholipid has been identified as a platelet-activating factor, which is responsible for platelet aggregation at concentra- ıs as low as 10^{-11}M. Recent chemical results have demonstrated that the active phospholipid molecule can be synthesized and degraded enzymatically in a variety of tissues.

Radiation and Nuclear Medicine Section

Nuclear Medicine

Clinical research at the division focuses on two important areas in nuclear medicine: (1) emission computerized tomography with positron-emitting radiopharmaceuticals and (2) computer applications in image analysis. Emphasis is on diagnostic applications of ^{11}C-labeled amino acids for the study of a variety of neoplastic lesions and of specific metabolic disorders in pancreas and brain. One of the important goals in this effort is to develop accurate quantitative lytical methods for the ECT nner for noninvasive (in vivo) studies of amino acid utilization and turnover in tumors and in normal tissue. ^{11}C-Labeled amino acids are an ideal choice for studying the metabolism of proteins since, unlike other isotopes, the ^{11}C label does not change the physical and chemical properties of macromolecules. During 1981 the emission computerized axial tomography (ECAT$_{EM}$) scanner will be updated to an ECAT-II system.

This past year the clinical evaluation of ^{11}C-labeled amino acids (DL-valine, DL-tryptophan, 1-aminocyclobutanecarboxylic acid, and 1-aminocyclopentanecarboxylic acid) continued. Results show that these amino acids have a high affinity for a variety of human neoplasms; for example, [^{11}C]1-aminocyclobutanecarboxylic acid had good uptakes in over 90 percent of the tumors studied. Experiments have also continued with ^{68}Ga-labeled radiopharmaceuticals for quantitative studies in patients with chronic fibrotic lung diseases. Preliminary results obtained with ^{68}Ga-labeled-albumin microspheres in clinical investigations with positron-ECT have shown the potential for improved quantitative perfusion studies in patients with regional lung disease; these experiments will be extended to the investigation of lung ventilation with a ^{68}Ga-labeled ethylenediaminetetraacetate aerosol.

Progress has also been made in computer applications. Detailed phantom studies with the ECAT scanner led to the discovery that image noise, which interferes with accurate determination of activity distributions in large volumes such as the trunk, presents itself as a convolving function. The mathematical solution of this problem was translated into the ECAT software system. By removing data noise before image reconstruction, computer quantitative analyses of ECAT scans with an error of less than 10 percent can be obtained.

Radiation Emergency Assistance Center/Training Site

The objectives of the REAC/TS program are to improve the medical care of persons injured by radiation and to learn more about the late effects and risk factors from exposures to relatively low doses of radiation in man. These objectives are approached in five specific tasks: (1) rendering assistance in the management of health problems associated with radiation accidents, (2) maintaining registries of radiation accidents and gathering follow-up information, (3) holding training courses for medical and paramedical personnel, (4) conducting research in radiopathology with emphasis on early detection of severe localized radiation injury, and (5) maintaining a cytogenetics program that conducts biological dosimetry and research in radiation and chemical effects on chromosomes.

REAC/TS continues to provide consultation services and assistance to physicians in the management of health problems of patients with suspected and sustained radiation injury. At the request of the Department of Energy, REAC/TS staff have given expert medical testimony and advice to the U.S. Department of Justice. The REAC/TS registries were restructured into the Control (comprehensive) Registry forming the basis for three specific registries: (1) The U.S. Radiation Accident Registry, (2) the DTPA Registry, and (3) the DOE Study ($\geq$ 5 rem) Registry. To date, 120 accidents have been recorded in the U.S. Radiation Accident Registry, 424 entries have been made in the DTPA Registry, and 3247 individuals have been recorded in the DOE Study Registry.

The REAC/TS training program includes formal courses and seminars; tours and informal discussions with professionals, students, and lay people; and information exhibits displayed at professional meetings. A total of 539 professionals have been formally trained at REAC/TS since June of 1976.

Highlights and special accomplishments of REAC/TS the past year are (1) management of an International Conference on the Medical Basis for Radiation Accident Preparedness in October 1979, attended by 234 people from 17 different countries, (2) development and publication of a manual entitled *Response to Radioactive Materials Transport Accidents* for the U.S. Department of Transportation, and (3) reorganization of the REAC/TS registry system.

Future plans for this program include expansion of training activities, a morbidity and mortality

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study on the ≥ 5-rem group, "first round" medical follow-up on all registered REAC/TS radiation accident cases, and completion of in vitro dose response studies on radiation-induced chromosome lesions. A special effort will be made to write training course manuals as requested by the Federal Emergency Management Agency for emergency medical technicians and nurses who need to be trained in the management of health problems of people exposed to radiation.

Cytogenetics

The two primary missions of the cytogenetics program are (1) to provide biological dosimetry and follow-up evaluations for persons referred to REAC/TS because of suspected exposures to ionizing radiation, and (2) to conduct basic research studies aimed at defining the risk of human exposure to environmental mutagens, including radiation and clastogenic chemicals.

During the past few years, research efforts have focused on assessing the effects of radiations possessing different linear energy transfer on the induction of chromosome lesions in human lymphocytes of normal and radiosensitive individuals. These results have led to the development of in vitro dose response curves for evaluating the induction of chromosome aberrations in first division metaphases of lymphocytes exposed to fission neutrons. These data will be used to assess the dose in persons involved in radiation accidents and to derive appropriate statistical models aimed at defining the effects of linear energy transfer on the kinetics of lesion induction and the distributions of lesions in exposed cells.

Investigations of chemical clastogenesis have been directed toward determining whether the frequencies of chromosome lesions induced in somatic cells by clastogenic chemicals can be used to predict the risk of transmission of chromosome damage to pre- and postimplantation embryos. These data have shown that the direct-acting chemical clastogen, streptonigrin, induces structural chromosome lesions in female germ cells that can be transmitted to the preimplantation embryo, and that the majority of the cytogenetically aberrant blastocysts do not survive implantation. Since the analysis of mutagen-induced sister chromatid exchanges is a more sensitive estimator of exposure to many chemical clastogens, this methodology is being used in the cytogenetic screening of exposed persons and in experimental procedures. An important recent observation is that sensitivity to mitomycin C induction of sister chromatid exchanges in the tamarin, *Saguinus oedipus oedipus*, may be genetically determined, and that increased sensitivity could be a genetic marker for individual tamarins at risk for adenocarcinoma of the colon.

Occupational Epidemiology

The epidemiology studies are concerned mainly with examining associations between occupational radiation exposure and subsequent mortality. The health effects of chemical toxicants, especially metals, are also under investigation as both primary and confounding (with respect to radiation exposure) occupational stresses. In the statistical and computer methodology sections, biostatisticians and computer scientists are developing improved methods for epidemiological analyses and data systems for merging large masses of data into analysis files.

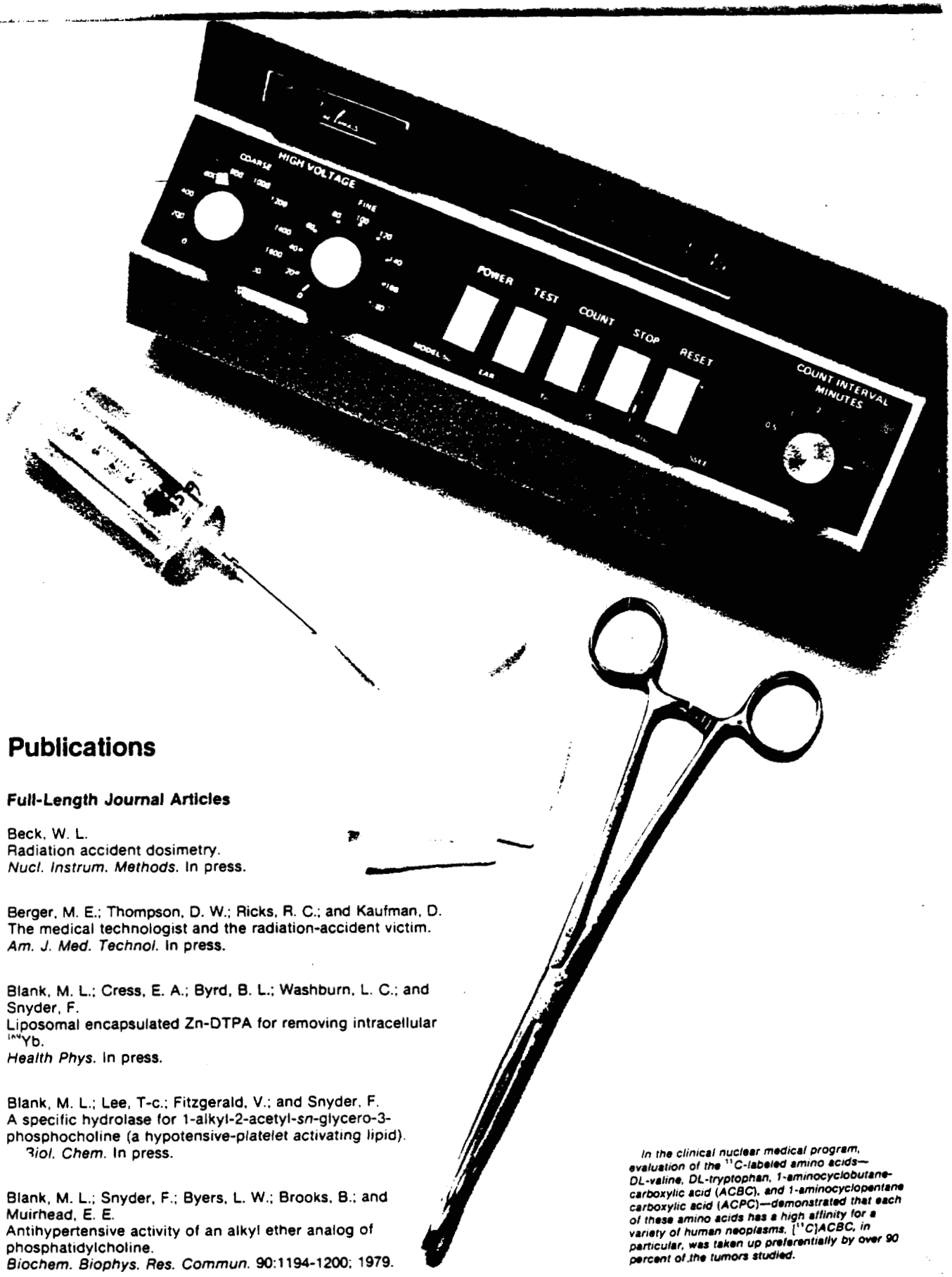
In the area of radiation effects, the epidemiology group is continually evaluating the mortality experience of workers within the Oak Ridge Operations Office area. This area includes workers at facilities run by Union Carbide Corporation, Nuclear Division, in Oak Ridge, Tennessee, and Paducah, Kentucky, and workers at the National Lead Company and the Mound Facility, both in Ohio. Editing and correction procedures have been developed for the data bases that extend back to the early 1940s. Preliminary mortality analyses have been completed on two groups of workers at the Oak Ridge Y-12 plant.

Another study, designed to examine possible health effects of occupational exposure to radiation equal to or greater than the current limit (set by the Nuclear Regulatory Commission and DOE) of 5 rem per year, is in the data collection and validation phase. We have constructed a roster of 3247 employees of DOE and its predecessor organizations who have been exposed to 5 or more rem per year. Preliminary analyses of mortality experience for this group will begin during 1981, once retrieval of death certificates has been completed.

Other studies in progress include the long-term health effects of exposure to metals and other toxic substances. A completed mortality study of Oak Ridge workers potentially exposed to metallic nickel is being updated to include more follow-up data. Mortality is being examined among welders of the Union Carbide Corporation, Nuclear Division, which contains a subgroup exposed to nickel vapor.

To facilitate these studies and to aid other DOE epidemiological studies (such as those of atomic energy workers at Hanford, Washington, Los Alamos, New Mexico, and Rocky Flats, Colorado), the epidemiology group has developed the DOE Death Certificates Retrieval Center to obtain information on the vital status of specific workers. These data are being accumulated through the cooperation of the Social Security Administration and the vital statistics departments in all 50 states.

1028456



In the clinical nuclear medical program, evaluation of the ^{11}C-labeled amino acids—DL-valine, DL-tryptophan, 1-aminocyclobutane-carboxylic acid (ACBC), and 1-aminocyclopentane carboxylic acid (ACPC)—demonstrated that each of these amino acids has a high affinity for a variety of human neoplasms. [^{11}C]ACBC, in particular, was taken up preferentially by over 90 percent of the tumors studied.

Publications

Full-Length Journal Articles

Beck, W. L.
Radiation accident dosimetry.
Nucl. Instrum. Methods. In press.

Berger, M. E.; Thompson, D. W.; Ricks, R. C.; and Kaufman, D.
The medical technologist and the radiation-accident victim.
Am. J. Med. Technol. In press.

Blank, M. L.; Cress, E. A.; Byrd, B. L.; Washburn, L. C.; and Snyder, F.
Liposomal encapsulated Zn-DTPA for removing intracellular ^{169}Yb.
Health Phys. In press.

Blank, M. L.; Lee, T-c.; Fitzgerald, V.; and Snyder, F.
A specific hydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive-platelet activating lipid).
Biol. Chem. In press.

Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; and Muirhead, E. E.
Antihypertensive activity of an alkyl ether analog of phosphatidylcholine.
Biochem. Biophys. Res. Commun. 90:1194-1200; 1979.

1028457

Buonocore, E., and Hübner, K. F.
Positron-emission computed tomography of the pancreas: a preliminary study.
Radiology 133:195-201; 1979.

Buonocore, E.; Hübner, K. F.; and Collmann, I. R.
Differentiation of retroperitoneal tumor using positron emission computed tomography.
J. Comput. Assist. Tomogr. 3:825-828; 1979.

Buyukmihci, N., and Richter, C. B.
Prevalence of ocular disease in a colony of tamarins and marmosets.
Lab. Anim. Sci. 29:800-804; 1979.

Cabot, M. C., and Lumb, R. H.
The activity of a low temperature lipase in the larvae of *Sarcophaga bullata* (Diptera: Sarcophagidae).
Comp. Biochem. Physiol. In press. Work performed at Western Carolina University, Cullowhee, North Carolina.

Cabot, M. C., and Snyder, F.
Manipulation of alkylglycerolipid levels in cultured cells. Fatty alcohol versus alkylglycerol supplements.
Biochim. Biophys. Acta 617:410-418; 1980.

Cabot, M. C.; Welsh, C. J.; Callaham, M. F.; and Huberman, E.
Alterations in lipid metabolism induced by 12-*O*-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells.
Cancer Res. In press.

Casey, D. L.; Digenis, G. A.; Wesner, D. A.; Washburn, L. C.; Chaney, J. E.; Hayes, R. L.; and Callahan, A. P.
Preparation and preliminary tissue studies of optically active C-11- D- and L- phenylalanine.
Int. J. Appl. Radiat. Isot. In press.

Chepenik, K. P.; Blank, M.; Snyder, F.; and Waite, B. M.
On the presence of plasmalogens in developing rat embryos.
Int. J. Biochem. 11:605-607; 1980.

DuFrain, R. J.; Heffernan, K.; Wilmer, J. L.; Batson, J.; and Littlefield, L. G.
Sister chromatid exchange and mitotic stimulation in lymphocytes from four species of the genus *Saguinus*.
J. Med. Primatol. 9: 233-239; 1980.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Frome, E. L.
Human cytogenetic dosimetry: a dose-response relationship for alpha particle radiation from ^{241}Am.
Health Phys. 37: 279-289; 1979.

DuFrain, R. J., Littlefield, L. G., and Wilmer, J. L.
Cyclophosphamide-induced sister chromatid exchanges in rabbit lymphocytes.
Environ. Mutagenesis 1:283-286; 1979.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
The effect of washing lymphocytes after in vivo treatment with streptonigrin on the yield of chromosome and chromatid aberrations in blood cultures.
Mutat. Res. 69:101-105; 1980.

Dupree, E. A., and Meyer, M. B.
Role of risk factors in complications of diabetes mellitus.
Am. J. Epidemiol. 112:100-112; 1980. Work performed at the Johns Hopkins University, Baltimore, Maryland.

Frome, E. L., and Khare, M.
Comments on Broksky's statistical methods for evaluating epidemiological results.
Health Phys. In press.

Frome, E. L., and Yakatan, G. J.
Statistical estimation of the pharmokinetic parameters in the one compartment open model.
Commun. Statist.-Simula. Computa. B9:201-222; 1980.

Fuhr, J. E., and Gengozian, N.
Fetal hemoglobin: in vivo stimulation by D-thyroxine in adult marmosets.
Blood Cells 5:471-474; 1979.

Gengozian, N.; Brewen, J. G.; Preston, R. J.; and Batson, J. S.
Presumptive evidence for the absence of functional germ cell chimerism in the marmoset.
J. Med. Primatol. 9:9-27; 1980.

Gengozian, N., and McLaughlin, C. L.
IgG+ platelets in the marmoset: their induction, maintenance and survival.
Blood 55:885-890; 1980.

Gengozian, N., and Ostby, D. A.
Antibodies selectively reactive to autologous and host-type platelets are obtained following interspecies immunizations in marmosets.
Clin. Exp. Immunol. In press.

Germain, G. S., and Arneson, R. M.
Effects of metal ions and selenoamino acids on induction of glutathione peroxidase in mouse neuroblastoma.
Enzyme 24:337-342; 1979. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

Godbold, J. H., Jr., and Tompkins, E. A.
A long-term mortality study of workers occupationally exposed to metallic nickel at the Oak Ridge Gaseous Diffusion Plant.
J. Occup. Med. 21:799-806; 1979.

Hayes, R. L.
The interaction of gallium with biological systems.
Int. J. Nucl. Med. Biol. In press.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; and Carlton, J. E.
The effect of scandium on the tissue distribution of ^{67}Ga in normal and tumor-bearing rodents.
*J. Nucl. Med.*21:361-365; 1980.

Hayes, R. L.; Rafter, J. J.; Byrd, B. L.; and Carlton, J. E.
Studies of the in vivo entry of ^{67}Ga into normal and malignant tissue.
J. Nucl. Med. In press.

1028458

Hodges, H. D., and Aldridge, R. E.
'diation safety in the nuclear medicine laboratory.
Nucl. Med. Technol. 8:87-89; 1980.

Lee, T-c., and Fitzgerald, V.
Phase transitions of alkyl ether analogs of phosphatidylcholine.
Biochim. Biophys. Acta 598:189-192; 1980.

Lee, T-c.; Fitzgerald, V.; Stephens, N.; and Snyder, F.
Activities of enzymes involved in the metabolism of ether-linked lipids in normal and neoplastic tissues of rat.
Biochim. Biophys. Acta 619:420-423; 1980.

Lee, T-c.; Hougland, A. E.; and Stephens, N.
Perturbation of lipid metabolism in L-M cultured cells by elaidic acid supplementation: formation of fatty alcohols.
Biochem. Biophys. Res. Commun. 91:1497-1503; 1979.

Leonard, R. B., and Ricks, R. C.
Emergency department radiation-accident protocol.
Ann. Emergency Med. In press.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Comparison of sister-chromatid exchanges in human lymphocytes after G_0 exposure to mitomycin in vivo vs. in vitro.
Mutat. Res. 69:191-197; 1980.

Dermott, C. A., and Gengozian, N.
.ıe effect of low-exposure-rate gamma irradiation on T and B lymphocyte function in the mouse.
Int. J. Radiat. Biol. 37:415-428; 1980.

Nickerson, D. A., and Gengozian, N.
Functional capabilities of marmoset T and B lymphocytes in primary in vitro antibody formation.
Cell. Immunol. In press.

Palisano, J. R.; Walne, P. L.; and Brown, D. H.
Ultrastructure and physical characteristics of proplastids and chloroplasts isolated by zonal centrifugation from *Euglena gracilis* var. *bacillaris*.
Eur. J. Cell Biol. 21:305-312; 1980.

Polednak, A. P.
Mortality among men occupationally exposed to phosgene in 1943-1945.
Environ. Res. 22:357-367; 1980.

Polednak, A. P., and Frome, E. L.
Mortality among men employed (between 1943 and 1947) at a uranium-processing plant.
J. Occup. Med. In press.

Renooij, W., and Snyder, F.
'synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine atelet activating factor and a hypotensive lipid) by cholinephosphotransferase in various rat tissues.
Biochim. Biophys. Acta. In press.

Renooij, W., and Van Golde, L. M. G.
Asymmetry in the renewal of molecular classes of phosphatidylcholine in the rat erythrocyte membrane.
Biochim. Biophys. Acta 558:314-319; 1979. Work performed at the State University of Utrecht, The Netherlands.

Sayer, A. M.; Littlefield, L. G.; DuFrain, R. J.; and Richter, C. B.
Analysis of mutagen-induced chromosome damage in a primate species (*Saguinus oedipus oedipus*) at risk for spontaneous adenocarcinoma of the colon.
Cancer Genet. Cytogenet. In press.

Schremmer, J. M.; Blank, M. L.; and Wykle, R. L.
Bradykinin-stimulated release of [^{3}H]arachidonic acid from phospholipids of $HSDM_1C_1$ cells: comparison of diacyl phospholipids and plasmalogens as sources of prostaglandin precursors.
Prostaglandins 18:491-505; 1979.

Scott, C. C.; Heckman, C. A.; and Snyder, F.
Regulation of ether lipids and their precursors in relation to glycolysis in cultured neoplastic cells.
Biochim. Biophys. Acta 575:215-224; 1979.

Swartzendruber, D. C., and Richter, C. B.
Mucous and argentaffin cells in colonic adenocarcinoma of tamarins and rats.
Lab. Invest. In press.

Urso, P., and Gengozian, N.
Nonspecific suppressor elements in murine allogeneic radiation chimeras.
Cell. Immunol. 47:19-31; 1979.

Urso, P., and Gengozian, N.
Depressed humoral immunity and increased tumor incidence in mice following in utero exposure to benzo(α)pyrene.
J. Toxicol. Environ. Health 6:569-576; 1980.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and Butler, T. A.
1-Aminocyclobutane[^{11}C]carboxylic acid, a potential tumor-seeking agent.
J. Nucl. Med. 20:1055-1061; 1979.

Wykle, R. L.; Kraemer, W. F.; and Schremmer, J. M.
Specificity of lysophospholipase D.
Biochim. Biophys. Acta 619:58-67; 1980.

Wykle, R. L.; Malone, B.; Blank, M. L.; and Snyder, F.
Biosynthesis of pulmonary surfactant: comparison of 1-palmitoyl-*sn*-glycero-3-phosphocholine and palmitate as precursors of dipalmitoyl-*sn*-glycero-3-phosphocholine in adenoma alveolar type II cells.
Arch. Biochem. Biophys. 199:526-537; 1980.

Wykle, R. L.; Malone, B.; and Snyder, F.
Acyl-CoA reductase specificity and synthesis of wax esters in mouse preputial gland tumors.
J. Lipid Res. 20:890-896; 1979.

Wykle, R. L.; Malone, B.; and Snyder, F.
Enzymatic synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a hypotensive and platelet aggregating lipid.
J. Biol. Chem. In press.

1028459

Chapters, Review Articles, and Other Publications

Andrews, G. A.; Hübner, K. F.; Fry, S. A.; Lushbaugh, C. C.; and Littlefield, L. G.
Report of 21-year medical follow-up of survivors of a major radiation accident.
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Arneson, R. M., and Wander, J. D.
Antioxidants in neoplastic cells: III. An antioxidant artifact in tissue culture media. Letter to the editor.
Lipids 15:203-204; 1980. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

Beck, W. L., Jr.; Berger, C. D.; Berger, J. D.; and Goans, R. E.
Instrumentation for detection and measurement of ionizing radiation
Instrumentation and Controls Handbook. New York: Marcel Dekker Publishing Co. In press.

Beck, W. L., and Holly, F. E.
Dosimetry studies for an industrial radiography accident (^{192}Ir).
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Frome, E. L.
In vitro human cytogenetic dose response systems.
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry, New York: Elsevier North-Holland. In press.

Frome, E. L., and Hudson, D. R.
A jackknife procedure for estimating standardized mortality ratios.
Proceedings of the Statistical Computing Section of the American Statistical Association, 1979, pp. 323-325. Washington, D.C.: 1980.

Frome, E. L., and Hudson, D. R.
A general statistical data structure for epidemiologic studies of DOE workers.
Proceedings of the 1980 DOE Statistical Symposium, Berkeley, California, 1980. In press.

Fry, S. A.
The U.S. radiation accident and other registries of the "REAC/TS" registry system: their function and current status.
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Gengozian, N.
The marmoset as a model for twinning, chimerism, and immunologic tolerance.
Spontaneous Animal Models of Human Disease, Vol. 1, pp. 222-224, ed. E. J. Andrews, B. C. Ward, and N. H. Altman. New York: Academic Press, 1979.

Hayes, R. L., and Byrd, B. L.
Transfer of ^{67}Ga from the hamster dam to fetus and offspring.
Proceedings of the Third International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, 1980, ed. E. E. Watson; A. Schlafke-Stelson; J. L. Coffey; and R. J. Cloutier. Rockville, Maryland: Bureau of Radiological Health, Food and Drug Administration. In press.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; Carlton, J. E.; and Coffey, J. L.
Ytterbium-169 citrate for detection of inflammatory lesions.
Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging, Heidelberg, Germany, 1980. In press.

Hempelmann, L. H., Lushbaugh, C. C., and Voelz, G. L.
What happened to the survivors of the early Los Alamos nuclear accidents?
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Hodges, H. D. [Reviewer].
NCRP Report No. 57: Instrumentation and monitoring methods for radiation protection.
J. Nucl. Med. Technol. 7:245; 1979.

Hübner, K. F.; Andrews, G. A.; Washburn, L.; Wieland, B. W.; Gibbs, W. D.; Hayes, R. L.; Butler, T. A.; and Winebrenner, J. D.
Tumor location with 1-aminocyclopentane [^{11}C]carboxylic acid; preliminary clinical trials with single-photon detection (Original publication: *J. Nucl. Med.* 18:1215-1221; 1977).
Year Book Cancer, 1979, pp. 237-239.

Hübner, K. F. and Fry, S. A. [Editors].
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979. New York: Elsevier North-Holland. In press.

Hübner, K. F.; King, P.; Gibbs, W. D.; Partain, C. L.; Washburn, L. C.; Hayes, R. L.; and Holloway, E.
Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases.
Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging, Heidelberg, Germany, 1980. In press.

Jay, M.; Digenis, G. A.; Chaney, J. E.; Washburn, L. C.; Byrd, B. L.; Hayes, R. L.; and Callahan, A. P.
Synthesis and brain uptake of carbon-11 phenethylamine.
J. Labelled Compd. Radiopharm. In press.

Kabalka, G. W.; Gooch, E. E.; Sun, T. T.; and Washburn, L. C.
A rapid and mild method for labeling functionally substituted molecules with iodine radionuclides.
J. Labelled Compd. Radiopharm. In press.

King, P. H., and Wieland, B. W.

1028460

Computer modeling of accelerator beam window failures. *J. Labelled Compd. Radiopharm.* In press.

Littlefield, L. G. [Contributor].
Chromosome aberrations in somatic cells.
Influence of Dose and Its Distribution in Time on Dose-Response Relationships for Low-LET Radiations. NCRP Report No. 64, pp. 23-37. Washington, D. C.: National Council on Radiation Protection and Measurements, 1980.

Littlefield, L. G.; Joiner, E. E.; DuFrain, R. J.; Hübner, K. F.; and Beck, W. L.
Cytogenetic dose estimates from in vivo samples from persons involved in real or suspected radiation exposures.
The Medical Basis for Radiation Accident Preparedness. Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Lushbaugh, C. C.; Fry, S. A.; Hübner, K. F.; and Ricks, R. C.
Total-body irradiation: a historical review and follow-up.
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Mauderly, J.; Hahn, F.; Martin, C. J.; and Richter, C.
Animal models of diseases of the human elderly: chronic bronchitis and emphysema.
Mammalian Models for Research on Aging, sponsored by the Institute of Laboratory Animal Resources (ILAR) Committee on Animal Models for Research on Aging, National Research Council. Washington, D. C.: U.S. Department of Health and Human Services. In press.

Parker, J. C., and Richter, C. B.
Respiratory (Sendai, PVM, K).
The Mouse in Biomedical Research, ed. H. L. Foster, J. D. Small, and J. G. Fox. New York: Academic Press. In press.

Polednak, A. P.
Long-range studies of uranium workers and the Oak Ridge radiation-worker population.
The Medical Basis for Radiation Accident Preparedness, Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Polednak, A. P., and Frome, E. L.
Mortality among men employed between 1943 and 1947 at a uranium-processing plant.
Proceedings of the 1979 DOE Statistical Symposium, Gatlinburg, Tennessee, 1979, CONF-791016, pp. 116-127, ed. D. A. Gardiner and T. Truett. Washington, D.C.: U.S. Department of Energy, 1980.

Richter, C. B.
Diagnostic Exercise [Mucinous adenocarcinoma of the descending colon].
Lab. Anim. Sci. In press.

Richter, C. B.; Lushbaugh, C. C.; and Swartzendruber, D. C.
Cancer of the colon in the cotton-topped tamarins.
Comparative Pathology of Zoo Animals, Proceedings of a Symposium, 1978, ed. R. Montali and G. Migaki. Washington, D.C.: The Smithsonian Press. In press.

Ricks, R. C.
A training course for radiation accident management in the health physics/medical environment.
Health Physics Training, Proceedings of the Health Physics Society 13th Midyear Topical Symposium. Honolulu, 1979, pp. 7-15, ed. W. L. Yanger and P. J. Manly. Honolulu: Hawaii Chapter, Health Physics Society, 1980.

Ricks, R. C.
REAC/TS: its role as a specialty referral center and training site.
The Medical Basis for Radiation Accident Preparedness. Proceedings of an International Conference, Oak Ridge, Tennessee, 1979, ed. K. F. Hübner and S. A. Fry. New York: Elsevier North-Holland. In press.

Ricks, R. C. [Reviewer].
NCRP Report No. 65: Management of persons accidentally contaminated with radionuclides.
Radiat. Res. In press.

Ricks, R. C.; Beck, W. L.; and Berger, J. D.
Response to Radioactive Materials Transport Accidents, DOT/RSPA/MTB-79/8 (Interim edition). Washington, D.C.: U.S. Department of Transportation, 1980.

Sagan, L. A., and Fry, S. A.
Radiation accidents: a conference review.
Nucl. Saf. In press.

Snyder, F.; Lee, T-c.; Blank, M. L.; and Moore, C.
In vivo modification of phospholipid polar head groups and alterations in membrane activities and cellular events.
Membrane Fluidity. Biophysical Techniques and Cellular Regulation, pp. 307-323, ed. M. Kates and A. Kuksis. Clifton, New Jersey: The Humana Press, Inc., 1980.

Swartzendruber, D. C.; Miller, P. N.; and Richter, C. B.
Ultrastructure of colonic neoplastic cells in metastases of tamarins.
Proceedings for the 38th Annual Meeting of the Electron Microscope Society of America, San Francisco, 1980, pp. 730-731, ed. C. J. Arceneaux. Baton Rouge, Louisiana: Claitor's Publishing Division, 1980.

Washburn, L. C.; Ringenberg, R. E.; Sun, T. T.; and Hayes, R. L.
^{11}C-Labeled 1-aminocyclohexanecarboxylic acid ([^{11}C]ACHC), a potential agent for studies of amino acid transport in the brain
J. Labelled Compd. Radiopharm. In press.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; Butler, T. A.; and Callahan, A. P.
High-level production of [^{11}C]carboxyl-labeled amino acids.
Radiopharmaceuticals II: Proceedings of the 2nd International Symposium on Radiopharmaceuticals, Seattle, 1979, pp. 767-777. New York: Society of Nuclear Medicine, 1979.

Wieland, B. W.; Schlyer, D. J.; Ruth, T. J.; and Wolf, A. P.
Deuteron beam penetration in a neon gas target for producing fluorine-18.
J. Labelled Compd. Radiopharm. In press.

1028461

Published Abstracts of Papers Presented at Meetings

Blank, M. L.; Snyder, F. L.; Byers, L. W.; Brooks, B.; Smith, K. A.; and Muirhead, E. E.
Similarities in the antihypertensive action of an alkyl ether analog of phosphatidylcholine and a phospholipid derived from the renal medulla.
Clin. Res. 27:781A; 1979.

Brown, A. J., and Snyder F.
Solubilization of alkyldihydroxyacetone-P synthase from Ehrlich ascites cell microsomal membranes.
Soc. Exp. Biol. Med. (S.E. Sect.) 4:17; 1979.

Brown, A. J., and Snyder, F.
Properties of membrane-bound and solubilized alkyldihydroxyacetone-P synthase.
Fed. Proc. 39:1993; 1980.

Cabot, M. C., and Welsh, C. J.
Alterations in lipid metabolism induced by 12-*O*-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia.
Fed. Proc. 39:2188; 1980.

DuFrain, R. J.; Frome, E. L.; and Littlefield, L. G.
Dose response, distribution, and dispersion analyses of streptonigrin-induced cytogenetic abnormalities in somatic cells of rabbit.
Environ. Mutagenesis 2:256-257; 1980.

DuFrain, R. J.; Frome, E. L.; Littlefield, L. G.; and Joiner, E. E.
Application of the generalized Poisson distribution to alpha particle-induced chromosome aberrations in human lymphocytes.
Radiat. Res. 83:486-487; 1980.

DuFrain, R. J.; Littlefield, L. G.; Frome, E. L.; Wilmer, J. L.; Huff, V.; Hutton, D.; and Morrison, W. D.
Cytogenetic evaluations of embryos and somatic cells from female rabbits treated with streptonigrin.
Mamm. Chromosomes Newslett. In press.

DuFrain, R. J.; Wilmer, J. L.; and Littlefield, L. G.
Analysis of cyclophosphamide-induced sister-chromatid exchange.
Environ. Mutagenesis 1:176; 1979.

Dupree, E. A.
Mortality among workers at a uranium refining and processing plant.
Am. J. Epidemiol. In press.

Ehrenkaufer, R. L. E.; Fowler, J. S.; MacGregor, R. R.; Ruth, T. J.; Wieland, B.; and Wolf, A. P.
Production of carrier free, anhydrous $H^{18}F$ by deuteron irradiation of H_2/Ne target gas.
Second Chemical Congress of the North American Continent, San Francisco, 1980, Abstracts of Papers, Pt. I, NUCL No. 89, Washington, D.C.: American Chemical Society, 1980.

Fishman, J. B.; Blank, M. L.; and Snyder F.
Accumulation of *O*-alkylacyl ethanolamine phospholipid in tumor tissues.
Soc. Exp. Biol. Med. (S.E. Sect.) 4:13; 1979.

Frome, E. L., and DuFrain, R. J.
Determination of overdispersion of dicentric chromosome aberrations that are assumed to follow Poisson distribution.
Biometrics. In press.

Fry, S. A.; Lushbaugh, C. C.; and Hübner, K. F.
An overview of radiation accidents and injuries.
Int. J. Nucl. Med. Biol. In press.

Gengozian, N.; Ostby, D. A.; and Rankin, J.
Active induction of immune thrombocytopenia in the marmoset.
Abstracts, Fourth International Congress of Immunology, International Union of Immunological Societies, Paris, France, 1980. Abstract 15.5.06. Paris: Société Francaise d'Immunologie, 1980.

Hayes, R. L.; Rafter, J. J.; and Carlton, J. E.
Toxicity and tissue distribution of ^{99m}Tc pertechnetate.
Proceedings of the 21st Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures). Nashville, Tennessee. In press.

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
The in vivo entry of ^{67}Ga into an experimental abscess.
Proceedings of the 21st Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures). Nashville, Tennessee. In press.

Hübner, K. F.; King, P.; Gibbs, W. D.; Washburn, L. C.; and Hayes, R. L.
Positron emission computerized tomography: a potential tool for in vivo quantitation of the distribution of radiopharmaceuticals.
Proceedings of the Third International Radiopharmaceutical Dosimetry Symposium, Oak Ridge, Tennessee, 1980, ed. E. E. Watson; A. Schlafke-Stelson; J. L. Coffey; and R. J. Cloutier. Rockville, Maryland: Bureau of Radiological Health, Food and Drug Administration. In press.

Hübner, K. F.; Partain, C. L.; Robertson, J.; Mahaley, M. S.; Purvis, J.; and Gibbs, W. D.
Brain tumor imaging by positron emission computerized tomography (ECT) using ^{11}C-labeled amino acids.
Proceedings of the 21st Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures). Nashville, Tennessee. In press.

Huff, V.; Hutton, D. G.; DuFrain, R. J.; and Littlefield, L. G.
Comparison of chromosome lesion frequency in rabbit bone marrow following a single or double injection of cyclophosphamide.
Soc. Exp. Biol. Med. (S.E. Sect.) 4:19; 1979.

Huff, V.; Hutton, D. G.; DuFrain, R.; Littlefield, L. G.; and Wilmer, J.
Streptonigrin- and cyclophosphamide-induced chromosome lesions in somatic cells vs. oocytes of the rabbit.
Environ. Mutagenesis 2:254; 1980.

1028462

Kabalka, G. W.; Gooch, E. E.; Sun, T. T.; and Washburn, ?.
...pid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology.
Second Chemical Congress of the North American Continent. San Francisco, 1980, Abstracts of Papers, Pt. I, NUCL No. 91, Washington, D.C.: American Chemical Society, 1980.

Lee, T-c., and Stephens, N.
Increased formation of fatty alcohols by *trans* fatty acid supplementation in cultured L-M cells.
Fed. Proc. 39:1724; 1980.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Sister-chromatid exchanges in human lymphocytes after G_0 exposure to mitomycin C (MMC): effects of concentration and exposure time.
Environ. Mutagenesis 2:262; 1980.

Littlefield, L. G.; DuFrain, R. J.; Joiner, E.; Colyer, S.; Batson, J.; and Welter, D. J.
Cytogenetic dose response kinetics in lymphocytes of persons with Down's syndrome following in vitro exposure to low LET radiation.
Mamm. Chromosomes Newslett. In press.

Moore, C., and Snyder, F.
S...nthesis of long chain alcohols by acyl-CoA reductase ... mouse preputial gland.
...c. *Exp. Biol. Med. (S.E. Sect.)* 4:15; 1979.

Morrison, W. D.; Huff, V.; Colyer, S.; Littlefield, G.; and DuFrain, R.
Cytogenetic effects of *cis*-platinum(II)diaminedichloride (*cis*-PPD) on rabbit and human cells.
Environ. Mutagenesis 2:298-299; 1980.

Partain, C. L., Hübner, K. F.; Mahaley, M. S.; Rollo, F. D.; Price, R. R.; and Scatliff, J. H.
Brain tumor imaging based on ^{11}C-labeled amino acid utilization using positron emission tomography.
Invest. Radiol. In press.

Partain, C. L.; Hübner, K. F.; Mahaley, M. S.; Rollo, F. D.; Price, R. R., and Scatliff, J. H.
Brain tumor imaging—positron emission tomography using ^{11}C-labeled amino acids.
J. Comput. Assist. Tomogr. In press.

Partain, C. L.; Hübner, K. F.; Staab, E. V.; Scatliff, J. H.; Miller, G. F.; and Gibbs, W. D.
CSF Kinetics: comparison of nuclear medicine, contrast enhanced CT, and positron emission tomography.
Invest. Radiol. 14:377; 1979.

...ain, C. L.; Price, R. R.; James, A. E. Jr.; Hübner, K. F.; Mahaley, M. S.; Scatliff, J. H.; and Robertson, J. T.
Positron emission tomography: brain tumor imaging using carbon-11 amino acids
Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging, Heidelberg, Germany, 1980. In press.

Polednak, A. P.
Studies of workers exposed to internal radiation (uranium and thorium).
Am. J. Epidemiol. In press.

Renooij, W.; Wykle, R. L.; Blank, M. L.; Lee, T-c.; Malone, B.; Fitzgerald, V.; and Snyder, F.
Metabolism of 1-alkyl-2-acetoyl-*sn*-glycero-3-phosphocholine, an antihypertensive phospholipid.
Fed. Proc. 39:2187; 1980.

Richter, C. B.; Swartzendruber, D. C.; and Littlefield, L. G.
Neoplastic disease of the large bowel of the cotton-topped tamarin.
J. Am. Med. Vet. Assoc. 176:880; 1980.

Sayer, A. M.; Littlefield, L. G.; DuFrain, R. J.; and Richter, C. B.
Mutagen-induced chromosome lesions in lymphocytes of *Saguinus oedipus*—A possible genetic marker for animals at risk for colon cancer.
Environ. Mutagenesis 2:297; 1980.

Sayer, A. M.; Littlefield, L. G.; Richter, C. B.; DuFrain, R. J.; and Batson, J. S.
Mutagen-induced chromosome lesions in lymphocytes of *Saguinus oedipus* and *Saguinus fuscicollis*.
Soc. Exp. Biol. Med. (S.E. Sect.) 4:18; 1979.

Urso, P., and Gengozian, N.
Suppression of humoral and cell-mediated immune response after in utero exposure to benzo(α)pyrene.
Fed. Proc. 39:775; 1980.

Urso, P., and Gengozian, N.
Depressed immunity and increased tumor incidence in progeny of pregnant mice exposed to benzo(α)pyrene.
Abstracts, Fourth International Congress of Immunology, International Union of Immunological Societies Paris, France, 1980. Abstract 17.1.41. Paris: Société Francaise d'Immunologie, 1980.

Washburn, L. C.; Dees, L.; Byrd, B. L.; Sun, T. T.; and Hayes, R. L.
^{11}C-Labeled α-aminoisobutyric acid, a potential agent for positron tomographic assessment of blood-brain barrier disruption.
Proceedings of the 21st Annual Meeting, Southeastern Chapter, Society of Nuclear Medicine (Continuing Education Lectures), Nashville, Tennessee. In press.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Hayes, R. L.
^{11}C-Labeled branched-chain amines, potential agents for positron tomographic lung function studies.
J. Nucl. Med. 21(6):P13; 1980.

1028463

Published Abstracts of Full-Length Publications

Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; and Muirhead, E. E.
Antihypertensive activity of an alkyl ether analog of phosphatidylcholine.
Biochem. Biophys. Res. Commun. 90:1194-1200; 1979.

An alkyl ether analog of phosphatidylcholine was prepared from choline plasmalogen of fresh beef heart by reducing the alk-1-enyl moiety to an alkyl group and by hydrolyzing the *sn*-2 acyl moiety and replacing it with an acetoyl group. The basis for preparing and testing this compound resulted from experience with antihypertensive lipids derived from the renal medulla and from experience with ether lipid biochemistry. The alkyl lipid analog was found to possess powerful antihypertensive properties in the one-kidney, one-clip hypertensive rats when given either intravenously or orally. This activity induced acute and prolonged depressor effects. These actions were similar to those of a glycerophosphate obtained from fresh renal medulla and designated as APRL (antihypertensive polar renomedullary lipid).

Buonocore, E., and Hübner, K. F.
Positron-emission computed tomography of the pancreas: a preliminary study.
Radiology 133:195-201; 1979.

Twenty-seven patients with suspected pancreatic disease were examined with emission computed tomography (ECT) with ^{11}C-labeled amino acids. Of 24 patients with known clinical outcome, there were one false positive and two false negative results. One finding of interest was the increased concentration of radiopharmaceutical that accurately delineated the presence and extent of four pancreatic carcinomas and one lymphoma. In the normal subjects, ECT reliably identified the pancreas. Disadvantages of ECT include the need for a cyclotron and high-quality hot chemistry laboratory, and the relatively long scanning time. The advantage of ECT is that it offers a unique opportunity to image biologic activity of the pancreas.

Buonocore, E.; Hübner, K. F.; and Collmann, I. R.
Differentiation of retroperitoneal tumor using positron emission computed tomography.
J. Comput. Asst. Tomogr. 3:825-828; 1979.

Positron emission computed tomography (ECT) with [^{11}C]DL-tryptophan and [^{11}C]aminocyclobutanecarboxylic acid ([^{11}C]ACBC) has produced some promising results in patients with retroperitoneal masses. This brief report gives an example of one patient in whom a retroperitoneal mass could not be differentiated from the pancreas by transmission computed tomography but was suspected to be of pancreatic origin based on arteriographic findings. The ECT scans with [^{11}C]DL-tryptophan showed a normal pancreas, whereas the tumor-localizing agent, [^{11}C]ACBC, concentrated in a tumor surrounding the tail and body of the pancreas. This approach with dual radiopharmaceuticals for the differentiation of metabolically active neoplasms and normal tissues warrants further investigation.

Buyukmihci, N., and Richter, C. B.
Prevalence of ocular disease in a colony of tamarins and marmosets.
Lab. Anim. Sci. 29:800-804; 1979.

In a large tamarin and marmoset experimental colony, 526 animals were examined by biomicroscopy and ophthalmoscopy for the presence of ocular disease. In 109 animals, there were 147 abnormalities involving the eyelids, cornea, iris, lens, retina, or optic nerve. Most abnormalities were unimportant in terms of ocular function, but a few did cause loss of vision and included diffuse, progressive retinal atrophy and a severely traumatized globe. The survey indicated that while usually minor, ocular disease in the tamarin and marmoset was widespread.

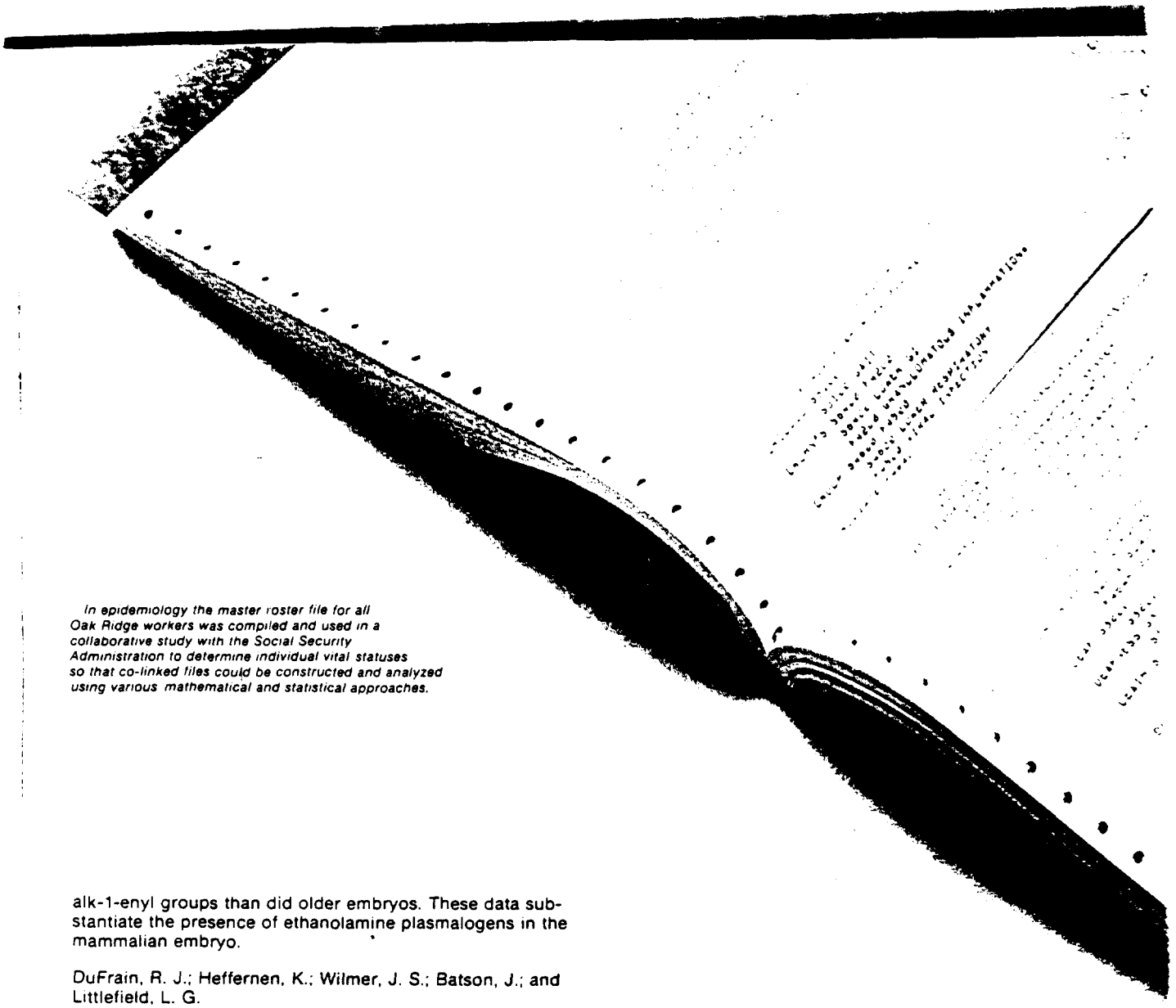
Cabot, M. C., and Snyder, F.
Manipulation of alkylglycerolipid levels in cultured cells. Fatty alcohol versus alkylglycerol supplements.
Biochim. Biophys. Acta 617:410-418; 1980.

Ehrlich ascites cells and monolayers of L-M cell fibroblasts were grown in medium containing either long-chain fatty alcohols or alkylglycerols. The cells were then analyzed to determine the contribution of these lipid precursors to the synthesis of complex lipids for the purpose of defining the most efficient system to elevate the levels of ether phospholipids. Label from high specific activity [1-^{14}C]hexadecanol, [1-^{14}C]octadecanol, and [1-^{14}C]octadecenol was incorporated into alkyl linkages (C-18:1>C-16:0>C-18:0); however, similar labeling of acyl groups occurred. Increasing the amount of hexadecanol in the growth medium resulted in a higher percentage of ^{14}C-labeled acyl groups than alkyl linkages at all concentrations of the alcohol supplement. Supplements of *rac*-[1-^{14}C]hexadecylglycerol to the growth media were assimilated into phospholipids, which significantly increased as a function of the amounts added. Mass determinations of the alkyl ether phospholipid content in L-M cells incubated for 24 hours with an alkylglycerol mixture (10.8 μg/ml) showed an approximate 70% increase over control levels; supplementation had only a slight effect on the alk-1-enyl content. The systems described will be useful for investigating biophysical and biochemical effects of alkyl ether enrichment in membranes.

Chepenik, K. P.; Blank, M.; Snyder, F.; and Waite, B. M.
On the presence of plasmalogens in developing rat embryos.
Int. J. Biochem. 11:605-607; 1980.

Ethanolamine plasmalogens were found to constitute as much as 30% of the total 1,2-diradyl-*sn*-glycero-3-phosphoethanolamine in the early rat embryo. Qualitative and quantitative analysis revealed that the relative amount of ethanolamine phospholipid present in the rat embryo as ethanolamine plasmalogen remained fairly constant during the period of organogenesis. Fetal brain was found to contain relatively more ethanolamine plasmalogen than did fetal liver. Ethanolamine plasmalogens extracted from early embryos contain relatively less 16:0 alk-1-enyl groups and relatively more 18:1

1028464



In epidemiology the master roster file for all Oak Ridge workers was compiled and used in a collaborative study with the Social Security Administration to determine individual vital statuses so that co-linked files could be constructed and analyzed using various mathematical and statistical approaches.

alk-1-enyl groups than did older embryos. These data substantiate the presence of ethanolamine plasmalogens in the mammalian embryo.

DuFrain, R. J.; Heffernen, K.; Wilmer, J. S.; Batson, J.; and Littlefield, L. G.
Sister chromatid exchange and mitotic stimulation in lymphocytes from four species of the genus *Saguinus*.
J. Med. Primatol. 9:233-239; 1980.

Lymphocytes from 18 tamarins of four species were cultured in bromodeoxyuridine supplemented medium, stained by a fluorescence plus Giemsa method, and evaluated for both sister chromatid exchange and mitotic stimulation. The overall sister chromatid exchange frequency was found to be 7.02 ± 3.45 per cell with individual cells containing from 0 to 33 spontaneous sister chromatid exchanges. As a measure of mitotic stimulation a total of 3200 metaphases from the 18 animals were evaluated for the number of DNA replications in vitro by the fluorescence plus Giemsa differential chromatid staining.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Frome, E. L.
man cytogenetic dosimetry: a dose-response relationship alpha particle radiation from ^{241}Am.
ealth Phys. 37:279-289; 1979.

Cytogenetic dosimetry estimates to guide treatment of persons internally contaminated with transuranic elements have not previously been possible because appropriate in vitro dose-response curves specifically for alpha particle irradiation of human lymphocytes do not exist. Using well-controlled cytogenetic methods for human lymphocyte culture, an experimentally derived dose-response curve for ^{241}Am alpha particle (5.49 and 5.44 MeV) radiation of G_0 lymphocytes was generated. Cells were exposed to 43.8, 87.7, 175.3, or 350.6 nCi/ml ^{241}Am for 1.7 hours giving doses of 0.85, 1.71, 3.42, or 6.84 rad. Based on dicentric chromosome yield, the linear dose-response equation is $Y = 4.90\ (\pm 0.42) \times 10^{-2} X$, with Y given as dicentrics per cell and X as dose in rads. The study also shows that the two-break asymmetrical exchanges in cells damaged by alpha particle radiation are overdispersed when compared to a Poisson distribution. An example is presented to show how the derived dose-response equation can be used to estimate the radiation dose for a person internally contaminated with an actinide. An experimentally derived relative biological effect value of 118 or 0.85 rad is calculated for the efficiency of

1028465

^{241}Am alpha particle induction of dicentric chromosomes in human G_0 lymphocytes as compared with the efficiency of ^{60}Co gamma radiation. The maximum theoretical value for the relative biological effect for cytogenetic damage from alpha irradiation was determined to be 278 at 0.1 rad or less, which is in marked contrast to previously reported relative biological effect values of approximately 20.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
Cyclophosphamide-induced sister chromatid exchanges in rabbit lymphocytes.
Environ. Mutagenesis 1:283-286; 1979.

An experiment was performed to determine if extrinsic influences during handling and culture affect sister chromatid exchange (SCE) frequency in mitogen-stimulated lymphocytes. SCE frequencies were determined in lymphocyte chromosomes from cultures from a female New Zealand white rabbit before and 24 hours after a single injection of 35 mg/kg cyclophosphamide. Pre- and posttreatment blood samples (0.8 ml) were either placed into culture or washed twice with media before culture. All cultures had 10 μM bromodeoxyuridine added either immediately or 20 hours after phytohemagglutinin stimulation. Following 4 hours in colcemid, all cultures were harvested at 72 hours. The sister chromatids were differentiated by fluorescence plus Giemsa staining. Results of cyclophosphamide treatment, washing lymphocytes before culture, and varying the bromodeoxyuridine exposure time were subjected to an analysis of variance test to determine if either of the postblood collection procedures significantly altered the SCE frequency in control or treated cells. Before analysis SCE/cell values for 21 metaphases from each of the 8 cultures were transformed to square roots to equalize the variances between pre- and posttreatment group. The only significant factor was the cyclophosphamide treatment; neither washing for bromodeoxyuridine exposure time caused significant ($P < .05$) differences in SCE. These findings support the theory that some lesions induced in vivo in the DNA of G_1 lymphocytes persist and give rise to SCE.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
The effect of washing lymphocytes after in vivo treatment with streptonigrin on the yield of chromosome and chromatid aberrations in blood cultures.
Mutat. Res. 69:101-105; 1980.

Cytogenetic analyses of cultured blood lymphocytes from streptonigrin-treated female rabbits demonstrated that the yield of chemically induced chromatid aberrations, especially exchanges, is influenced by the method of blood handling before in vitro culture. When blood cells from treated animals were washed with tissue-culture medium before culture, chromatid breaks were observed at the same level as in the controls, and no chromatid exchanges were found. However, when duplicate aliquots of blood were inoculated directly into culture medium, without washing, elevated levels of chromatid breaks were observed and chromatid exchanges were found. These findings indicate that the clastogen, streptonigrin, was carried into the culture medium with the blood-cell inoculum.

Dupree, E. A., and Meyer, M. B.
Role of risk factors in complications of diabetes mellitus.
Am. J. Epidemiol. 112:100-112; 1980. Work performed at the Johns Hopkins University, Baltimore, Maryland.

To compare antecedent and concurrent factors in diabetics and matched controls, and to measure the role played by these factors in mortality, 371 "diabetics" aged 20-75 years were identified from a source population of 26,000 participants in a community-wide health survey carried out in Washington County, Maryland, in 1974. ("Diabetics" were those who reported on the survey questionnaire that they had taken antidiabetic medications in the previous 48 hours.) Two sex- and age-matched controls for each diabetic were selected from the survey population. Comparison characteristics included blood pressure measured at the time of the survey; marital status; grades of school completed; cigar, cigarette, and pipe smoking; use of aspirin, oral contraceptives, and female hormones; and treatment for hypertension. Deaths were ascertained prospectively for 39 months following the 1974 survey. Diabetics had higher systolic blood pressures than controls, the distribution for diabetics resembling that of controls who were 10 years older. More diabetics reported treatment for hypertension, and diabetics under treatment for hypertension had higher systolic blood pressures than treated controls. Both male and female diabetics treated with insulin or oral agents alone showed similar patterns, with orally treated females showing the largest differences between cases and controls. Orally treated females had over twice the rate of systolic blood pressure over 160 mmHg or of reported treatment for hypertension than did controls. It was also found that controls had completed more years of school, reported more use of oral contraceptives and female hormones, and used more aspirin than diabetics. Diabetics were more likely to have been married. Smoking habits did not differ between diabetics and controls except for more cigar smoking among diabetics. Diabetics had significantly higher mortality rates than controls in the 39 months after the survey. For all causes of death, diabetic rates were 2.5 times control rates. The increase was 1.5 times for orally treated and 8 times for insulin-treated diabetics compared with their own controls. For vascular causes, the mortality risk for diabetics was increased 4.8 times overall, 2.5 times for the orally treated, and 14.2 times for the insulin-treated compared with their own controls.

Frome, E. L., and Yakatan, G. J.
Statistical estimation of the pharmokinetic parameters in t. one compartment open model.
Commun. Statist.—Simula. Computa. B9:201-222; 1980.

The kinetics of a pharmaceutical formulation can be determined by modeling the relation between concentration of drug in the plasma and the time elapsed since administration. The resulting concentration-time curve reflects the absorption, distribution, and elimination of the drug. The simplest model that represents this kind of data is the one compartment open model. In many situations, this serves as a convenient model for estimating the bioavailability parameters—areas under the concentration-time curve, time to peak concentration, and peak concentration.

The one compartment open model is nonlinear in the unknown parameters and is usually fit using least squares. Gradient methods often fail for real world data, and we have found that the Nelder-Mead simplex algorithm provides a useful alternative. Further, we propose that least absolute values criteria be used as a resistant alternative to least squares. Both least absolute values and least square estimates can be obtained using an iterative weighted least squares algorithm. The iterative weighted least squares procedure is also used to obtain maximum quasilikelihood estimates and resistant estimates using the sine weight function. A numerical example is presented.

Fuhr, J. E., and Gengozian, N.
Fetal hemoglobin: in vivo stimulation by D-thyroxine in adult marmosets.
Blood Cells 5:471-474; 1979.

Reactivation of fetal hemoglobin gene expression was studied in New World monkeys (marmosets). After a series

1028466

of intraperitoneal injections of D-thyroxine, fetal hemoglobin production was increased in three experimental animals. Control animals were unaffected by the injection of the solvent. Because the percentage of cells containing fetal hemoglobin exceeded the total percentage of fetal hemoglobin, it was concluded that thyroid hormone influenced the synthesis of fetal hemoglobin rather than the development of a population of fetal cells.

Gengozian, N.; Brewen, J. G.; Preston, R. J.; and Batson, J. S.
Presumptive evidence for the absence of functional germ cell chimerism in the marmoset.
J. Med. Primatol. 9:9-27; 1980.

Presumptive evidence for functional germ cell chimerism in the marmoset was evaluated by calculating the offspring sex ratio of young derived from animals in which the blood chimerism status was known. In the four possible mating combinations involving chimeric and nonchimeric marmosets, there was no apparent deviation from the anticipated 1:1 sex ratio. Analysis of 2500 primary spermatocytes from 10 known blood chimeras failed to reveal a single cell in which an unequivocal XX bivalent could be identified. The combined breeding and cytogenetic data offer presumptive evidence against functional germ cell chimerism in the marmoset.

Gengozian, N., and McLaughlin, C. L.
IgG+ platelets in the marmoset: their induction, maintenance, and survival.
Blood 55:885-890; 1980.

Immunization of marmosets with platelets from another species of marmoset leads to antibody formation to the donor platelets, deposition of IgG on the host's platelets, and thrombocytopenia. This disease closely resembles post-transfusion purpura of man, which may develop after one or two transfusions of whole blood. The mode of immunization in the marmoset was found to be important: intravenous inoculations were without effect, while intramuscular immunizations led to the disease. Intramuscular inoculations were characterized by formation of 7S antibodies, as measured by indirect immunofluorescent and complement-dependent platelet cytotoxicity tests; in contrast, intravenous immunizations, while leading to 7S antibodies by the immunofluorescent test, yielded only 19S antibodies reactive in the platelet cytotoxicity assay. The titers were also consistently higher with intramuscular immunizations. Antibody was not limited to the donor platelets, but auto- or host-type reactivity was also present; this antibody was in very low titer and could be found only when the animal was thrombocytopenic. A primary finding was the ability to maintain increased deposition of IgG on the host's platelets in the absence of thrombocytopenia by biweekly or monthly inoculations of the donor platelet antigen. The amount of IgG found on platelets of normal and immunized marmosets was comparable to that reported for normal humans and patients with clinical immune thrombocytopenia. Finally, platelet survival studies in animals with IgG+ platelets and normal platelet counts indicated a rapid turnover, suggesting operation of a compensatory mechanism to maintain platelet levels.

...main, G. S., and Arneson, R. M.
Effects of metal ions and selenoamino acids on induction of glutathione peroxidase in mouse neuroblastoma.
Enzyme 24:337-342; 1979. Work performed at the University of Tennessee Center for the Health Sciences, Memphis.

The induction of glutathione peroxidase in mouse neuroblastoma cells by selenite is enhanced by equimolar amounts of arsenate, arsenite, molybdate, chromic, or dichromic ions. At equimolar selenium concentration, selenite, selenocystine, and selenomethionine induced glutathione peroxidase activities having the ratios 4:4:1. Protein synthesis inhibitors prevented the induction of glutathione peroxidase by selenite indicating that de novo protein synthesis is required.

Godbold, J. H., Jr., and Tompkins, E. A.
A long-term mortality study of workers occupationally exposed to metallic nickel at the Oak Ridge Gaseous Diffusion Plant.
J. Occup. Med. 21:799-806; 1979.

This study was undertaken to determine whether mortality from respiratory cancer among workers occupationally exposed to metallic nickel at the Oak Ridge Gaseous Diffusion Plant differed from that of workers at the same plant with no record of occupational exposure to metallic nickel or any nickel compound. A cohort of 814 nickel-exposed workers and one of 1600 controls were identified. The members of both cohorts had a minimum follow-up period of 19 years. Mortality from respiratory cancer and from other causes was examined in both groups. The data showed no evidence of an increased risk of mortality due to respiratory cancer among the nickel-exposed workers. The exposed cohort experienced lower mortality than the controls, both in deaths due to respiratory cancer and in deaths due to all causes, although neither of these differences was statistically significant.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; and Carlton, J. E.
The effect of scandium on the tissue distribution of ^{67}Ga in normal and tumor-bearing rodents.
J. Nucl. Med. 21:361-365; 1980.

In rats and mice the intravenous administration of scandium before or with ^{67}Ga produces in rats an increase in ^{67}Ga excretion and bone deposition, coupled with pronounced decreases in the uptake of ^{67}Ga in soft tissues. These effects result from the blocking by scandium of ^{67}Ga plasma-protein binding sites, which forces ^{67}Ga into an unbound or loosely bound state. This increases ^{67}Ga excretion and bone deposition, which in turn act to produce greatly reduced ^{67}Ga uptake in soft tissues. When tumor-bearing rats and mice are administered scandium, similar effects occur; but the uptake of ^{67}Ga by tumor tissue remains unchanged. This suggests that ^{67}Ga enters tumor and normal soft tissues by different routes. With tumor, an unbound or loosely bound form of gallium is primarily involved, whereas with normal soft tissues this route is apparently of minor importance.

Hodges, H. D., and Aldridge, R. E.
Radiation safety in the nuclear medicine laboratory.
J. Nucl. Med. Technol. 8:87-89; 1980.

Those of us who are actively engaged in handling and manipulation of radioactivity in either solid, liquid, or gaseous forms must strive constantly to maintain the delicate balance between providing maximum efficiency and preventing error. Technologists who work in the nuclear medicine laboratory preparing doses, assaying samples, or doing radioimmunoassay procedures should not concentrate only on accident prevention but should know also the procedures to be followed in the event of a radiation spill in their laboratory. We review and discuss six aspects directly related to radiation spills and suggest appropriate actions that could be taken should an accident occur.

1028467

Lee, T-c., and Fitzgerald, V.
Phase transitions of alkyl ether analogs of phosphatidylcholine.
Biochim. Biophys. Acta 598:189-192; 1980.

The phase-transition temperatures of aqueous dispersions of diester, monoether, and diether analogs of phosphatidylcholine were determined using *trans*-parinaric acid as a fluorescent probe. The diether analog of phosphatidylcholine has a higher phase-transition temperature, whilst the monoether analog has a lower phase-transition temperature than the diester counterpart.

Lee, T-c.; Fitzgerald, V.; Stephens, N.; and Snyder, F.
Activities of enzymes involved in the metabolism of ether-linked lipids in normal and neoplastic tissues of rat.
Biochim. Biophys. Acta 619:420-423; 1980.

We have compared the activities of three enzymes (acyl-CoA reductase, fatty alcohol:NAD^+ oxidoreductase, and alkyl synthase) involved in the metabolism of ether lipids in rat liver and two tumors that contained various levels of ether lipids. The activity of fatty alcohol:NAD^+ oxidoreductase was high and the activities of acyl-CoA reductase and alkyldihydroxyacetone-*P* synthase were low in liver, which has low amounts of ether glycerolipids. On the other hand in Fischer R-3259 sarcoma, where high concentrations of ether-linked lipids were present, the reverse pattern was observed. In Morris hepatomas 5123C, with an intermediate value of ether lipids, the activities of all three enzymes ranged between those found in liver and Fischer sarcomas.

Lee, T-c.; Hougland, A. E.; and Stephens, N.
Perturbation of lipid metabolism in L-M cultured cells by elaidic acid supplementation: formation of fatty alcohols.
Biochem. Biophys. Res. Commun. 91:1497-1503; 1979.

Supplementation of culture medium with elaidic acid (400 g/flask) in L-M cells results in the formation of an otherwise undetected lipid component. We have identified this lipid component to be a mixture of free fatty alcohols containing primarily elaidyl alcohol with cetyl, stearoyl, and oleoyl alcohols as minor constituents. Formation of fatty alcohols by fatty acid supplementation seems to be specific with *trans* fatty acids (i.e., elaidate, *trans* vaccenate, and linolelaidate); addition of stearate and oleate to the L-M cells does not produce fatty alcohols. The fatty alcohols accumulated by the *trans* fatty acid supplementation are associated with both the particulate and supernatant fractions of the cells.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Comparison of sister-chromatid exchanges in human lymphocytes after G_0 exposure to mitomycin in vivo vs. in vitro.
Mutat. Res. 69:191-197; 1980.

Sister-chromatid exchanges (SCE) were evaluated in cultured lymphocytes from a cancer patient 1, 4, and 8 days after he received a single 36-mg bolus of Mutamycin. Extensive SCE (>40 SCE/metaphase) were observed in standard autologous plasma cultures from the patient, as well as in preparations initiated after extensive washing of his lymphocytes. Statistical analyses of these data demonstrated no significant differences in SCE frequencies between cultures initiated in autologous versus homologous plasma, nor between cultures initiated 1, 4, or 8 days after treatment with Mutamycin. SCE frequencies in lymphocytes from this patient were comparable to those observed in control mphocytes following a 2-hour G_0 exposure to mitomycin C .5-1.5 μg/ml). These findings suggest that mitomycin C is equally effective in inducing lesions in DNA that can be expressed as SCE in human lymphocytes exposed in vivo versus in vitro and demonstrate that no diminution in mean SCE frequencies nor shift in distribution of SCE occur within the first 8 days after Mutamycin treatment.

McDermott, C. E., and Gengozian, N.
The effect of low exposure-rate gamma irradiation on T and B lymphocyte function in the mouse.
Int. J. Radiat. Biol. 37:415-428; 1980.

The effect of chronic irradiation on T and B cell numbers and function was studied in mice. Cobalt-60 gamma radiation at 6 R/hr reduced the numbers of anti-sheep red blood cell plaque-forming cells in the spleen, with minimal levels recorded after total exposures of 1000-2000 R. Recovery was incomplete after 1000 R, reaching only 40-50% of normal in 4 months and remaining at that level for the animal's lifetime. The long-term deficiency in plaque-forming cell formation was not due to a quantitative lack of T or B cells since normal cell numbers were observed in the spleen 60-144 days after 1000 R. Adoptive transfer studies with combinations of bone marrow and thymus cells, or of splenic T and B cells, from normal and irradiated mice, revealed functional defects in both cell compartments during the first 2 months. Normal and near normal function of T and B cells occurred 100 days postirradiation, a time when the splenic in vivo response was still only 50% of the controls. The latter observation suggests that the microenvironment of the chronically irradiated spleen alters factors regulating T and B cell interactions in response to a T-dependent antigen.

Palisano, J. R.; Walne, P. L.; and Brown, D. H.
Ultrastructure and physical characteristics of proplastids and chloroplasts isolated by zonal centrifugation from *Euglena gracilis* var. *bacillaris*.
Eur. J. Cell Biol. 21:305-312; 1980.

Proplastids at various stages of development and mature chloroplasts were isolated by zonal centrifugation in a double-sigmoid, sucrose-sorbitol gradient from *Euglena gracilis* var. *bacillaris* grown in darkness and subjected subsequently to various periods of illumination with white light. Computer programs were used in characterizing physical properties of the isolated organelles and in predicting the location of plastids of different developmental stages in the gradient. The technique permits computation of these parameters in any gradient material. The convoluted nature of the early developing plastids, revealed by electron microscopy, suggests that the frictional coefficients should be taken into account in describing the physical constants of the organelles, since their sedimentation coefficients are lower than would be expected for spherical particles of the same size and density.

Polednak, A. P.
Mortality among men occupationally exposed to phosgene in 1943-1945.
Environ. Res. 22:357-367; 1980.

Mortality and causes of death as reported on death certificates were described among white male workers exposed to phosgene in 1943-1945 at a uranium-processing plant in Tennessee. Standardized mortality ratios were calculated by using death rates for United States white males. As of 1974, standardized mortality ratios for all causes and for various selected causes were similar in 699 chemical workers chronically exposed to low levels of phosgene (and to daily episodes of levels above 1 ppm) and in 9352 controls who worked at the same plant. Standardized mortality ratios for diseases of the respiratory system were 78 and 113 in the chemical workers and controls, respectively. A

1028468

group of 106 male chemical and other workers was acutely exposed to higher levels of phosgene (i.e., above 50 ppm, ased on clinical symptoms), and 25 had X-ray evidence of acute pneumonitis. Among the 106 men, one death from pulmonary edema due to phosgene poisoning occurred less than 24 hours after exposure, and a total of 30 deaths occurred as of 1974 (standardized mortality ratio = 113); there were no deaths from lung cancer, but three deaths (versus 1.37 expected) were due to respiratory diseases. Further follow-up of these groups is needed, including data on morbidity as well as mortality.

Polednak, A. P., and Frome, E. L.
Mortality among men employed (between 1943 and 1947) at a uranium-processing plant.
Proceedings of the 1979 DOE Statistical Symposium, Gatlinburg, Tennessee, 1979, CONF-791016, pp. 116-127, ed. D. A. Gardiner and T. Truett. Washington, D. C.: U. S. Department of Energy, 1980.

Mortality is described in a cohort of 19,500 white males who were employed at the Tennessee Eastman Corporation in Oak Ridge, Tennessee (in the period between 1943 and 1947), where uranium was enriched by an electromagnetic separation process. As of January 1, 1974, some 5500 men had died, according to records of the Social Security Administration; copies of death certificates were obtained for most decedents. Expected numbers of deaths from specific causes were calculated on the basis of person-years of follow-up from year of first employment and applying death rates for U.S. white males specific for age (5-year groups), cause of death, and calendar year (5-year intervals). The standardized mortality ratio for all causes of death s less than 1.00 (i.e., 0.88), and few of the standardized ortality ratios for 59 causes or groups of causes were significantly different from 1.00. Mortality from cancer, especially cancer of the lung, was of particular interest since uranium dust was the major potential health hazard. Some workers in the first stage of the electromagnetic separation process were exposed to average air levels of uranium dust that were several times past our present maximum permissible levels. There was no evidence for significantly higher mortality from lung cancer or other cancers in comparisons by subgroups based on departments of employment (corresponding to reported average levels of uranium dust) or length of employment in those departments.

Follow-up of this cohort is continuing, and analyses of mortality until the end of 1978 (i.e., 31 to 35 years after employment) are in progress. Various methods of follow-up and ascertainment of deaths are being utilized.

Renooij, W., and Van Golde, L. M. G.
Asymmetry in the renewal of molecular classes of phosphatidylcholine in the rat erythrocyte membrane.
Biochim. Biophys. Acta 558:314-319; 1979. Work performed at the State University of Utrecht, The Netherlands.

Rat erythrocytes labeled in vivo with [^{32}P]phosphate were treated with phospholipase A_2 and sphingomyelinase to discriminate between the labeling patterns of the phospholipids from the inner and outer layer of the membrane. The specific activities of the more unsaturated classes of phosphatidylcholine were higher in the outer layer ie membrane than in the inner layer. The disaturated class, however, had the highest specific activity in the inner layer. The labeling pattern recovered in the molecular classes of plasma phosphatidylcholine labeled by exchange with erythrocyte phosphatidylcholine was very similar to that of the phosphatidylcholines in the outer layer of the erythrocyte membrane. It is proposed that the exchange of phosphatidylcholine between plasma and erythrocytes is mainly responsible for the renewal of the unsaturated phosphatidylcholines of the erythrocyte, and that the acylation activity of the erythrocyte is directed toward the formation of disaturated phosphatidylcholines at the inside of the membrane.

Ricks, R. C.
A training course for radiation-accident management in the health physics/medical environment.
Health Physics Training, Proceedings of the Health Physics Society 13th Midyear Topical Symposium, Honolulu, 1979, pp. 7-15, ed. W. L. Yanger and P. J. Manly. Honolulu: Hawaii Chapter, Health Physics Society.

Since the birth of the nuclear age, the availability of radiation-accident management training courses for the health or medical physicist performing in a medical environment has been limited. This paper describes an extensive, medically oriented, radiation-accident management training course for the health physicist who desires such experience. The course is taught at the Radiation Emergency Assistance Center/Training Site (REAC/TS) in Oak Ridge, Tennessee. REAC/TS, operated by Oak Ridge Associated Universities for the U.S. Department of Energy, is fully equipped to respond to the medical emergency/decontamination needs of radiation accident victims, thus complementing its use as a training facility. The health physics training course includes a brief health physics procedures review, radiation-accident history, medical consequences of radiation accidents, decontamination procedures, bioassay, accident dosimetry, and emergency procedures. The role of the health physicist in directing the radiological aspects of an emergency medical team is stressed. Instruction is complemented with radiation-accident drills emphasizing total response and realism. A thorough critique of the radiation-accident drill evaluates the participants' response to health physics problems and their interaction with the medical team, news media, the public, etc. Techniques will be presented for providing realistic training in a technology where experience is so important but difficult to obtain other than by simulation and drills.

Schremmer, J. M.; Blank, M. L.; and Wykle, R. L.
Bradykinin-stimulated release of [^{3}H]arachidonic acid from phospholipids of $HSDM_1C_1$ cells: comparison of diacyl phospholipids and plasmalogens as sources of prostaglandin precursors.
Prostaglandins 18:491-505; 1979.

Ethanolamine plasmalogens (1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamines) of many tissues contain high levels of arachidonate at their 2-position, and in certain tissues have been implicated as possible donors of arachidonate required in the synthesis of prostaglandins (PGs) and thromboxanes. In the present study, [^{3}H]-arachidonate-labeled phospholipids of $HSDM_1C_1$ cells, a cell line derived from a mouse fibrosarcoma, were examined to determine the donor of the arachidonic acid released upon bradykinin stimulation of the synthesis of PGE_2. $HSDM_1C_1$ cells labeled with [^{3}H]arachidonic acid for 24 hours in serum-free medium were used in most of the experiments and had the following distribution of label among the cellular lipids: phosphatidylcholine (33%), phosphatidylinositol (20%), diacyl-*sn*-glycero-3-phosphoethanolamine (15%), ethanolamine plasmalogen (15%), and less polar lipids (16%). Bradykinin treatment stimulated a rapid hydrolysis of [^{3}H]arachidonate from the cellular lipids and conversion of the released acid to PGE_2, which was secreted into the medium. The label was released

1028469

predominantly from phosphatidylinositol and possibly from phosphatidylcholine with no detectable change in the labeling of diacyl- or 1-alk-1'-enyl-2-acyl-*sn*-glycero-phosphoethanolamine. The ethanolamine plasmalogens, therefore, do not appear to be involved in the stimulated release of arachidonate in the $HSDM_1C_1$ cells. Indomethacin blocked the bradykinin-stimulated synthesis of PGE_2 and to a lesser degree inhibited the release of [^{3}H]arachidonate from the cellular lipids into the medium.

Scott, C. C.; Heckman, C. A.; and Snyder, F.
Regulation of ether lipids and their precursors in relation to glycolysis in cultured neoplastic cells.
Biochim. Biophys. Acta 575:215-224; 1979.

Tumors typically show high rates of glycolysis and elevated levels of ether lipids, particularly the alkyldiacylglycerols; thus, we investigated the relationship between ether lipid accumulation and glucose metabolism in a neoplastic cell line (B2-1). The B2-1 cells grown in 5.5 mM galactose in the absence of glucose produced very low levels of alkyldiacylglycerols, triacylglycerols, lactic acid, and dihydroxyacetone-P. Increasing concentrations of glucose caused a progressive increase in lactic acid, dihydroxyacetone-P, and up to a 10-fold increase in alkyldiacylglycerols and triacylglycerols. Glucose supplements also caused an increased incorporation of [9,10-^{3}H]palmitic acid into alkyldiacylglycerols and triacylglycerols. These metabolic changes appeared to be independent of altered growth rates of the cells. The addition of hexadecanol along with glucose to the cultures resulted in a shorter lag and a more rapid rate of accumulation of alkyldiacylglycerols; hexadecanol supplements alone had no effect. The extent of uptake and oxidation of hexadecanol was similar in both the glucose- and galactose-grown cells. These results indicate that the levels of alkyldiacylglycerols in neoplastic cells can be regulated by the extent to which their precursors are formed from glucose.

Snyder, F.; Lee, T-c.; Blank, M. L.; and Moore, C.
In vivo modification of phospholipid polar head groups and alterations in membrane activities and cellular events.
Membrane Fluidity: Biophysical Techniques and Cellular Regulation, pp. 307-323, ed. M. Kates and A. Kuksis. Clifton, New Jersey: The Humana Press, Inc., 1980.

Assessment of the functional role of the polar head group portion of membrane phospholipids in vivo is a difficult task. Our laboratory has approached this problem by using lipid analog precursors to alter the composition of the polar head groups. We have shown that *N*-isopropylethanolamine (IPE), a competitive inhibitor of choline, can be incorporated into 1,2-diacyl-*sn*-glycero-3-phosphoisopropylethanolamine (phosphatidyl-IPE) of membranes from cultured L-M cells and rat liver. After IPE treatment, we observed profound inhibition of certain enzymatic activities (e.g., Δ9 acyl-desaturase and 5'-nucleotidase), but not in others (e.g., NADH-cytochrome *c* reductase, NADPH-cytochrome *c* reductase, and succinic dehydrogenase). The changes in enzyme activities do not appear to be directly related to membrane fluidity or the formation of phosphatidyl-IPE.

Urso, P., and Gengozian, N.
Nonspecific suppressor elements in murine allogeneic radiation chimeras.
Cell. Immunol. 47:19-31; 1979.

Spleen cells from long-term mouse allogeneic radiation chimeras were tested for their ability to modulate the graft-versus-host (GvH) or plaque-forming cell (PFC) response of normal lymphocytes transplanted in lethally X-irradiated recipients. In vivo GvH proliferation of normal lymphocytes (syngeneic to donor cells of the chimera) against antigens of host type in which the chimeric state had been established was reduced by chimera cells. Inhibition varied, some chimeras suppressing GvH more than others and a few not suppressing at all. The suppressive effect was abrogated if the chimera cells were treated with anti-Θ; treatment with anti-IgM did not eliminate this activity. When mixtures of normal donor lymphocytes and chimera cells were given to irradiated recipients genetically different from host or donor, reduction of donor cell GvH also occurred. Further, chimera cells reduced the GvH activity of normal host cells in irradiated recipients differing from the host at one H-2 locus and from the donor at minor histocompatibility loci. The modulating effect of spleen cells from chimeras on the PFC response by normal lymphocytes also varied. Six chimeras induced a 25-90% suppression, two enhanced the response, and one showed no effect. Where suppression occurred, treatment of chimera cells with anti-Θ most often, but not always, restored PFC production. Our results show that the suppressive action of splenic lymphoid cells by chimeras is highly nonspecific and variable in expression. We suggest that tolerance in chimeras may be mediated by nonspecific suppressor elements leading to unresponsiveness to a variety of antigens including sheep red blood cells.

Urso, P., and Gengozian, N.
Depressed humoral immunity and increased tumor incidence in mice following in utero exposure to benzo(α)pyrene.
J. Toxicol. Environ. Health 6:569-576; 1980.

Exposure of mice to a single small dose of benzo(α)pyrene in utero leads to a severe suppression of the anti-sheep erythrocyte plaque-forming response shortly after birth that persists into later life. There is also an 11- to 13-fold increase in the frequency of tumors in these mice at 56-78 weeks of age compared to the untreated control group. Mice exposed to 150-R X-irradiation in utero, after an early immune suppression, show recovery of the plaque-forming response with no increase in tumor incidence. The sustained suppression in immune status induced in mice by exposure to benzo(α)pyrene during fetal life may be related to an altered immune surveillance mechanism and a consequent increased susceptibility to tumor development.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; and Butler, T. A.
1-Aminocyclobutane[^{11}C]carboxylic acid, a potential tumor-seeking agent
J. Nucl. Med. 20:1055-1061; 1979.

1-Aminocyclobutane [^{14}C]carboxylic acid ([^{14}C]ACBC) was incorporated preferentially by several tumor types in rats and hamsters. The agent was cleared rapidly from rat blood, attaining its maximum tissue concentrations within 30 minutes after intravenous injection. Carrier ACBC had little effect on the tissue distribution of [^{14}C]ACBC. This agent showed no affinity for a *Staphylococcus aureus* abscess in rats. The total excretion was low, 3.6% in 2 hours.

[^{11}C]ACBC was synthesized in amounts up to 415 mCi (55% chemical yield) using our modified Bücherer-Strecker technique. Forty minutes were required for the two-step synthesis and chromatographic purification. ACBC was found to be nontoxic in three animal species. The radiation dose from [^{11}C]ACBC should be minimal. [^{11}C]ACBC thus appears to have good potential as a tumor-seeking agent, particularly when used with a positron emission computed tomograph.

1028470

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Hayes, R. L.; Butler, T. A.; and Callahan, A. P.
gh-level production of [^{11}C]carboxyl-labeled amino acids.
Radiopharmaceuticals II: Proceedings of the 2nd International Symposium on Radiopharmaceuticals, Seattle, 1979, pp. 767-777. New York: The Society of Nuclear Medicine, 1979.

^{11}C-Labeled amino acids have significant potential as agents for positron tomographic functional imaging. We have developed a rapid, high-temperature, high-pressure modification of the Bücherer-Strecker amino acid synthesis and found it to be quite general for the production of [^{11}C]carboxyl-labeled neutral amino acids. Production of [^{11}C]carboxyl-labeled DL-tryptophan requires certain modifications in the procedure. Twelve different amino acids have been produced to date by this technique. Synthesis and chromatographic purification require approximately 40 minutes and [^{11}C]carboxyl-labeled amino acids have been produced in yields of up to 425 mCi. Two [^{11}C]carboxyl-labeled amino acids are being investigated clinically for tumor scanning and two others for pancreatic imaging. Over 120 batches of the various agents have been produced for clinical use over a 3-year period.

Wykle, R. L.; Kraemer, W. F.; and Schremmer, J. M.
Specificity of lysophospholipase D.
Biochim. Biophys. Acta 619:58-67; 1980.

The specificity of lysophospholipase D (1-alkyl-*sn*-glycero-3-phosphoethanolamine ethanolaminehydrolase, EC 3.1.4.39, also works on choline analogs) for 1-alkyl- and 1-acyl-linked substrates was examined using rat liver microsomes. The microsomes were treated with diisopropylphosphorofluoridate to inhibit the hydrolysis of acyl chains from the acyl-linked compounds (1-palmitoyl-*sn*-glycero-3-phosphocholine and 1-palmitoyl-*sn*-glycero-3-phosphoethanolamine) and were treated with *p*-bromophenacyl bromide to block acylation of the compounds tested. In the presence of the inhibitors, 1-alkyl-*sn*-glycero-3-phosphocholine and 1-alkyl-*sn*-glycero-3-phosphoethanolamine were hydrolyzed extensively by lysophospholipase D but the corresponding 1-acyl-linked analogs were only negligibly hydrolyzed. Lysophospholipase D therefore appears to be specific for the ether-linked compounds. 1-Alk-1'-enyl-*sn*-glycero-3-phosphoethanolamine (lyso plasmalogen) was also tested as a substrate, but a plasmalogenase in the rat liver microsomes rapidly hydrolyzed the compound and we were unable to determine whether it is a substrate for lysophospholipase D. Alkyl-linked substrates containing long-chain acyl groups at the 2-position are not hydrolyzed by the enzymes. We tested 1-alkyl-2-acetoyl-*sn*-glycero-3-phosphocholine and 1-alkyl-2-acetoyl-*sn*-glycero-3-phosphoethanolamine to determine if the less bulky, more hydrophilic acetate group would permit hydrolysis by lysophospholipase D; the derivatives did not appear to be attacked, except after hydrolysis of the acetate group. However, in the absence of inhibitors, the acetate groups were rapidly hydrolyzed by microsomal preparations.

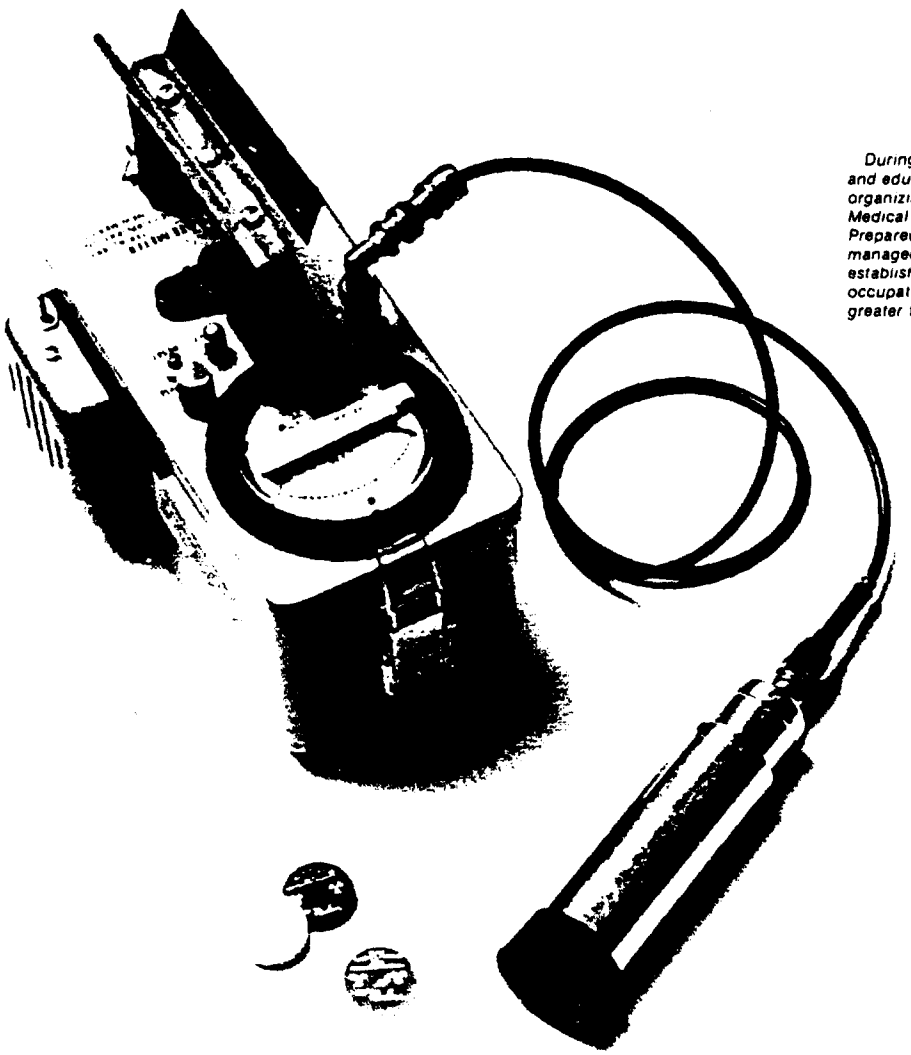
Wykle, R. L.; Malone, B.; Blank, M. L.; and Snyder, F.
Biosynthesis of pulmonary surfactant: comparison of 1-palmitoyl-*sn*-glycero-3-phosphocholine and palmitate as precursors of dipalmitoyl-*sn*-glycero-3-phosphocholine in adenoma alveolar type II cells.
Arch. Biochem. Biophys. 199:526-537; 1980.

Urethan-induced pulmonary adenomas of mice are composed of cells that appear to be morphologically identical to alveolar type II cells and synthesize disaturated diacyl-*sn*-glycero-3-phosphocholine (GPC), the major component of pulmonary surfactant. 1-[1-^{14}C]Palmitoyl-GPC and [1-^{14}C]palmitic acid were compared as precursors of disaturated diacyl-GPC in the adenoma type II cells by incubating both substrates with whole adenomas. When the precursors were compared at equal concentrations (100μM) in the presence of albumin (1 mg/ml), the rates of incorporation of 1-[1-^{14}C]palmitoyl-GPC and [1-^{14}C]palmitic acid into diacyl-GPC were 5.2 and 2.9 nmol/min/g tissue, respectively. The concentration of monoacyl-GPC (lysolecithin) in the blood plasma of BALB/c mice was 150 μM. In short-term labeling experiments, the label in disaturated diacyl-GPC was equally distributed between *sn*-1 and *sn*-2 positions when 1-[1-^{14}C]-palmitoyl-GPC was the precursor, whereas 75 to 80% was in the *sn*-2 position when [1-^{14}C]palmitic acid was the precursor. The ratios are consistent with incorporation of 1-palmitoyl-GPC via the lysolecithin:lysolecithin transacylase reaction and incorporation of palmitate via acylation of 1-palmitoyl-GPC by acyl-CoA:lysolecithin acyltransferase. 1-[1-^{14}C]Palmitoyl-*sn*-glycero-3-phospho-[^{3}H-*methyl*]choline was incorporated into total cellular diacyl-GPC with an isotope ratio similar to that of the precursor; the disaturated species was more enriched in ^{14}C. These findings indicate the cells take up intact monoacyl-GPC and incorporate it into diacyl-GPC. The ability of the cells to utilize intact lysophosphoglycerides for synthesis of cellular lipids was further demonstrated by showing that ether analogs, 1-alkyl-GPC and 1-alkyl-*sn*-glycero-3-ethanolamine, are taken up and acylated by the cells. Activities of lysolecithin:lysolecithin transacylase and acyl-CoA:lysolecithin acyltransferase were measured in subcellular fractions of the adenoma type II cell; the specific activities of the enzymes were 2.1 nmol/min/mg microsomal protein and 21 nmol/min/mg protein, respectively. The total activity of the acyltransferase in the cell fractions was about fourfold higher than the activity of the transacylase. Characteristics of the two enzymes were studied and are discussed. The findings indicate that exogenous 1-palmitoyl-GPC and palmitic acid both serve as efficient precursors of disaturated diacyl-GPC in the adenoma alveolar type II cells.

Wykle, R. L.; Malone, B.; and Snyder, F.
Acyl-CoA reductase specificity and synthesis of wax esters in mouse preputial gland tumors.
J. Lipid Res. 20:890-896; 1979.

Long-chain alcohols are synthesized in the mouse preputial gland tumor (ESR-586) by NADPH:acyl-CoA oxidoreductase. In this study, a series of labeled acids was tested as substrates for the oxidoreductase in a cell-free system from the tumor, and the distribution of label into alcohols, waxes, and other products was determined. The system contained the labeled acid, an acyl-CoA-generating system, an NADPH-generating system, and tumor homogenate. The highest rates of alcohol synthesis were obtained with palmitic (16:0), heptadecanoic (17:0), stearic (18:0), myristic (14:0), elaidic (18:1 *trans*), and linoleic (18:2) acids, which yielded, respectively, 151, 124, 102, 76, 65, and 35 pmol alcohol/min/mg protein. Decanoic (10:0), lauric (12:0), oleic (18:1 *cis*), linolenic (18:3), arachidonic (20:4), and behenic (22:0) acids all gave lower activities. Acyl-CoA formation did not appear to be rate limiting with any of the substrates tested except behenic acid. In addition to the fatty alcohol product, a small amount of fatty aldehyde was formed in the system. Incorporation of the labeled fatty acids into wax esters was examined, and the distribution of label between the alcohol and acid components of the waxes was determined. Incubation of [1-^{14}C]palmitic acid yielded 3.4% free alcohol, 8.3% alcohol esterified in waxes, and 7.7% palmitoyl groups esterified into waxes; whereas, at the other extreme, [1-^{14}C]linolenic acid yielded 0.8%, 0.6%, and 38%, respectively, into the homologous components.

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During 1980 REAC/TS expanded their training and educational activities and was responsible for organizing an International Conference on the Medical Basis of Radiation Accident Preparedness. As a part of the registry systems managed by REAC/TS, a follow-up study was established for 3247 radiation workers, with occupational radiation exposures equal to or greater than 5 rem.

Training Programs

The Medical and Health Sciences Division maintains an active training program in biomedical research. Graduate students from the University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences; the University of Tennessee (Knoxville and the UT Center for the Health Sciences, Memphis); and the University of North Carolina, Chapel Hill, have completed their doctoral dissertations at ORAU in biochemir and immunological research. Th. emerging epidemiology program has also recently recruited gradu-

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ate students from the University of North Carolina. Stipends for the ıpetitive fellowships awarded .hese students have been obtained from a National Cancer Institute (NCI) training grant (CA-09104) at Oak Ridge National Laboratory (ORNL), University Programs at ORAU (through DOE), the University of Tennessee (Hilton A. Smith Fellowship), individual research grants, and operational subcontracts.

Postdoctoral fellows also contribute to the various research activities of the division. In the past, funds for support of postgraduate training have been awarded to fellows from the Damon Runyon-Walter Winchell Cancer Fund (DRG-127-FT), an NCI Training in Carcinogenesis Research (CA-05296) at ORNL, and division funds. During 1980, a special committee was formed to help increase the breadth of this postdoctoral training program.

Summer research participation sponsored by University Programs at ORAU makes it possible for temic staff and seniors from eges and universities throughout the United States to partake in the type of laboratory studies described in this report. Training experiences are also available for high school students as secretaries and laboratory aides through vocational training programs at Clinton and Oak Ridge high schools.

Short-term courses in the management of health problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of 10 REAC/TS courses were given during 1980.

Some of the senior staff at the division conduct special courses and give lectures at the UT, Oak Ridge Graduate School of Biomedical Sciences and at the Univ y of Tennessee's Knoxville a Memphis campuses, where they have joint appointments and act as advisors to graduate students who pursue their research studies at ORAU. Three staff members are adjunct professors at the University of North Carolina.

The following individuals have participated in the high school, graduate, and postgraduate training programs during the past 12 months:

1. **High school**
   - Aleisa Cummins, Clinton Senior High School (Advisors—E. Cress and L. Woods, Biochemistry)
   - Kevin Hurt, Oak Ridge High School (Advisors—E. Cress and L. Woods, Biochemistry)
   - Tammy Kidwell Hill, Clinton Senior High School (Advisor—D. Vola, Biochemistry)
   - Janie Saunders, Clinton Senior High School (Advisor—D. Vola, Biochemistry)

2. **College student research participation**
   - Mark Clapp, Vanderbilt University, Tennessee (Advisor—D. Swartzendruber)
   - Sandra C. Crowder, Taylor University, Indiana (Advisor—C. Richter)
   - Linda Dees, Cumberland College, Kentucky (Advisor—L. Washburn)
   - Thomas M. Duncan, Clemson University, South Carolina (Advisor—T-c. Lee)
   - Timothy J. Garrand, St. Lawrence College, New York (Advisor—R. DuFrain)
   - Edward T. A. Fry, Washington University, Missouri (Advisor—D. Robie)
   - Thomas B. Whittle, Pomona College, California (Advisor—M. Blank)
   - Mark Worrell, Middle Tennessee State University, Murfreesboro (Advisor—M. Wray)

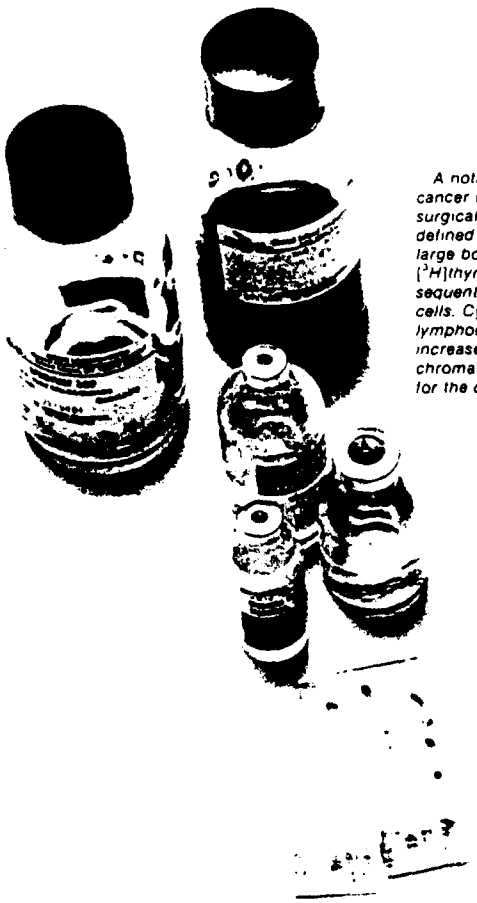
3. **Master's degree program**
   - Randall Rossi, University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences (Advisor—N. Gengozian)
   - Anne Sayer, University of Tennessee (Advisor—G. Littlefield)

4. **Predoctoral students (Ph.D)**
   - William Brawner, Auburn University, Alabama (Advisor—K. Hübner)
   - Alex Brown, University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences (Advisor—F. Snyder)
   - Diane Cookfair, University of North Carolina, Chapel Hill (Advisor—C. Lushbaugh)
   - Suzette Davis, Michigan State University (Advisor—C. Richter)
   - Patsy Fowlkes, University of Tennessee, Memphis (Advisor—F. Snyder)
   - Meena Khare, University of Tennessee (Advisor—E. Frome)
   - Claudia Moore, University of Tennessee, Oak Ridge Graduate School of Biomedical Sciences (Advisor—F. Snyder)
   - Jefferson Surles, University of North Carolina, Chapel Hill (Advisor—F. Snyder)
   - Jerome Wilson, University of North Carolina, Chapel Hill (Advisor—C. Lushbaugh)

5. **Postgraduate**
   - Christine McDermott, University of Scranton, Pennsylvania (Advisor—N. Gengozian)
   - Arthur E. Hougland, East Tennessee State University, Johnson City (Advisor—T-c. Lee)
   - Margaret Hougland, East Tennessee State University, Johnson City (Advisor—D. Swartzendruber)
   - Akira Imahori, Juntendo University, Tokyo (Advisor—K. Hübner
   - Edward N. Lambremont, Louisiana State University, Baton Rouge (Advisor—R. Wykle)
   - Willem Renooij, State University of Utrecht, The Netherlands (Advisor—F. Snyder)
   - Kolam Mani, sited at the University of North Carolina, Chapel Hill (Advisor—C. Lushbaugh)

6. **Predoctoral graduate students** sited at the University of North Carolina, Chapel Hill, but collaborating with the ORAU occupational epidemiology group
   - Naima Abd-Elghany (Epidemiology)
   - Bruce Parrish (Environmental Science)
   - Dan Strom (Environmental Science)

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A notable achievement of the marmoset colon cancer task group was the development of a surgical technique that makes possible carefully defined pulse labeling of specific segments of the large bowel of cancer-prone tamarins with [^{3}H]thymidine. Labeled segments are used for sequential biopsies and kinetic studies of mucous cells. Cytogenetic evaluations in cultured lymphocytes of tamarins suggested that increased sensitivity to mutagen-induced sister chromatid exchanges may be a genetic marker for the cancer-prone animals.

Collaborative Research

M. C. Cabot
Lipid metabolism in TPA-differentiating human leukemia
Oak Ridge National Laboratory, E. Huberman

M. C. Cabot
Alterations in lipid metabolism during oncogenic transformation of cultured fibroblasts
University of Southern California, C. Heidelberger

R. J. DuFrain
Human cytogenetic dosimetry for neutron radiation
Oak Ridge National Laboratory, H. Dickson; University of California, Los Angeles, A. Norman

R. J. DuFrain
Cytogenetic effect studies on chlorinated biphenol degradation products
University of Tennessee, G. Sayler

R. J. DuFrain
Gene mapping the laboratory rabbit
Oak Ridge National Laboratory, P. Lalley

E. L. Frome
Evaluation of record-linkage methods for use in DOE health and mortality studies
Emory University, B. Blumenstein

N. Gengozian
Fetal hemoglobin synthesis in the marmoset
University of Tennessee Memorial Research Center, J. Fuhr

N. Gengozian
Immunocontraception in the marmoset
University of Tennessee, C. Shivers

N. Gengozian
Radiation sensitivity (cytogenetic) of the marmoset
Oak Ridge National Laboratory, R. Preston

N. Gengozian
Thrombopoiesis in the marmoset
University of Tennessee Memorial Research Center, T. McDonald

R. Hayes
Collaborative studies in radiopharmaceutical development
Oak Ridge National Laboratory, F. Knapp

R. Hayes
Synthesis of labeled compounds using organoborane techniques
University of Tennessee, G. Kabalka

K. F. Hübner
Comparative efficiency of diagnostic methods for detection of pancreatic disease
University of Tennessee Memorial Research Center, R. Collmann

K. F. Hübner
Differentiation of glioblastomas and meningiomas with ^{11}C-labeled amino acids and positron emission computed tomography
Vanderbilt University, C. L. Partain; University of Tennessee Center for the Health Sciences, J. T. Robertson; University of North Carolina, Chapel Hill, M. S. Mahaley, Jr.; Fort Sanders Presbyterian Hospital, Knoxville, J. T. Purvis

K. F. Hübner
Radiation pneumonitis, a model for early detection of pulmonary disease using perfusion and ventilation techniques
University of Tennessee Memorial Research Center, F. Comas and T. Sullivan

L. G. Littlefield
Analyses of radiosensitivity in human B lymphocytes, T lymphocytes, and lymphoblastoid lines of persons with ataxia telangiectasia
Northwestern University Medical School, M. M. Cohen

L. G. Littlefield
Cytogenetic dose response kinetics in G-trisomic lymphocytes exposed to low LET radiation.
Medical College of Georgia, D. Welter

L. G. Littlefield
Cytogenetic follow-up studies in survivors of the LA-0 and [illegible] accidents

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University of Rochester, Tempelmann

L. G. Littlefield
Sister chromatid exchange analysis in coke oven workers
National Institute of Occupational Safety and Health, G. Taylor

C. C. Lushbaugh
Comprehensive epidemiology of DOE atomic workers
University of North Carolina, M. Ibrahim, C. Shy, H. Tyroler

C. C. Lushbaugh
Diethylenetriaminepentaacetate (DTPA) project
U.S. Department of Energy contractors, 40 co-investigators

C. C. Lushbaugh
United States Transuranium Registry
Hanford Environmental Health Foundation, B. Breitenstein

C. B. Richter
Microbiology and pathology of *Klebsiella* infections in marmosets and tamarins
Center for Disease Control, Atlanta, J. Farmer

C. B. Richter
Pathology of respiratory viruses in mice and the pathology of Sendai virus vaccination in mice
Microbiological Associates, J. Parker

C. B. Richter
Saturine gout using *Callithrix jacchus* as a model
University of Tennessee Memorial Research Center, W. Farcus

C. B. Richter
Studies on the skeletal maturation and aging in marmosets and tamarins
University of Tennessee, F. Smith and D. Glassman

F. Snyder
Effect of NO_2 and other toxicants on the lung surfactant system
Oak Ridge National Laboratory, W. Dalbey

F. Snyder
Mass spectrometry of unique lipids
Oak Ridge National Laboratory, Rainey

F. Snyder
Nuclear magnetic resonance studies of unique lipids
Oak Ridge National Laboratory, B. Benjamin

F. Snyder
Role of ether lipids in growth, cell differentiation, and neoplasia
Oak Ridge National Laboratory, C. Heckman

F. Snyder
Synthesis of lipid analogs and their precursors
University of North Carolina, Chapel Hill, C. Piantadosi and K. Ishaq

F. Snyder
Biologically active phospholipids
Schering Ag, Berlin, E. Schillinger; University of Alabama, Birmingham, J. B. Smith; University of California, San Francisco, J. A. Clements

L. C. Washburn
Pharmacological studies using ^{11}C-labeled compounds
University of Kentucky, G. Digenis

L. C. Washburn and K. F. Hübner
WR-2721 as an adjunct to radiotherapy
University of New Mexico, J. Yuhas

B. W. Wieland
Measurement of the p,n cross sections for production of ^{11}C, ^{13}N, and ^{18}F using the ORNL EN Tandem Van de Graaff accelerator
Oak Ridge National Laboratory, P. Miller and J. Bair

B. W. Wieland
Research on targetry and automated radiochemistry systems for the production of ^{11}C, ^{13}N, ^{15}O, and ^{18}F
Brookhaven National Laboratory, A. Wolf

B. W. Wieland and K. F. Hübner
Development of software for quantitative imaging of gamma emitters and for simulation of accelerator target systems
Vanderbilt University, P. King

Grant-Funded Research

R. Arneson
Antioxidants in mouse neuroblastoma
National Cancer Institute
$38,688* (December 1, 1979, to November 30, 1980)

N. Gengozian
"Autoimmune" thrombocytopenia in the marmoset
National Heart, Lung, and Blood Institute
$70,181* (July 1, 1980, to June 30, 1981)

N. Gengozian
Development of markers for assessment of damage to the immune system
Environmental Protection Agency
$328,000** (October 1, 1978, to September 30, 1980)

G. Littlefield
Evaluation of induced cytogenetic lesions in oocytes
National Institute of Child Health and Human Development
$85,385* (June 1, 1980, to May 31, 1981)

C. C. Lushbaugh
Biomedical research support grant
National Institutes of Health, Division of Research Resources
$56,000** (April 1, 1980, to March 31, 1981)

C. Richter
Marmoset breeding facility
National Institutes of Health, Division of Research Resources
$1,212* (September 1, 1980, to August 31, 1981)

R. Ricks
"Responder's Guidebook for Radioactive Materials Transportation Incidents"
Department of Transportation
$18,500** (April 1, 1979, to September 30, 1980)

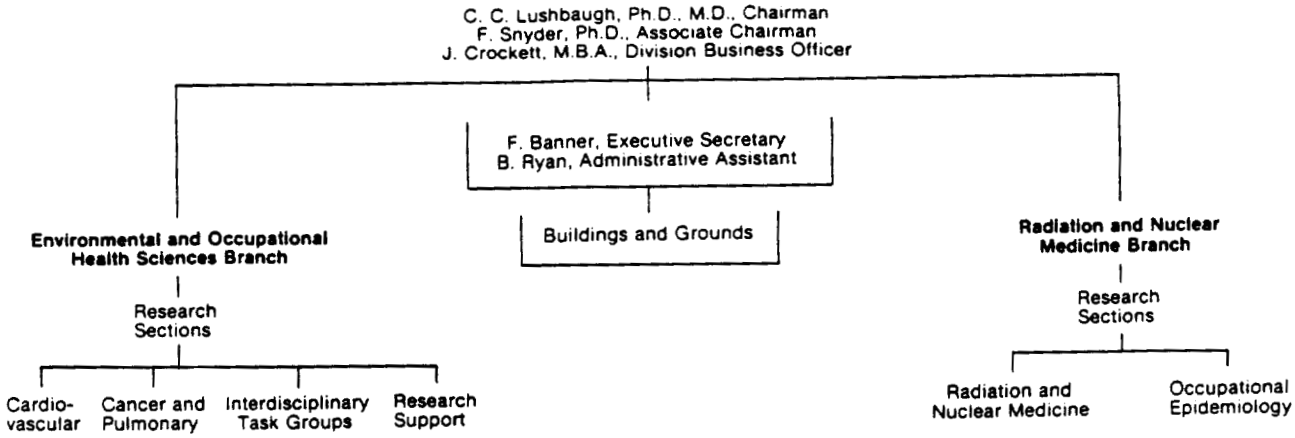
F. Snyder
Ether lipids in cancer: enzyme mechanisms and inhibitors
National Cancer Institute
$54,200* (May 1, 1980, to April 30, 1985)

F. Snyder
Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells
American Cancer Society
$63,250** (December 1, 1979, to November 30, 1980)

*Direct costs (1 year)
**Total costs (1 year)

1028475

Organization and Staff



Environmental and Occupational Health Sciences Research Branch

Fred Snyder, Ph.D., Chief

Interdisciplinary Task Group Section

Marmoset Colon Cancer
C. Richter, V.M.D.
L. Borman, Ph.D.
N. Gengozian, Ph.D.
T-c. Lee, Ph.D.
G. Littlefield, Ph.D.
D. Swartzendruber, Ph.D.

^{11}C Lung Tomography
M. Cabot, Ph.D.
R. Hayes, Ph.D.
L. Washburn, Ph.D.
K. Hübner, M.D.
T. Sun, M.S.
C. Welsh, M.S.
W. Gibbs, B.S.

Benzene Toxicity
N. Gengozian, Ph.D.
F. Snyder, Ph.D.
D. Swartzendruber, Ph.D.
P. Urso, Ph.D.
C. Richter, V.M.D.
M. Blank, B.S.

DTPA Project
L. Washburn, Ph.D.
S. Fry, M.B., Ch.B.
M. Blank, B.S.

Cardiovascular Research Section

Hypotensive-Platelet Activating Lipid Project
F. Snyder, Ph.D.
T-c. Lee, Ph.D.
M. Blank, B.S.
E. Cress, B.S.

Thrombocytopenia Project
N. Gengozian, Ph.D.
D. Rice, M.S.
S. Wierwille, B.S.

Cancer and Pulmonary Research Section

Immune Surveillance of Carcinogens
N. Gengozian, Ph.D.
L. Fox, Ph.D.
D. Rice, M.S.
S. Wierwille, B.S.
P. Urso, Ph.D.
D. Hadden, M.A.
R. Gibson, B.A.

Cancer and Pulmonary Biochemistry
T-c. Lee, Ph.D.
V. Fitzgerald, B.A.
B. Malone, B.S.
N. Stephens, B.S.
R. Arneson, Ph.D.
P. Kirk, B.S.
M. Cabot, Ph.D.
C. Welsh, M.S.
M. Blank, B.S.
E. Cress, B.S.

Radiopharmaceuticals Design and Testing
R. Hayes, Ph.D.
B. Wieland, Ph.D.
E. Carlton, B. S.
J. Rafter, B.S.

Radiopharmaceutical Synthesis
L. Washburn, Ph.D.
T. Sun, M.S.
B. Byrd, B.S.

Pulmonary and Cancer Cell Biology
D. Swartzendruber, Ph.D.
P. Miller
L. Borman, Ph.D.
D. Oakley, B.A.

Inhalation Toxicology
Recruitment position

Research Support Section*

Experimental Pathology and Veterinary Services
C. Richter, V.M.D.
G. Humason, M.A.
W. Tankersley, M.S.
B. Gangaware, B.A.
R. Carson
M. Ruffner
W. Arndt
J. Bates
I. Caldwell
J. Graham
R. Rice
J. Suttles
A. Webster

Library
M. Garber, B.S.L.S.
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1028477

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1028478

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1028480

Research Report 1980-81

Medical and Health Sciences Division

Oak Ridge Associated Universities



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The Medical and Health Sciences Division conducts biomedical research and training programs in studies of respiratory and gastrointestinal diseases and cancers related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Major program areas responsible for the research and training activities are biological chemistry, radiopharmaceutical chemistry, nuclear medicine, experimental pathology, cytogenetics, and epidemiology. Predoctoral and postdoctoral training programs are provided in each of these areas. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a demonstration and training unit for medical preparedness in radiation accidents.

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ORAU-191, October 1981
Distribution list: ORAU/MHSD-1981
This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

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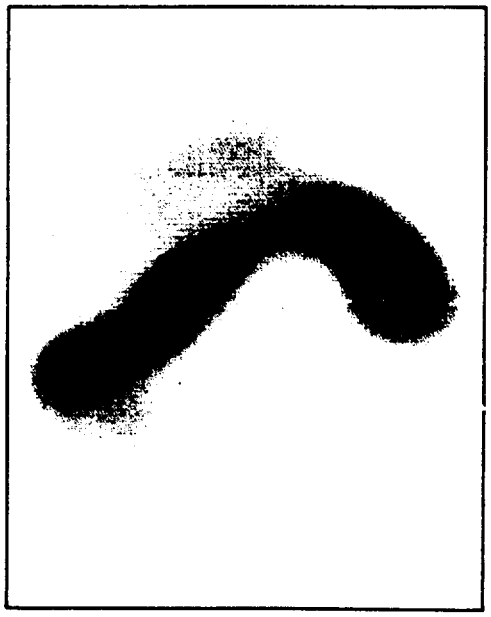
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Research Report 1980-81

Medical and Health Sciences Division

Oak Ridge Associated Universities



A tomographic scan of a patient. The scan was made with the Emission Computerized Axial Tomograph (ECAT-II) scanner located at the Medical and Health Sciences Division. A new grant in Nuclear Medicine and Radiopharmaceuticals, designed to

1028484

Table of Contents

Preface 3

Scope of Research Programs 4
- Biochemistry 4
- Radiopharmaceutical Design, Synthesis, and Testing 6
- Nuclear Medicine 8
- Immunobiology 9
- Radiation Emergency Assistance Center/Training Site 10
- Cytogenetics 11
- Occupational Epidemiology . . 12
- DTPA Task Group 13
- ^{11}C-Lung Tomography Task Group 13

Publications 14
- Full-Length Journal Articles . . 14
- Chapters, Review Articles, and Other Publications 16
- Published Abstracts of Papers Presented at Meetings 18

Summaries of Full-Length Publications 20

Training Programs 26

Collaborative Research 28

Grant-Funded Research 29

Organization and Staff 30

1028485

Preface

This year's research report describes significant progress in all of the ongoing research activities of the Medical and Health Sciences Division. The success of the achievements are reflected by the recent funding of two new grants and three renewals in the areas of cytogenetics, nuclear medicine, and biochemistry. On the front cover and elsewhere in this report, we have illustrated some of the different types of research activities that can be identified with these projects. The grants were awarded by the National Institutes of Health and the American Cancer Society; however, the funding for division-wide programs is primarily from the Office of Health and Environmental Research, U.S. Department of Energy (DOE).

Both the basic and applied research programs are directed toward solving health problems related to occupational hazards. The staff responsible for conducting this research represents a diverse group of scientific disciplines that include epidemiology, biochemistry, radiopharmaceuticals, nuclear medicine, cytogenetics, pathology, and until the 1982 fiscal year, immunobiology. Cancer, radiation injury, and diseases involving the lung, pancreas, gastrointestinal tract, and cardiovascular system are the targeted areas of the specific experimental projects. The long-range goals of these research efforts are (1) to determine the basic mechanisms of biochemical disease processes, (2) to develop useful agents and tests for clinical applications in nuclear medicine, and (3) to evaluate occupational health risks through epidemiological and cytogenetic studies. The division also manages a Radiation Emergency Assistance Center/Training Site for DOE; this facility provides assistance and medical care in radiation emergencies and also gives regularly scheduled training courses related to health care in radiation accidents.

This report describes the scope of our research and training activities, in addition to providing a listing and synopsis of all publications for the period from October 1, 1980, through September 30, 1981. Reprints of specific publications can be obtained by sending a request to one of the authors or to the chairman's office.

C. C. Lushbaugh, Chairman
Fred Snyder, Associate Chairman
October 1981

1028486

Scope of Research Programs

Biochemistry

Research in the biochemistry program consists of three primary projects: (1) basic membrane studies on lipid metabolism and regulatory processes in relation to cancer, (2) biologically active phospholipids and their cellular functions, especially those affecting the cardiovascular system, and (3) biochemical mechanisms associated with toxicant responses in pulmonary cells. All three projects have a strong emphasis on lipid metabolism (both cellular and enzymatic) and membrane function.

Basic Membrane Studies

This project concentrates on a group of lipids that contains ether linkages (characteristically elevated in cancer cells) and on the enzymes responsible for their metabolism. Unlike the ester-type pathway, the enzymes that synthesize the alkyl ether linkage are located in the internal matrix of microsomal membranes; the ether phospholipid products serve as structural components of membranes and as precursors of biologically active forms of phospholipids (a newly detected acetylated type). Current emphasis is on the purification and characterization of the properties of alkyl synthase, the enzyme that synthesizes the ether bond in lipids. An important accomplishment this past year has been the partial purification of alkyl synthase. The properties of the purified alkyl synthase are now under investigation. Results from this project are expected to contribute to our understanding of (1) how the ether lipid levels are regulated, (2) the precise chemical mechanism responsible for the formation of ether linkages in lipids, and (3) why such linkages are so prominent in lipids of cancer cells.

Effects of tumor promoters and other inducers of cellular differentiation and how changes in the differentiation process relate to membrane lipid metabolism are also under investigation; the goal is to pinpoint the lipid involvement in neoplastic transformation and in the cell differentiation process. Recent results further substantiate that the accumulation of ether lipids in cancer cells is closely associated with neoplastic transformation.

Biologically Active Phospholipids

A major discovery at ORAU in 1979 demonstrated that a novel group of glycerolipids (alkylacetyl phospholipids), hitherto undetected, possessed potent biological activities that could elicit hypotensive, platelet, and inflammatory responses. Investigations of the metabolism, biochemical mechanisms that accompany the responses, and the regulation of the alkylacetyl phospholipids are now the major research thrusts. We have identified the enzymes responsible for the biosynthesis and catabolism of the bioactive phospholipid molecules and have shown them to be distributed ubiquitously in mammalian cells; therefore, the active lipid appears to have an important functional role in most mammalian cells. Specific enzymatic steps in the activation (acetyltransferase and cholinephosphotransferase) and inactivation (acetylhydrolase) of these biologically potent phospholipids have been identified in our recent studies, and it will be the details of these enzymatic control points that will receive major attention in the future. An important observation has been that the active acetylated phospholipid can greatly stimulate the influx of calcium ions across

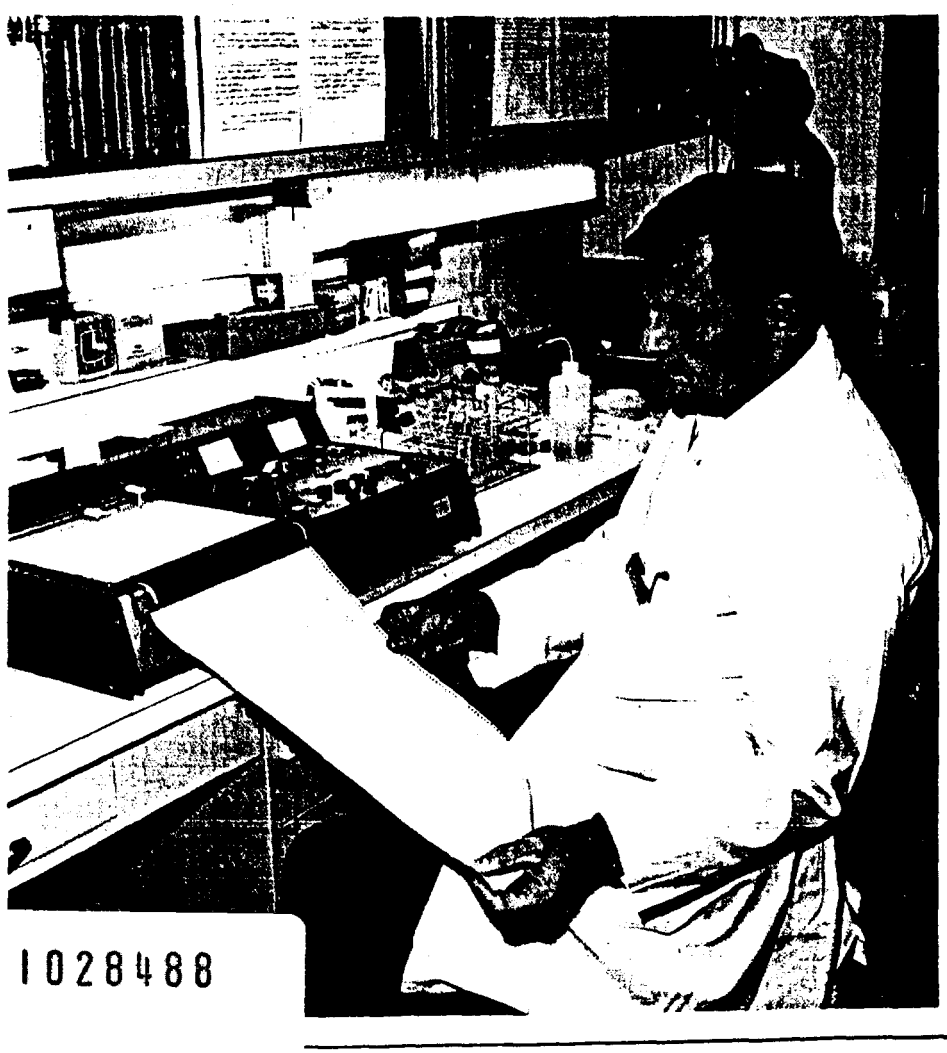
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platelet membranes, which suggests that 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine could serve as a physiological ionophore.

Biochemical Basis of Toxicant Responses in Pulmonary Cells

These studies are designed to probe the biochemical mechanisms that explain how energy-related toxicants (NO_2, O_3, and various classes of hydrocarbons) produce their devastating biological consequences in the respiratory tract. Up to now the experimental approach has centered on the basic mechanisms of pulmonary surfactant synthesis and how oxidant gases such as NO_2 and O_3 affect this crucial surface-active system at the alveolar-air interface. Earlier results from this project have clearly shown that the primary pathway for surfactant synthesis in a model system of alveolar type II cells is by a highly specific deacylation-reacylation reaction; the molecular restructuring occurs on preformed phospholipid molecules in the type II cells. Our data show that exposures to NO_2 and O_3 elevate surfactant levels in rat lung and that the effect appears to be at least partially explained by an increased activity in the synthetic enzymes. Interestingly, repeated exposures of rats to NO_2 cause an adaptation response, whereby the lung becomes resistant to further damage by NO_2. Although the reason for the adaptive response is still unclear, there seems to be a more oxidant-resistant type I cell formed during the recovery phase.

Catabolic pathways for surfactant lipids will be investigated in alveolar macrophages as the model system since the increased levels of surfactant found in the lungs after O_3 or NO_2 exposures could also be due to a decreased clearance rate. Previous evidence has indicated alveolar macrophages are important in the regulation of surfactant at the lung surface; the high content of phospholipases and related enzymes in macrophages further implicate the very important role of this cell type in lipid metabolism of the lung. In addition, macrophages have been chosen as the model system since they appear to be activated by toxic substances and by lipid analogs that have shown selective cytotoxicity in cancer cells. Ultimately, these studies are expected to examine the role of various cellular lipid mediators (prostaglandins and alkylacetyl phospholipids) as the responsible agents for altered pulmonary cell function by energy-related toxicants.



Analyzing the role of a potent new class of biologically active phospholipids in platelet aggregation. A new grant from the National Heart, Lung, and Blood Institute was awarded to study the biochemical properties and mechanism of action of a phospholipid responsible for platelet aggregation, antihypertensive, inflammatory, and allergic responses in various mammalian species. Also, two separate grants from the National Cancer Institute and the American Cancer Society were renewed for conducting basic biochemical studies on the enzymology and function of ether-linked lipids in cancer cells.

Radiopharmaceutical Design, Synthesis, and Testing

The objectives of the preclinical nuclear medicine program are (1) to identify, prepare, and test in animal models potential new radiopharmaceutical agents for the diagnosis of human diseases, particularly those diseases that are produced or aggravated by environmental pollutants, (2) to supply experimental radiopharmaceutical agents for clinical trials, and (3) to study the mechanisms involved in radiopharmaceutical localization as an aid to the development of even more effective agents.

Emphasis is on the development and production of positron-emitting radiopharmaceuticals. Positron decay produces annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions from various angles with a positron-emission computerized tomograph (ECT), it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of such radiopharmaceuticals. The images and information obtained are similar to those resulting from autoradiographic techniques except that the positron-ECT technique is noninvasive. The images obtained by positron-ECT reflect differences in tissue function rather than differences in tissue density as do conventional X-ray images. Thus, they can be used to detect metabolic alterations associated with disease processes.

Physiologic studies of the pancreas and brain are feasible using ^{11}C-labeled amino acids in conjunction with positron-ECT. Several years ago we developed a general method for the synthesis and purification of racemic mixtures of ^{11}C-labeled amino acids (mixtures of the naturally occurring L-isomer and the unnatural D-isomer). For physiologic studies, the ^{11}C-labeled L-amino acid must be separated from the mixture by a process that is compatible with the 20.4-minute half-life of carbon-11. Preparative resolution of three amino acids of nuclear medical interest, DL-[^{11}C] valine, DL-[^{11}C] tryptophan, and DL-[^{11}C] leucine, has been accomplished by high-performance liquid chromatography. Enzymatic resolution methods are also being investigated in collaboration with Dr. George Digenis of the University of Kentucky. Carbon-11-labeled L-amino acids have potential for differential diagnosis of pancreatic disease (L-[^{11}C] tryptophan and L-[^{11}C] valine) and for measurement of regional brain protein synthesis (L-[^{11}C] valine and L-[^{11}C] leucine). We are currently supplying L-[^{11}C] valine for clinical trials; clinical evaluation of L-[^{11}C] tryptophan will begin shortly.

Carbon-11-labeled alicyclic α-amino acids are effective tumor-localizing agents ([^{11}C] 1-aminocyclobutanecarboxylic acid, [^{11}C] ACBC, and [^{11}C] 1-aminocyclopentanecarboxylic acid, [^{11}C] ACPC). In addition, they are not metabolized and therefore have potential for positron-ECT measurements of brain amino acid transport. In a study of the effect of structure on brain uptake, 1-aminocyclohexanecarboxylic acid (ACHC) was found to have the highest affinity for brain. ACHC is now being tested for toxicity.

A comparison of ^{68}Ga-EDTA ($T_{1/2}$ = 68 min), [^{11}C] α-aminoisobutyric acid, [^{11}C] AIB, and [^{99m}Tc] pertechnetate for assessing breakdown of the blood-brain barrier (BBB) showed that ^{68}Ga-EDTA is superior to the other two agents. The commonly used agent, [^{99m}Tc] pertechnetate, was least effective. Although [^{11}C] AIB uptake was not as good as ^{68}Ga-EDTA in the experimental BBB lesion, the combination of its tumor-localizing and BBB properties may make [^{11}C] AIB useful for imaging brain tumors. Studies were also initiated to determine the

1028489



potential of ^{11}C-labeled amino acids for assessment of regenerating myocardial infarctions.

In collaboration with Dr. Digenis, the pharmacology in the brain of [^{11}C] phenethylamine, an amphetamine analog, is being studied. Another collaborative project, with Dr. George Kabalka of the University of Tennessee, is investigating the potential of organoboranes as precursors for incorporating radionuclides into organic and biochemical compounds of nuclear medical interest. Radioiodinated and radiobrominated estrogens and fatty acids have been produced by this technique.

Investigations with gallium-67 have shown it is preferentially taken up by malignancies and inflammatory lesions. The extent to which ^{67}Ga binds to plasma proteins (principally transferrin) is an important determinant in the biodistribution of this radiopharmaceutical. Blocking the binding of ^{67}Ga to plasma proteins with various agents reduces the uptake of ^{67}Ga by normal soft tissue and inflammatory processes but does not affect its uptake in tumors. On the other hand, experimentally increasing the binding of ^{67}Ga to plasma proteins decreases ^{67}Ga uptake by tumors, whereas the uptake in normal soft tissue and inflammatory lesions is increased. The uptake process involved in the concentration of ^{67}Ga in normal soft tissues and in inflammatory lesions may thus be similar, possibly involving micropinocytosis. Uptake of ^{67}Ga by tumor tissue, however, appears to occur by an entirely different process. This may involve mainly diffusion of uncharged $Ga(OH)_3$ molecules through the plasma and lysosomal membranes followed by binding of ^{67}Ga to lysosomal proteins due to the lower intralysosomal pH and the consequent production of $Ga(OH)^{2+}$ and $Ga(OH)_2^+$ ions. The uptake process in tumor cells would thus be rendered more or less irreversible.

Comparison of ^{169}Yb ($T_{1/2}$ = 32 days) and ^{67}Ga ($T_{1/2}$ = 3.3 days) in rats bearing experimental abscesses has suggested that ^{169}Yb may be more effective than ^{67}Ga for the detection of inflammatory lesions, particularly at early time periods (e.g., 4 hours). Ytterbium-169 is especially attractive as a potential agent for such studies because it is reactor produced (^{67}Ga is cyclotron produced) and has a long shelf life. An Investigational New Drug (IND) application prepared by the division for clinical testing of ^{169}Yb has been approved by the U.S. Food and Drug Administration (FDA).

A hydrous ferric oxide colloid labeled with the positron emitter ^{68}Ga has been developed by our program. The colloid particles have a mean diameter of approximately 10 nm and are prepared by heat hydrolysis of a dilute solution of ferric chloride containing [^{68}Ga] gallium chloride. The binding of ^{68}Ga to the colloid is essentially 100%. This preparation is expected to be very useful in positron-ECT detection of deep-seated lesions in the liver and spleen.

Emission computed tomography ... for conducting studies of physiologically ... agents labeled with positron ... quantitative methods for the assessment of ... and tumor detection. A ... National Institutes of Health grant in Nuclear Medicine emphasizes the use of ... acids to evaluate pancreatic ... disease.

1028490

The staff of the clinical nuclear medicine project, which is closely related to the radiopharmaceutical development program, continued to apply positron-emission computerized tomography (ECT) to (1) evaluate the utility of positron-emitting radiopharmaceuticals for clinical and research applications and (2) improve image and data analysis capabilities of the emission computerized axial tomographic scanner ($ECAT_{TM}$) for more accurate quantitative evaluation of the in vivo distribution of positron-emitting radiopharmaceuticals. Nuclear medical investigations included ECT studies using ^{11}C-labeled amino acids in patients with pancreatic disease, neoplasms, and nonneoplastic brain diseases. Amino acids with carbon-11 substituted for carbon-12 offer distinct advantages for studying protein synthesis or protein turnover in vivo because ^{11}C-labeled agents are indistinguishable physiologically and chemically from their unlabeled counterparts.

Progress made in previous years and during the past year in ECT pancreas studies, i.e., visualization of the normal pancreas and pancreatic cancers after injection of ^{11}C-labeled DL-valine, DL-tryptophan, or aminocyclobutane carboxylic acid, has provided essential supporting material for a grant proposal to the National Pancreatic Cancer Project of the National Cancer Institute. This organization has funded the pancreas-related research of the ^{11}C-amino acid project at a level of $243,000 for a 3-year period. An IND for comparative studies using DL-valine and L-valine in patients with pancreatic diseases has been approved by the FDA, and the first clinical studies have been done.

This past year we have concluded preliminary clinical investigation of amino acid ECT scans of brain tumors, especially malignant gliomas. The results indicate that both DL-[^{11}C] valine and DL-[^{11}C] tryptophan localize at higher concentrations in malignant gliomas than in normal brain tissue and that ECT is a potential tool for measuring amino acid utilization of brain tumors for diagnostic purposes and intervention studies.

Also during the past year, a new project on brain metabolism has been started in collaboration with the University of Michigan. This project focuses on measuring intracerebral valine distribution as a gauge for assessing the rate of protein synthesis in the brain of patients who have phenylketonuria, Huntington's chorea, cerebrovascular accidents, or cerebral palsy. So far, the most significant result obtained in this study is the in vivo demonstration of the fact that cerebral valine utilization is greatly reduced in patients who have phenylketonuria.

In order to apply radiopharmaceuticals in meaningful clinical quantitative studies, improved computer applications are needed for data analysis. In the past year, the ECAT-I system has been updated, with the full support of DOE, to an ECAT-II system with increased computer memory, improved data acquisition, and better image noise removal capability needed for quantitative in vivo examinations. Software programs originally written for ECAT-I have been translated for the ECAT-II system; similar programs are also being used to analyze data of images collected by the Pho-gamma camera, an instrument primarily used in ongoing pulmonary studies.

In continued joint efforts, especially in the area of chemistry and radiopharmaceutical development and instrumentation, a significant contribution has been made towards developing positron tomography as a new and important clinical diagnostic tool in nuclear medicine.

1028491

Immunobiology

The program in immunobiology has focused on the effects of environmental pollutants on immune processes and the basic immune mechanisms involved in the active induction of an autoimmune disease. The former study was conducted in the laboratory mouse, and the latter utilized the marmoset, a small, South American primate.

The focus of our study on environmental pollutants has been the susceptibility of the developing fetus to benzo[α]pyrene (BP), a pervasive toxicant known to have potent carcinogenic activity. Exposure of pregnant mice at varying stages of gestation has demonstrated a unique sensitivity of the immune system of the resulting progeny, with a lowered immune capacity persisting through 18 months of age. Coincidental with this has been a high frequency of tumors, with malignancies of the reproductive tract prominent in females and tumors of the liver most evident in males. Both humoral and cell-mediated immune responses were compromised, suggesting a relationship between the immunosuppressed state and tumor development. Further, dose-response studies with BP have shown the developing fetus to be far more sensitive to the immunosuppressive and tumorigenic properties of BP than neonatal or adolescent animals. Exposure of the fetal system may therefore reveal a potentially deleterious environmental toxicant at a level of sensitivity not encountered in the young adult animal.

In vitro studies on chemical immunosuppression were in progress, with the aim to determine whether short-term immunologic assays may be predictive of the potential immunosuppressive action of various chemical agents in vivo. Known to alter the metabolism of human lymphocytes under in vitro conditions, BP interferes with in vitro humoral antibody formation by splenic cells and lowers the proliferative response of T cells to foreign antigenic stimuli and mitogens. These effects were observed under conditions in which the chemical is nontoxic to the responding cells, an indication of a specific action on cell metabolism. The relationship of the in vitro immunosuppressive effects to the carcinogenic action of BP in vivo is not known but it may be noted that benzo(e)pyrene, the noncarcinogenic analog of benzo[α]pyrene, is not immunosuppressive in animals and has shown no inhibitory effects in the in vitro immunologic assays.

Active induction of immune thrombocytopenia in the marmoset was obtained readily by an interspecies platelet inoculation. The disease syndrome closely resembles posttransfusion purpura of man and has many features in common with other forms of clinical immune thrombocytopenia. Major efforts this year have centered on the identification of strong platelet antigens within one species so that intraspecies immunizations may be used to induce thrombocytopenia. This approach was initiated in animals sensitized to interspecies platelets since previous studies had shown the formation of antibody towards "self-type" platelets during such immunizations. Provisional platelet antigen identification with these antibodies in three marmoset species, *S. fuscicollis, S. o. oedipus,* and *C. jacchus,* permitted intraspecies inoculations for induction of the disease. Antisera obtained from sensitized animals showed a greater specificity and reactivity for platelet antigens; e.g., testing of members of each species with the reagents, in addition to demonstrating a clear differentiation among members of each species, also permitted visualization of platelet chimerism by immunofluorescent microscopy. Acute thrombocytopenia was subsequently realized by an intraspecies platelet inoculation in *S. fuscicollis* marmosets based on the antigenic differences. Recent adaptation of an in vitro assay, antibody-dependent cellular cytotoxicity, to the study of clinical immune thrombocytopenia in humans. greater insight into the cellular and humoral mechanisms leading to destruction of platelets in vivo. Significantly, the same assay has also been found to be applicable to the study of clinical immune thrombocytopenia in humans.

1028492

Radiation Emergency Assistance Center/ Training Site

The REAC/TS facility, a treatment center and training unit, continued to be available for rendering assistance in the management of health problems associated with radiation accidents and for formal teaching of medical and paramedical personnel. The REAC/TS staff's overall objective is to improve the medical care of persons exposed to high or low levels of radiation. The programs designed to meet this objective are (1) training in the management of health problems related to radiation accidents, (2) gathering information about radiation accidents through maintaining registries and data bases, (3) conducting training courses for medical-emergency and occupational medical personnel, (4) doing research in radiation pathology, especially in the area of high dose localized tissue injury, and (5) utilizing cytogenetic criteria as a basis for evaluating radiation dosimetry and chemical effects on chromosomes.

REAC/TS has a continued commitment to be available and ready to assist U.S. Government nuclear facilities and private nuclear power and nuclear fuel processing plants in the event of nuclear emergencies. During the past year, letter agreements with private companies have increased by 8 to a total of 48. Over 150 former REAC/TS trainees are now employed at these sites, and continued testing of the emergency response system is done through REAC/TS involvement in drills and exercises at these sites. In February, REAC/TS also accepted, at the request of the World Health Organization, the responsibility to provide assistance and collaborative services to all countries of the Americas.

During the past year, REAC/TS has been contacted for assistance 37 times. In three instances a serious radiation overexposure was diagnosed, and REAC/TS staff were consulted concerning the medical management of these patients. Bilateral bronchogenic pneumonia was the cause of death in one accident victim approximately eight months after an acute, nonuniform, approximately 350-rad dose to the whole body and many tens of thousands of rad to the chest, believed due to contact with a sealed radiation source. In the two other accidents, two of five persons involved incurred radiation injuries to the hands as the result of high-dose local exposure to radiation devices. In addition, cytogenetic dosimetry services were rendered for 32 persons with proven or suspected overexposure to radiation.

A total of 50 events of interest to REAC/TS programs were entered into the REAC/TS Registry System. Direct and indirect long-term follow-up of victims of earlier accidents represented continued efforts, including the determination of vital status by the Social Security Administration.

Coinvestigators on the DOE/ORAU FDA/IND for DTPA reported treatment of a total of 17 persons (6 facilities) between July 1980 and June 1981; although no problems were encountered, a metallic taste and dryness of the throat was reported by two persons treated with Zn-DTPA in the aerosol form. The coinvestigators reported follow-up information for 63% of the cases treated with DTPA before 1980; no unusual pattern of late effects has been identified in this population so far, but follow-up studies are continuing.

Progress continued in the development of the registry-based study of DOE workers exposed to $\geq$5 rem/year. A preliminary mortality study is in progress based on approximately 350 (11%) deaths identified in the population by the Social Security Administration. Alternate methods are being used to determine the vital status of approximately 600 participants whose vital status was unknown to the Social Security Administration. A comprehensive screening questionnaire has been developed in collaboration with the Epidemiology Group at Los Alamos National Laboratory for submission for approval by the Office of Management and Budget. The questionnaire aims to avoid multiple contacts of workers whose employment experiences cause them to be included in more than one study cohort. Officials at the 40 sites with reported $\geq$5 rem exposures are collaborating with registry staff to locate former DOE workers for purposes of the health status follow-up interview.

The REAC/TS courses and training program continued to be

1028493

in great demand during the past year. Participants included 92 physicians, 24 health physicists, and 63 paramedical professionals from nine different countries (one Fulbright Fellow studied at REAC/TS from August 1980 to August 1981). Less formalized training and information was provided through talks, a special exhibit presented by REAC/TS staff at professional meetings, and through publication of a quarterly newsletter that has an annual distribution of 9140. Special accomplishments include publication of the proceedings of an international conference entitled "Medical Basis for Radiation Accident Preparedness" (close to 1,500 copies sold) and of writing manuals for teaching emergency medical care of patients involved in radiation accidents; the latter is under a special contract with the Federal Emergency Management Agency.

Cytogenetics

The primary missions of the REAC/TS Cytogenetics Program are (1) to provide biological dosimetry and follow-up evaluations for persons having known or suspected exposures to ionizing radiation, (2) to improve the use of cytogenetic methods as a means for quantifying dose in persons for whom physical dose estimates are questionable or lacking, and (3) to conduct basic research aimed at defining the risk of human exposure to environmental mutagens, including physical and chemical clastogens.

Since the inception of the biological dosimetry program in 1976, the REAC/TS Cytogenetics Group has provided dose estimates for over a hundred persons having suspected radiation exposures. In many instances these evaluations provided the only means for determining whether a significant radiation dose was actually received. As an adjunct to these dosimetry activities, the cytogenetics staff organized and served as host for a two-day work session on the applications and limitations of cytogenetic techniques in biological dosimetry that was held in Gatlinburg in October 1980. Invited participants included radiation cytogeneticists from several DOE laboratories, the National Radiological Protection Board and Nuclear Fuels Limited in Great Britain, and York University, Ontario, Canada. The purpose of the work session was to provide a forum for informal discussions of the experiences of various laboratories in biological dose assessment and for discussions of the implications of cytogenetic findings as predictors of biological risk.

Our basic research efforts in radiation biology have focused on assessing the effects of various qualities of radiation on the induction of chromosome aberrations in cells of normal and radiosensitive individuals. The analysis of radiation-induced chromosome damage in cultured lymphocytes is the most sensitive biological technique currently available for assessing radiation dose in persons with whole body exposures to ionizing radiation.

During the last year, techniques were developed for assessing the cytogenetic effects of in vitro exposure of human lymphocytes to positrons emitted by carbon-11. This methodology will be used in future studies of the cytogenetic and cell-killing effects of other particle emitters including americium-241, tritium, and gallium-68. The data will be used to assess dose in exposed persons and to derive appropriate statistical models for defining the effects of radiation quality on the kinetics of lesion induction and the distributions of lesions in exposed cells.

This year we also extended our earlier studies of the kinetics of lesion induction in somatic cells of persons with increased radiation sensitivity to include evaluations in

1028494

lymphocytes from persons with Huntington's chorea (HC) and ataxia telangiectasia (AT). While increased cytogenetic radiation sensitivity was not observed in cultured lymphocytes from two patients with HC, lymphoblastoid cells from patients with AT were 2- to 3-fold more radiosensitive than lymphocytes from normal persons, with the maximum increase in sensitivity at the lower dose range.

Our studies of chemical clastogenesis have been directed toward determining whether the frequencies of chromosome lesions induced in somatic cells by chromosome-damaging chemicals can be used to predict the risk of induction of transmissible lesions in female germ cells. Our earlier studies with female rabbits have shown that the direct-acting chemical clastogen, streptonigrin, induces structural chromosome lesions in female germ cells that can be transmitted to the preimplantation embryo; the majority of the cytogenetically aberrant blastocysts do not survive implantation. In current studies we are evaluating cytogenetic damage in chromosomes of female germ cells in metaphase I of meiosis with the aim of determining whether female germ cells and somatic cells are equally at risk to the induction of chromosome damage after in vivo exposures to clastogenic chemicals.

Occupational Epidemiology

The epidemiology studies are concerned mainly with examining associations between occupational radiation exposure and subsequent mortality. The health effects of chemical toxicants (especially metals) are also under investigation as both primary and confounding (with respect to radiation exposure) occupational stresses. In the statistical and computer methodology sections, biostatisticians and computer scientists are developing improved statistical methods for epidemiological analyses and data systems for merging large masses of data into analysis files.

In the area of radiation effects, we are continually evaluating the mortality experience of workers within the Oak Ridge Operations Office area. This area includes workers at facilities run by Union Carbide Corporation, Nuclear Division, in Oak Ridge, Tennessee, and Paducah, Kentucky, and workers at the National Lead Company and the Mound Laboratory, both in Ohio. Editing and correction procedures have been developed for the data bases that extend back to the early 1940s. Preliminary mortality analyses have been completed on two groups of workers at the Oak Ridge Y-12 plant.

Another study, designed to examine possible health effects of occupational exposure to radiation equal to or greater than the current limit (set by the Nuclear Regulatory Commission and DOE) of 5 rem per year, is in the data collection and validation phase. We have constructed a roster of 3200 DOE (and predecessor organizations) employees who have been exposed to 5 or more rem per year. Preliminary analyses of mortality experience for this group will be initiated when death certificate retrieval has been completed in 1981.

Other studies in progress include the long-term health effects of exposure to metals and other toxic substances. A completed mortality study of Oak Ridge workers potentially exposed to metallic nickel is being updated to include more follow-up data and a comparison with mortality among welders of the Union Carbide Corporation, Nuclear Division, which contains a subgroup exposed to nickel vapor.

To facilitate these studies and to aid other DOE epidemiological studies, such as those of atomic energy workers at Hanford, Washington; Los Alamos, New Mexico; and Rocky Flats, Colorado; we have developed the DOE Death Certificate Retrieval Center to obtain information on the vital status of specific workers. These data are being accumulated through the cooperation of the Social Security Administration and the vital statistics departments in all 50 states.

1028495

DTPA Task Group

The objective of this program is to coordinate the national use of DTPA (diethylenetriaminepentaacetic acid) chelation drugs for decorporation therapy of plutonium and other actinides. Both Ca-DTPA (the calcium salt of DTPA) and Zn-DTPA (the analogous zinc salt) have been approved for human use under Investigational New Drug (IND) status. The INDs for both drugs are managed by our DTPA task group for DOE. Approximately 40 physicians, mostly at DOE contractor sites are coinvestigators on the INDs. The program also maintains a national DTPA registry, a computer-assisted collection of case histories of DTPA-treated individuals which assists in the determination of the safety and efficacy of DTPA drugs and facilitates long-term medical follow-up.

New developments in chelation therapy, involving either revised protocols for the existing drugs or research on potentially improved chelating agents, are monitored, evaluated, and in some cases, tested experimentally. When indicated, the appropriate IND amendments or new IND applications are submitted through DOE to FDA. We are currently evaluating the use of liposome-encapsulated Ca- or Zn-DTPA for decorporation of intracellular actinide contaminants. Although the preliminary decorporation results in animals were promising, we have not been able to overcome the splenomegaly that results from administration of the lipophilic chelator.

^{11}C-Lung Tomography Task Group

The objective of this interdisciplinary group is to seek new radiopharmaceutical compounds that can be employed for positron-emission computerized tomographic studies of pulmonary function. Initial studies have centered on the uptake and organ distribution of ^{14}C-labeled aliphatic amines in animal models. Chemical synthesis and rapid purification procedures have been established for ^{11}C-labeling of the nitrogen-containing compounds, and methyl-substituted analogs have been synthesized. The branched chain prototypes demonstrate longer pulmonary residence because they are metabolically more inert when compared to the parent straight-chain amines. Other compounds under investigation include the basic amines, chloropromazine and imipramine. Compounds possessing a high affinity for the pulmonary endothelium could be useful for evaluating the endothelial integrity in disease states.

A collaborative arrangement was established with researchers at the University of Alberta interested in testing the potential of ^{11}C-labeled branched-chain primary aliphatic amines as agents for studying lung metabolism. They have established dog models that simulate various pathologic conditions in the lung and are, therefore, essential for this work. The first model selected for study involves the production of localized damage to lung endothelial cells by administration of oleic acid to one area of the lung.

In order to prepare for in vivo applications of ^{11}C-labeled aliphatic amines in positron-ECT studies, special computer applications for image noise reduction and attenuation correction have been developed. For conventional nuclear scintigraphy, computer software programs have been written to analyze activity distribution in regions of interest for more accurate assessment of global lung perfusion and ventilation. The software is compatible with the updated ECAT-II system.

No

1028496

Publications

Full-Length Journal Articles

Arneson, R. M.; Hinds, J. L.; and Wander, J. D.
Antioxidants in neoplastic cells: IV. Evidence that the phenolic antioxidant A274 is 9-(4-hydroxyphenyl)xanthene, an artifact in tissue culture media.
Lipids 16: 82-86; 1981.

Bair, J. K.; Miller, P. D.; and Wieland, B. W.
Neutron yields from 4- to 12-MeV proton bombardment of ^{11}B, ^{13}C, and ^{18}O as related to the production of ^{11}C, ^{13}N, and ^{18}F.
Int. J. Appl. Radiat. Isot. 32: 389-395; 1981.

Beck, W. L.
Radiation accident dosimetry.
Nucl. Instrum. Methods 175: 150-152; 1980.

Berger, M. E.; Thompson, D. W.; Ricks, R. C.; and Kaufman, D.
The medical technologist and the radiation-accident victim.
Am. J. Med. Technol. In press.

Blank, M. L.; Cress, E. A.; Whittle, T.; and Snyder, F.
In vivo metabolism of a new class of biologically active phospholipids: 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a platelet-activating, hypotensive phospholipid.
Life Sci. 29: 769-775; 1981.

Blank, M. L.; Dalbey, W.; Cress, E. A.; Garfinkel, S.; and Snyder, F.
Pulmonary NO_2 toxicity: Phosphatidylcholine levels and incorporation of [^3H] thymidine into DNA.
Environ. Res. In press.

Blank, M. L.; Lee, T-c.; Fitzgerald, V.; and Snyder, F.
A specific hydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive, platelet-activating lipid).
J. Biol. Chem. 256: 175-178; 1981.

Blank, M. L.; Cress, E. A.; Byrd, B. L.; Washburn, L. C.; and Snyder, F.
Liposomal encapsulated Zn-DTPA for removing intracellular ^{169}Yb.
Health Phys. 39: 913-920; 1980.

Borman, L. S.
Modulation of mammalian cell growth by a choline analog, *N*-isopropylethanolamine.
In Vitro. In press.

Cabot, M. C., and Goucher, C. R.
Chaulmoogric acid: Assimilation into the complex lipids of mycobacteria.
Lipids 16: 146-148; 1981.

Cabot, M. C., and Lumb, R. H.
The activity of a low temperature lipase in the larvae of *Sarcophaga bullata* (Diptera:Sarcophagidae).
Comp. Biochem. Physiol. 68B: 325-328; 1981.
Work performed at Western Carolina University, Cullowhee, North Carolina.

bot, M. C., and Welsh, C. J.
.ner lipid studies in mouse C3H/10T1/2 cells and a 3-methylcholine transformed clone.
Arch. Biochem. Biophys. In press.

Cabot, M. C., and Welsh, C. J.
Fatty acid metabolism in phorbol ester-differentiating human leukemia cells.
Cancer Res. In press.

Cabot, M. C.; Welsh, C. J.; Callaham, M. F.; and Huberman, E.
Alterations in lipid metabolism induced by 12-*O*-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells.
Cancer Res. 40: 3674-3679; 1980.

Casey, D. L.; Digenis, G. A.; Wesner, D. A.; Washburn, L. C.; Chaney, J. E.; Hayes, R. L.; and Callahan, A. P.
Preparation and preliminary tissue studies of optically active D- and L-[^{11}C] phenylalanine.
Int. J. Appl. Radiat. Isot. 32: 325-330; 1981.

DuFrain, R. J., and Garrand, T. J.
The influence of incorporated halogenated analogues of thymidine on the sister chromatid exchange frequency in human lymphocytes.
Mutat. Res. 91: 233-238; 1981.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. I. The test system and the effects of streptonigrin.
Mutat. Res. 85: 147-160; 1981.

Fox, L. L.; Gengozian, N.; Shivers, C. A.; and Sieg, P.
Marmoset response to immunization with porcine zonae pellucidae: Measurement of antibody activity with an enzyme-linked immunosorbent assay.
J. Med. Primatol. In press.

Frome, E. L.
Fisher's exact variance test for the Poisson distribution.
Appl. Stat. In press.

Fry, S. A.; Lushbaugh, C. C.; and Hübner, K. F.
An overview of radiation accidents and injuries.
Int. J. Nucl. Med. Biol. In press.

Gengozian, N., and Ostby, D. A.
Antibodies selectively reactive to autologous and host-type platelets are obtained following interspecies immunizations in marmosets.
Clin. Exp. Immunol. 43: 128-134; 1981.

Gengozian, N., and Rice, D.
Antibody-dependent cellular cytotoxicity with platelets as the target cell: Potential application to the study of immune thrombocytopenia.
Clin. Exp. Immunol. In press.

Hayes, R. L.
The interaction of gallium with biological systems.
Int. J. Nucl. Med. Biol. In press.

Hayes, R. L.; Carlton, J. E.; and Kuniyasu, Y.
A new method for labeling microspheres with ^{68}Ga.
Eur. J. Nucl. Med. In press.

Hayes, R. L.; Rafter, J. J.; Byrd, B. L.; and Carlton, J. E.
Studies of the in vivo entry of ^{67}Ga into normal and malignant tissue.
J. Nucl. Med. 22: 325-332; 1981.

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
Studies of the in vivo uptake of ^{67}Ga by an experimental abscess.
J. Nucl. Med. In press.

1028497

Hübner, K. F.; Krauss, S.; Washburn, L. C.; Gibbs, W. D.; and Holloway, E. C.
Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [^{11}C] carboxylic acid using positron emission computerized tomography.
Clin. Nucl. Med. 6: 249-252; 1981.

Hübner, K. F.; Purvis, J. T.; Mahaley, S. M., Jr.; Robertson, J. T.; Rogers, S.; Partain, C. L.; Gibbs, W. D.; and King, P.
Brain tumor imaging by positron emission computerized tomography (ECT) using ^{11}C-labeled amino acids (preliminary results).
J. Comput. Assist. Tomogr. In press.

Huff, V.; Littlefield, L. G.; DuFrain, R. J.; and Wilmer, J. L.
Factors affecting the sensitivity of rabbit lymphoid and bone marrow cells to streptonigrin-induced chromosome aberrations.
Cytogenet. Cell Genet. 30: 15-24; 1981.

Khare, M., and Federer, W. T.
A simple construction procedure for resolvable incomplete block designs for any number of treatments.
Biom. J. 23: 121-132; 1981.

King, P. H.; Hübner, K.; Gibbs, W.; and Holloway, E.
Noise identification and removal in positron imaging systems.
IEEE Trans. Nucl. Sci. NS-28: 148-151; 1981.

Lee, T-c., and Snyder, F.
Phase transition of an unnatural analog of phosphatidylcholine: Phosphatidyl-*N*-isopropylethanolamine.
Lipids 16: 75-76; 1981.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Substrate specificity in the biocleavage of the *O*-alkyl bond: 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid) and its metabolites.
Arch. Biochem. Biophys. 208: 353-357; 1981.

Lee, T-c.; Malone, B.; Blank, M. L.; and Snyder, F.
1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) stimulates calcium influx in rabbit platelets.
Biochem. Biophys. Res. Commun. In press.

Leonard, R. B., and Ricks, R. C.
Emergency department radiation-accident protocol.
Ann. Emerg. Med. 9: 462-470; 1980.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Physical, chemical, and biological factors affecting sister chromatid exchange induction in human lymphocytes exposed to mitomycin C prior to culture.
Mutat. Res. 81: 377-386; 1981.

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; DuFrain, R. J.; and Cohen, M. M.
Chromosomal radiation sensitivity in ataxia telangiectasia long-term lymphoblastoid cell lines.
Cytogenet. Cell Genet. In press.

Morrison, W. D.; Huff, V.; Colyer, S. P.; DuFrain, R. J.; and Littlefield, L. G.
Cytogenetic effects of *cis*-platinum(II)diaminedichloride in vivo.
Environ. Mutagen. 3: 265-274; 1981.

Nickerson, D. A., and Gengozian, N.
Functional capabilities of marmoset T and B lymphocytes in primary in vitro antibody formation.
Cell. Immunol. 57: 408-419; 1981.

1028498

Poledna, A. P.
Mortality among welders, including a group exposed to nickel oxides.
Arch. Environ. Health. In press.

Poledna, A. P., and Frome, E. L.
Mortality among men employed between 1943 and 1947 at a uranium-processing plant.
J. Occup. Med. 23: 169-178; 1981.

Poledna, A. P., and Keane, A. T.
Long half-life component of uranium retention: Implications for potential health effects among early uranium workers.
Health Phys. In press.

Poledna, A. P.; Keane, A. T.; and Beck, W. L.
Estimation of radiation doses to the lungs of early uranium-processing plant workers.
Environ. Res. In press.

Renooij, W., and Snyder, F.
Biosynthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor and a hypotensive lipid) by choline-phosphotransferase in various rat tissues.
Biochim. Biophys. Acta 663: 545-556; 1981.

Renooij, W., and Snyder, F.
Transmembrane orientation of palmitoyl-CoA:lysophosphatidylcholine acyltransferase in microsomes isolated from adenoma alveolar type II cells and rat liver.
Biochim. Biophys. Acta. In press.

Sayer, A. M.; Littlefield, L. G.; DuFrain, R. J.; and Richter, C. B.
Analysis of mutagen-induced chromosome damage in a primate species (*Saguinus oedipus oedipus*) at risk for spontaneous adenocarcinoma of the colon.
Cancer Genet. Cytogenet. 3: 161-169; 1981.

Swartzendruber, D. C., and Richter, C. B.
Mucous and argentaffin cells in colonic adenocarcinomas of tamarins and rats.
Lab. Invest. 43: 523-530; 1980.

Tardif, S. D., and Richter, C. B.
Competition for a desired food in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*).
Lab. Anim. Sci. 31: 52-55; 1981.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Callahan, A. P.
Production of L-[carboxyl-^{11}C] valine by HPLC resolution.
J. Nucl. Med. In press

Wykle, R. L.; Malone, B.; and Snyder, F.
Enzymatic synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a hypotensive and platelet-aggregating lipid.
J. Biol. Chem. 255: 10256-10260; 1980.

Chapters, Review Articles, and Other Publications

Andrews, G. A.; Hübner, K. F.; Fry, S. A.; Lushbaugh, C. C.; and Littlefield, L. G.
Report of 21-year medical follow-up of survivors of the Oak Ridge Y-12 accident.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 59-79, New York: Elsevier North-Holland, 1980.

Beck, W. L., Jr.; Berger, C. D.; Berger, J. D.; and Goans, R. E.
Instrumentation for detection and measurement of ionizing radiation.
In *Analytical Measurements and Instrumentation for Process and Pollution Control*, edited by P. N. Cheremisinoff and H. J. Perlis, pp. 223-272, Ann Arbor, Michigan: Ann Arbor Science Publishers, 1981.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Frome, E. L.
In vitro human cytogenetic dose response systems.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 357-374, New York: Elsevier North-Holland, 1980.

Frome, E. L.
PREG: A Computer Program for Poisson Regression Analysis, ORAU-178, Oak Ridge, Tennessee: Oak Ridge Associated Universities, 1981.

Frome, E. L., and Hudson, D. R.
Interval estimation for X predictions when the dependent variable follows the Poisson distribution.
In *1980 Proceedings of the Statistical Computing Section of the American Statistical Association*, Houston, Texas, pp. 294-297, Washington, D.C.: American Statistical Association [1981].

Frome, E. L., and Hudson, D. R.
A general statistical data structure for epidemiologic studies of DOE workers.
In *1980 DOE Statistical Symposium*, Berkeley, California, edited by T. Truett, D. Margolies, and D. W. Mensing, CONF-80145, pp. 206-218, Washington, D.C.: Department of Energy, 1981.

Frome, E. L., and Khare, M.
Comments on Brodsky's statistical methods for evaluating epidemiological results (Letter to the Editor), *Health Phys.* 39: 695-697; 1980.

Fry, S. A.
The United States radiation accident and other registries of the REAC/TS registry system: Their functions and current status.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 451-468, New York: Elsevier North-Holland, 1980.

Fry, R. J. M., and Fry, S. A.
Effects on humans of exposure to radioactive materials: Late somatic effects.
In *Encyclopedia of Materials Science and Engineering*, New York: Pergamon Press. In press.

Hansard, M. S., and Wray, M. L.
A SAS job stream generator.
In *Proceedings of Statistical Analysis System Users Group*, International Conference, 1981, Orlando, Florida, pp. 473-476, Cary, North Carolina: SAS Institute, Inc., 1981.

Hayes, R. L., and Byrd, B. L.
Transfer of ^{67}Ga from hamster dam to fetus and offspring.
In *Third International Radiopharmaceutical Dosimetry Symposium*, Oak Ridge, Tennessee, 1980, edited by E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, HHS Publication FDA 81-8166, pp. 447-453, Rockville, Maryland: U.S. Bureau of Radiological Health, 1981.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; Carlton, J. E.; and Coffey, J. L.
Ytterbium-169 citrate for detection of inflammatory lesions.
In *Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging*, 1980, Heidelberg, Germany. In press.

Hemplemann, L. H.; Lushbaugh, C. C.; and Voelz, G. L.
What happened to the survivors of the early Los Alamos nuclear accidents?
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 17-32, New York: Elsevier North-Holland, 1980.

Hodges, H. D. [Reviewer].
Perceptions of Risk, Proceedings No. 1, NCRP, Washington, D.C., 1979.
J. Nucl. Med. Technol. 9: 55; 1981.

Holly, F. E., and Beck, W. L.
Dosimetry studies for an industrial radiography accident.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 265-277, New York: Elsevier North-Holland, 1980.

Hübner, K. F., and Fry, S. A., eds.
The Medical Basis for Radiation Accident Preparedness, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, New York: Elsevier North-Holland, 1980.

Hübner, K. F., and Watson, E. E. [Reviewers].
NCRP Report No. 65: Management of persons accidentally contaminated with radionuclides.
J. Nucl. Med. 22: 397; 1981.

Hübner, K. F.; Fry, S. A.; and Lushbaugh, C. C.
Biological effects of radiation in man.
In *Funk & Wagnalls New Encyclopedia*, edited by R. S. Phillips. New York: Funk & Wagnalls. In press.

Hübner, K. F.; King, P.; Gibbs, W. D.; Washburn, L. C.; and Hayes, R. L.
Positron emission computerized tomography: A potential tool for in vivo quantitation of the distribution of radiopharmaceuticals.
In *Third International Radiopharmaceutical Dosimetry Symposium*, Oak Ridge, Tennessee, 1980, edited by E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, HHS Publication FDA 81-8166, pp. 77-87, Rockville, Maryland: U.S. Bureau of Radiological Health, 1981.

Hübner, K. F.; King, P.; Gibbs, W. D.; Partain, C. L.; Washburn, L. C.; Hayes, R. L.; and Holloway, E.
Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases.
In *Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging*, 1980, Heidelberg, Germany. In press.

Janardan, K. G.; Schaeffer, D. J.; and DuFrain, R. J.
A stochastic model for the study of the distribution of chromosome aberrations in human and animal cells exposed to radiation or chemicals.

1028499

In *Proceedings of the International Summer School on Statistical Distributions in Scientific Work*, 1980, edited by G. Tallie, G. P. Patil, and B. Baldessari, Boston: D. Reidel Publishing Co. In press.

Kabalka, G. W.; Gooch, E. E.; Hsu, H. C.; Washburn, L. C.; Sun, T. T.; and Hayes, R. L.
Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology.
In *Applications of Nuclear and Radiochemistry*, New York: Pergamon Press. In press.

Littlefield, L. G.
Effects of DNA-damaging agents on sister chromatid exchange.
Sister Chromatid Exchange; Progress and Topics in Cytogenetics, edited by A. A. Sandberg. New York: Alan R. Liss Inc. In press.

Littlefield, L. G.; Joiner, E. E.; DuFrain, R. J.; Hübner, K. F.; and Beck, W. L.
Cytogenetic dose estimates from in vivo samples from persons involved in real or suspected radiation exposures.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 375-390, New York: Elsevier North-Holland, 1980.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
The impact of estimates of human radiation tolerance upon radiation emergency management.
In *Proceedings of the NCRP-FEMA Symposium: Control of Exposure from Ionizing Radiation*, 1981, Washington, D.C. In press.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
Effects of high doses of radiation.
In *Proceedings of the Eighth Life Sciences Symposium, Radiation and Its Effects*, 1980, Los Alamos, New Mexico. In press.

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Beck, W. L.
Effects on humans of exposure to radioactive materials: Acute effects.
In *Encyclopedia of Materials Science and Engineering*, New York: Pergamon Press. In press.

Lushbaugh, C. C.; Fry, S. A.; Hübner, K. F.; and Ricks, R. C.
Total-body irradiation: A historical review and follow-up.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 3-15, New York: Elsevier North-Holland, 1980.

Mauderly, J.; Hahn, F.; Martin, C. J.; and Richter, C. B.
Animal models of diseases of the human elderly: Chronic bronchitis and emphysema.
In *Mammalian Models for Research on Aging*, Institute of Laboratory Animal Resources (ILAR) Committee on Animal Models for Research on Aging, National Research Council, pp. 365-372, Washington, D.C.: National Academy Press, 1981.

Parker, J. C., and Richter, C. B.
Respiratory (Sendai, PVM, K).
In *The Mouse in Biomedical Research*, edited by H. L. Foster, J. D. Small, and J. G. Fox, New York: Academic Press. In press.

Partain, C. L.; Hübner, K. F.; Price, R. R.; Patton, J. A.; Erickson, J. J.; Pickens, D. R.; Hayes, R. L.; and James, A. E., Jr.
Functional mapping of the brain: Positron emission tomography and nuclear magnetic resonance imaging.
In *Proceedings of the Computer and Instrumentation Council of the Society of Nuclear Medicine*, 1981, New Orleans, Louisiana. In press.

Polednak, A. P.
Long-range studies of uranium workers and the Oak Ridge radiation worker population.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 401-409, New York: Elsevier North-Holland, 1980.

Richter, C. B.
Diagnostic Exercise [Mucinous adenocarcinoma of the descending colon].
Lab. Anim. Sci. 31: 137-138; 1981.

Richter, C. B.; Lushbaugh, C. C.; and Swartzendruber, D. C.
Cancer of the colon in the cotton-topped tamarins.
In *The Comparative Pathology of Zoo Animals*, edited by R. J. Montali and G. Migaki, pp. 567-571, Proceedings of a Symposium, 1978, Washington, D.C.: Smithsonian Institution Press, 1980.

Ricks, R. C.
REAC/TS: Its role as a specialty referral center and training site.
In *The Medical Basis for Radiation Accident Preparedness*, Proceedings of the REAC/TS International Conference, 1979, Oak Ridge, Tennessee, edited by K. F. Hübner and S. A. Fry, pp. 291-296, New York: Elsevier North-Holland, 1980.

Ricks, R. C. [Reviewer].
NCRP Report No. 65: Management of persons accidentally contaminated with radionuclides.
Radiat. Res. 85: 215-216; 1981.

Ricks, R. C.; Beck, J.; and Berger, J.
Radioactive Materials Transportation Information and Incident Guidance.
DOT/RSPA/MTB-81/4, various paging, Washington, D.C.: Department of Transportation, 1981.

Sagan, L. A., and Fry, S. A.
Radiation accidents: A conference review.
Nucl. Saf. 21: 562-569; 1980.

Wieland, B. W.
Target development problem areas in the accelerator production of ^{11}C, ^{13}N, ^{15}O, and ^{18}F for application in diagnostic nuclear medicine.
In *Proceedings of the 1980 World Conference of International Nuclear Target Development Society*, Gatlinburg, Tennessee, special edition of *Nuclear Instruments and Methods*, edited by E. H. Kobisk and H. L. Adair, New York: Elsevier North-Holland. In press.

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Published Abstracts of Papers Presented at Meetings

)d Elghany, N.; Lushbaugh, C. C.; and Checkoway, H.
.n epidemiologic study of cancer mortality among workers exposed to occupational low levels of ionizing radiation.
Am. J. Public Health. In press.

Beck, W. L.; Stansbury, P. S.; Watson, J. E; Parrish, B. R.; and Strom, D. J.
Dose assessment for health and mortality studies.
In *Twenty-Sixth Annual Meeting of the Health Physics Society,* Louisville, Kentucky, 1981, abstracts of papers presented at the meeting, p. 8, Abstract P/31, 1981.

Blank, M. L.; Lee, T-c.; Cress, E. A.; Whittle, T.; Fitzgerald, V.; and Snyder, F.
In vivo metabolism of a new class of phospholipid, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a platelet-activating, hypotensive lipid.
Fed. Proc. 40: 1806 (Abstract 1534); 1981.

Borman, L. S.; Swartzendruber, D. C.; and Littlefield, L. G.
Establishment of a cell system from rat colonic carcinoma.
In Vitro 17: 258 (Abstract 237); 1981.

Brown, A. J., and Snyder, F.
Partial purification of alkyl synthase.
Fed. Proc. 40: 1806 (Abstract 1535); 1981.

.bot, M. D., and Welsh, C. J.
Fatty acid metabolism in tetradecanoylphorbol-13-acetate-treated human leukemia cells.
Fed. Proc. 40: 1590 (Abstract 290); 1981.

Cookfair, D.; Frome, E.; and Patten, B.
Methodologic problems in occupational studies of female workers.
Am. J. Epidemiol. 114: 438; 1981.

DuFrain, R. J.
A conformation based hypothesis for the mechanism of sister chromatid exchange formation.
Mamm. Chromosomes Newslett. 22: 9; 1981.

DuFrain, R. J.
The influence of streptonigrin exposure on the SCE frequency in rabbit lymphocytes.
Environ. Mutagen. 3: 382-383; 1981.

DuFrain, R. J.; Littlefield, L. G.; Frome, E. L.; Wilmer, J. L.; Huff, V.; Hutton, D.; and Morrison, W. D.
Cytogenetic evaluation of embryos and somatic cells from female rabbits treated with streptonigrin.
Mamm. Chromosomes Newslett. 21: 17; 1980.

)ree, E. A.
)rtality among workers at a uranium refining and processing plant.
Am. J. Epidemiol. 112: 446; 1980.

Frome, E. L., and DuFrain, R. J.
Determination of overdispersion of dicentric chromosome aberrations that are assumed to follow Poisson distribution.
Biometrics 37: 183; 1981.

Fry, S. A.; Lushbaugh, C. C.; and Hübner, K. F.
An overview of radiation accidents and injuries.
Int. J. Nucl. Med. Biol. In press.

Hayes, R. L.; Rafter, J. J.; and Carlton, J. E.
Toxicity and tissue distribution of [^{99}Tc] pertechnetate.
In *Proceedings of the 21st Annual Meeting and Continuing Education Lectures,* Southeastern Chapter of the Society of Nuclear Medicine, [Abstract Section] p. 8. Nashville, Tennessee: 1980.

Hayes, R. L.; Hübner, K. F., and Gibbs, W. D.
Use of positron tomography for in vivo quantitation of the tissue distribution of radiopharmaceuticals.
In *Advances in Radiopharmacology*, edited by L. G. Colombetti, pp. 104-105, Chicago, Illinois: International Association of Radiopharmacology, 1981.

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
The in vivo entry of ^{67}Ga into an experimental abscess.
In *Proceedings of the 21st Annual Meeting and Continuing Education Lectures,* Southeastern Chapter of the Society of Nuclear Medicine, [Abstract Section] p. 1. Nashville, Tennessee: 1980.

Hübner, K. F.; Partain, C. L.; Robertson, J.; Mahaley, M. S.; Purvis, J.; and Gibbs, W. D.
Brain tumor imaging by positron emission computerized tomography (ECT) using ^{11}C-labeled amino acids.
In *Proceedings of the 21st Annual Meeting and Continuing Education Lectures,* Southeastern Chapter of the Society of Nuclear Medicine, [Abstract Section] p. 2. Nashville, Tennessee: 1980.

Hudson, D. R.; Frome, E. L.; Dupree, E. A.; Hansard, M. S.; Beck, W. L.; and Lushbaugh, C. C.
Exploratory analysis of mortality in a cohort of nuclear material workers.
Am. J. Epidemiol. 114: 446; 1981.

Jay, M.; Digenis, G. A.; Chaney, J. E.; Washburn, L. C.; Byrd, B. L.; Hayes, R. L.; and Callahan, A. P.
Synthesis and brain uptake of carbon-11 phenethylamine.
J. Labelled Compd. Radiopharm. 18: 237; 1981.

Kabalka, G. W.; Gooch, E. E.; Sun, T. T.; and Washburn, L. C.
A rapid and mild method for labeling functionally substituted molecules with iodine radionuclides.
J. Labelled Compd. Radiopharm. 18: 117-118; 1981.

King, P. H., and Wieland, B. W.
Computer modeling of accelerator beam window failures.
J. Labelled Compd. Radiopharm. 18: 225-226; 1981.

Littlefield, L. G.; DuFrain, R. J.; Joiner, E. E.; and Colyer, S. P.
Overdispersion of two-break chromosome lesions in cultured lymphocytes from a person internally contaminated with ^{241}Am.
Environ. Mutagen. 3: 363; 1981.

1028501

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; DuFrain, R. J.; and Cohen, M. M.
Chromosomal radiation sensitivity in lymphoblastoid cell lines from patients with ataxia telangiectasis.
Mamm. Chromosomes Newslett. 22: 20; 1981.

Littlefield, L. G.; DuFrain, R. J.; Joiner, E.; Colyer, S.; Batson, J.; and Welter, D. J.
Cytogenetic dose response kinetics in lymphocytes of persons with Down's syndrome following in vitro exposure to low LET radiation.
Mamm. Chromosomes Newslett. 21: 16; 1980.

Lushbaugh, C. C.
Pathophysiology of ionizing radiation injuries.
J. Occup. Med. 22: 276; 1980.

Lushbaugh, C. C.; Beck, W. L.; Dupree, A. E.; Frome, E. L.; Fry, S. A.; Hansard, S. A.; Hudson, D. R.; and Polednak, A. P.
Health and mortality of DOE workers.
Trans. Am. Nucl. Soc. In press.

Partain, C. L.; Hübner, K. F.; Mahaley, M. S.; Rollo, F. D.; Price, R. R.; and Scatliff, J. H.
Brain tumor imaging based on ^{11}C-labeled amino acid utilization using positron emission tomography.
Invest. Radiol. 15: 380; 1980.

artain, C. L.; Hübner, K. F.; Mahaley, M. S.; Rollo, F. D.; Price, R. R.; and Scatliff, J. H.
Brain tumor imaging: Positron emission tomography using ^{11}C-labeled amino acids.
J. Comput. Assist. Tomogr. 4: 702; 1980.

Partain, C. L.; Price, R. R.; James, A. E., Jr.; Hübner, K. F.; Mahaley, M. S.; Scatliff, J. H.; and Robertson, J. T.
Positron emission tomography: Brain tumor imaging using carbon-11 amino acid.
In *Proceedings of the IAEA International Symposium on Medical Radionuclide Imaging,* 1980, Heidelberg, Germany. In press.

Polednak, A. P.
Studies of workers exposed to internal radiation (uranium and thorium).
Am. J. Epidemiol. 112: 445; 1980.

Richter, C. B.; Tankersley, W. G.; Davis-Tardif, S.; and Carson, R. L.
Preference for high sugar diets by the common marmoset.
Am. J. Primatol. In press.

Rudnick, S.; Shy, C. M.; Fry, S.; and Robie, D.
Health effects in DOE workers occupationally exposed to five or more rem per year.
Am. J. Epidemiol. 114: 448-449; 1981.

Urso, P., and Gengozian, N.
In utero exposure to benzo[a] pyrene (BP) decreases the immune response and increases sensitivity to tumor induction.
Fed. Proc. 40: 1037; 1981.

Washburn, L. C.; Ringenberg, R. E.; Sun, T. T.; and Hayes, R. L.
^{11}C-Labeled 1-aminocyclohexanecarboxylic acid ([^{11}C] ACHC), a potential agent for studies of amino acid transport in the brain.
J. Labelled Compd. Radiopharm. 18: 13-14; 1981.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Callahan, A. P.
Resolution of DL-[^{11}C] valine by high-performance liquid chromatography.
J. Nucl. Med. 22: P74; 1981.

Washburn, L. C.; Dees, L.; Byrd, B. L.; Sun, T. T.; and Hayes, R. L.
Comparison of ^{68}Ga EDTA, [^{11}C] α-aminoisobutyric acid, and [^{99m}Tc] pertechnetate for diagnosis of blood-brain barrier disruption.
J. Nucl. Med. 22: P75; 1981.

Washburn, L. C.; Dees, L.; Byrd, B. L.; Sun, T. T.; and Hayes, R. L.
^{11}C-Labeled α-aminoisobutyric acid, a potential agent for positron tomographic assessment of blood-brain barrier disruption.
In *Proceedings of the 21st Annual Meeting and Continuing Education Lectures,* Southeastern Chapter of the Society of Nuclear Medicine, [Abstract Section] p. 2. Nashville, Tennessee: 1980.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Ringenberg, R. E.; and Hayes, R. L.
^{11}C-Labeled amino acids for the study of brain function.
In *Proceedings of the 182nd ACS National Meeting,* Abstract NUCL-45, New York: American Chemical Society, 1981.

Wieland, B. W.; Schlyer, D. J.; Ruth, T. J.; and Wolf, A. P.
Deuteron beam penetration in a neon gas target for producing fluorine-18.
J. Labelled Compd. Radiopharm. 18: 27-29; 1981.

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Summaries of Full-Length Publications

Arneson, R. M.; Hinds, J. L.; and Wander, J. D.
Antioxidants in neoplastic cells: IV. Evidence that the phenolic antioxidant A274 is 9-(4-hydroxyphenyl)xanthene, an artifact in tissue culture media.
Lipids 16: 82-86; 1981.

An extensively conjugated phenol (A274), recently shown to be present as a common trace constituent in randomly selected batches of commercial tissue culture media, was diazomethylated; the monomethyl derivative so prepared was shown to exhibit gas chromatographic and mass spectrometric properties identical to those observed for synthetically prepared 9-(4-methoxyphenyl)-xanthene but different from those of several isomeric compounds. The structure 9-(4-hydroxyphenyl)xanthene is thus proposed for A274.

Bair, J. K.; Miller, P. D.; and Wieland, B. W.
Neutron yields from 4- to 12-MeV proton bombardment of ^{11}B, ^{13}C, and ^{18}O as related to the production of ^{11}C, ^{13}N, and ^{18}F.
Int. J. Appl. Radiat. Isot. 32: 389-395; 1981.

Total neutron yields resulting from the proton bombardment of infinitely thick targets of ^{11}B, ^{NAT}C, and $^{28}Si^{NAT}O_2$ have been measured for bombarding proton energies below 12 MeV. From the ^{NAT}C data, the yield from ^{13}C has been derived. From the $^{28}Si^{NAT}O_2$ data, the yield for ^{NAT}O has been determined. The measured yields are compared to those calculated from the known thin-target cross sections. The relationship of these neutron yields to the production of the pharmaceutically portant short-lived positron emitters, ^{11}C, ^{13}N, and ^{18}F, scussed.

Beck, W. L.
Radiation accident dosimetry.
Nucl. Instrum. Methods 175: 150-152; 1980.

Thermoluminescence dosimeters have been used to determine the dose to victims of two serious external radiation accidents. Although these accidents differed significantly in source activity, geometry, exposure time, dose variation (10 to about 10^6 rad) to the victim, etc., the properties of the LiF dosimetry system were ideally suited for these studies.

Blank, M. L.; Cress, E. A.; Whittle, T.; and Snyder, F.
In vivo metabolism of a new class of biologically active phospholipids: 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a platelet-activating, hypotensive phospholipid.
Life Sci. 29: 769-775; 1981.

This report describes the in vivo metabolism of a new class of naturally occurring biologically active phospholipids (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines) that can cause hypotension and platelet aggregation. After intravenous injection in male rats, the acetylated ether phospholipid (1-[1′2′-^{3}H]alkyl) is rapidly cleared ($T_{1/2} \cong 30$ s) from blood, and its metabolites are found in a variety of tissues. The tissues containing the highest levels of radioactivity are lung, liver, spleen, and kidney. Chromatographic results showed that a considerable portion of the active lipid is not readily catabolized in some of the major tissues examined; however, inactive metabolites were also found, nly 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine and 1-alky-2-l-*sn*-glycero-3-phosphocholine; the latter has a long-chain fatty acid at the *sn*-2 position instead of the acetate. The findings are consistent with our earlier data that show these same tissues have the most active enzyme systems for metabolizing 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine.

Blank, M. L.; Lee, T-c.; Fitzgerald, V.; and Snyder, F.
A specific acetylhydrolase for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid).
J. Biol. Chem. 256: 175-178; 1981.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a phospholipid with platelet-activating and hypotensive properties, has an extremely labile acetate grouping. The acetate group is obviously important in the expression of the biological properties of this unique derivative of plasmanic acid since once it is hydrolyzed from the parent compound to form the lyso product, all biological activity is lost. Our studies show that the enzyme responsible for the hydrolysis of the acetate moiety, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine: acetylhydrolase, occurs in the cytosolic fraction of a variety of tissues and has a pH optimum of 7.5 to 8.5. Effects of calcium, magnesium, EDTA, dithiothreitol, deoxycholate, and diisopropylfluorophosphate on the enzyme activity and the fact that egg phosphatidylcholine was not inhibitory indicate the acetylhydrolase activity has different properties from those normally associated with the phospholipase A_2 that utilizes phospholipids with two long-chain acyl groups. The highest specific activity of the acetylhydrolase occurred in kidney; lung and brain were also good sources of the enzyme. The soluble fraction from the kidney cortex had an apparent K_m and V_{max} of 3.1 μM and 17.8 nmol/min/mg of protein, respectively. Our results indicate that acetylhydrolase plays a significant role in the catabolism of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine.

Blank, M. L.; Cress, E. A.; Byrd, B. L.; Washburn, L. C.; and Snyder, F.
Liposomal encapsulated Zn-DTPA for removing intracellular ^{169}Yb.
Health Phys. 39: 913-920; 1980.

Multilamellar liposomes possessing neutral, positive, or negative charges were tested for their capacity to encapsulate sodium ethylenediaminetetraacetate (EDTA) and for their selectivity in depositing in specific tissues after being injected into rats. Negative-charged liposomes had the greatest trapping efficiency over a wide range of lipid-to-aqueous phase ratios. In contrast, except for lung, liposomal charge had no significant effect on the tissue distribution of encapsulated EDTA; liver and spleen exhibited the highest uptake with all preparations. The proportion of encapsulated EDTA taken up by the liver decreased as the amount of injected liposomes was increased.

Free zinc diethylenetriaminepentaacetate (Zn-DTPA) and multilamellar liposomes containing entrapped Zn-DTPA were administered to rats that had been injected with ^{169}Yb-citrate 24 hr earlier. At doses of 14 mg Zn-DTPA per kg body weight, both free Zn-DTPA and the liposomal-bound Zn-DTPA caused increased removal of ^{169}Yb from animals. However, treatment with the liposomal Zn-DTPA caused significantly more of the ^{169}Yb to be removed than did the free Zn-DTPA treatment by itself. Our data indicate that lipophilic forms of chelators can effectively increase the removal rates of heavy metal contamination in tissues.

Cabot, M. C., and Goucher, C. R.
Chaulmoogric acid: Assimilation into the complex lipids of mycobacteria.
Lipids 16: 146-148; 1981.

Lipid analysis of *Mycobacterium vaccae*, grown in the presence of chaulmoogric acid, demonstrates that this cyclopentenyl fatty acid is taken up by the organism and incorporated into cellular phospholipids and triacylglycerols. As cell growth is retarded by the addition of chaulmoogric acid to the growth medium, it is possible that the antimicrobial properties of this compound result from a perturbation of membrane processes.

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Cabot, M. C., and Lumb, R. H.
The activity of a low temperature lipase in the larvae of Sarcophaga bullata (Diptera:Sarcophagidae).
Comp. Biochem. Physiol. 68B: 325-328; 1981.
Work performed at Western Carolina University, Cullowhee, North Carolina.

Minced larval tissues of the dipteran flesh fly, *Sarcophaga bullata*, were stored at −20°C for periods up to 14 days. After cold storage, analysis of lipid classes revealed a decrease in the amount of triacylglycerols and an increase in the amount of free fatty acids. The change in the proportions of larval triacylglycerols and fatty acids was shown to be the result of enzymatic activity persisting at −20°C. The enzyme shows a high degree of specificity for triacylglycerols containing unsaturated acyl moieties.

Cabot, M. C.; Welsh, C. J.; Callaham, M. F.; and Huberman, E.
Alterations in lipid metabolism induced by 12-*O*-tetradecanoylphorbol-13-acetate in differentiating human myeloid leukemia cells.
Cancer Res. 40: 3674-3679, 1980.

Several aspects of lipid metabolism were evaluated in differentiating human myeloid leukemia (HL-60) cells after treatment with the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate. Modifications accompanying the phorbol ester-induced differentiation include an increase in the incorporation of acetate into free fatty acids and neutral lipids, an increase in the amount of neutral glycerolipids, and a selective incorporation of long-chain fatty alcohols into triacylglycerols and ether-linked alkyldiacylglycerols. Additionally, an enhanced stimulation of phospholipid metabolism, as measured by the incorporation of ^{32}P and labeled precursors of the polar head groups, could be detected within 4 hr after treatment of cells with the tumor promoter. *O*-Methyltetradecanoylphorbol-13-acetate, an analog with poor tumor-promoting activity, failed to elicit any activity on phospholipid metabolism.

Casey, D. L.; Digenis, G. A.; Wesner, D. A.; Washburn, L. C.; Chaney, J. E.; Hayes, R. L.; and Callahan, A. P.
Preparation and preliminary tissue studies of optically active D- and L-[^{11}C] phenylalanine.
Int. J. Appl. Radiat. Isot. 32: 325-330; 1981.

A rapid method for the preparation of L-[1-^{11}C] phenylalanine and D-[1-^{11}C] phenylalanine is described. DL-[1-^{11}C] Phenylalanine (375 mCi) was synthesized from [^{11}C] cyanide (2.4 Ci) by a modified Bücherer-Strecker reaction with a chemical yield of 65% in 40 min (including purification). The resolution of the D- and L-isomers was accomplished in 35 min (including purification) by oxidative deamination using immobilized L- and D-amino acid oxidase, respectively; the yields for D- and L-[1-^{11}C] phenylalanine were 19 mCi and 27 mCi. Preliminary tissue distribution studies of these labeled isomers in the rat showed that the pancreas-to-liver ratio for the L-isomer increased throughout the first hour of observation following i.v. administration while that of the D-isomer decreased after 30 min. Whole body retention data revealed that the loss of radioactivity from DL- or D-[1-^{11}C] phenylalanine was less than 2% during the first hour observation after i.v. administration.

DuFrain, R. J., and Garrand, T. J.
The influence of incorporated halogenated analogues of thymidine on the sister chromatid exchange frequency in human lymphocytes.
Mutat. Res. 91: 233-238; 1981.

The DNA of PHA-stimulated human lymphocytes was substituted with three different halogenated analogues of thymidine (bromodeoxyuridine, chlorodeoxyuridine, and iododeoxyuridine) by culture in fluorodeoxyuridine supplemented medium. The metaphases from these blood cell cultures were differentiated by a modified fluorescence plus Giemsa method, and cell division rates as well as sister chromatid exchange frequencies were determined. The results of the study indicated that the three different analogues gave three different spontaneous sister chromatid exchange rates. This is interpreted to indicate that the degree of molecular conformation alteration in cellular DNA has a significant influence on the formation of sister chromatid exchanges.

DuFrain, R. J.; Littlefield, L. G.; and Wilmer, J. L.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. I. The test system and the effects of streptonigrin.
Mutat. Res. 85: 147-160; 1981.

An appropriate method for evaluating transmissible genetic damage in female germ cells is presented. Analysis of karyotype preparations from preimplantation (6-day) rabbit embryos for consistent structural chromosome abnormalities is described as a sensitive way in which these determinations can be made. A table prepared from summarized research reports shows that less than 1 in 2000 rabbit blastocysts karyotyped thus far has a consistent structural chromosome abnormality. In the new data presented in this study, none of the 90 control blastocysts karyotyped has consistent structural chromosome abnormalities while 13 of 278 from female rabbits treated with streptonigrin (NSC-45383) before conception did. These findings demonstrate that karyotype analysis for consistent chromosome lesions in preimplantation rabbit embryos from females exposed to chemical clastogens can serve as a sensitive and quantitive means of estimating effects on oocytes. The results of the study also show that the consistent structural chromosome lesions in the blastocyst karyotypes are what would be predicted from the mode of action of streptonigrin and the segregation of chromosomes during meiosis. The relative efficiency of this system based on the rabbit as compared to another recently described system based on the hamster is also discussed.

Frome, E., and Hudson, D.
Interval estimation for X predictions when the dependent variable follows the Poisson distribution.
In *1980 Proceedings of the Statistical Computing Section of the American Statistical Association*, Houston, Texas, pp. 294-297, Washington, D.C.; American Statistical Association, 1981.

Linear and quadratic regression models (dose-response curves) are used to establish standard curves, which are then used to obtain point and interval estimates of an unknown X value. The dependent variable is assumed to follow the Poisson distribution for both the dose-response curves and the unknown exposure. Poisson regression analysis is used to obtain maximum-likelihood estimates of the dose-response curve parameters. A procedure is described that can be used to obtain point and interval estimates for an unknown dose. The procedure requires estimates of the variances of the maximum-likelihood estimators and assumes that the yield for the unknown follows the Poisson distribution. A computer simulation is used to evaluate the effectiveness of the procedure for different sample sizes.

Frome, E. L., and Hudson, D. R.
A general statistical data structure for epidemiologic studies of DOE workers.
In *1980 Statistical Symposium*, Berkeley, California, edited by T. Truett, D. Margolies, D. W. Mensing, CONF-80145, pp. 206-218, Washington, D.C.: Department of Energy, 1981.

Epidemiologic studies to evaluate the occupational risks associated with employment in the nuclear industry are currently being conducted by DOE. Data (work history, demography, medical history, vital status, and hazardous exposure history) that have potential value in evaluating any long-term health effects of occupational exposure to low levels of radiation are

obtained for each individual at a given facility. We propose a general data structure for statistical analysis that is used to define transformations from the data management system into the data analysis system. The general data structure consists of a list of variables $\{V_{ij}, X_{ik}(t), t \in T(i), (j = 1, \ldots, J), (k = 1, \ldots, K), 1, \ldots, N)\}$, where N is the number of individuals, K is the number of time-dependent variables, J is the number of fixed variables, and $T(i)$ is a set of positive integers that indicate the time periods for which the X's are available for each individual. The resulting Master Statistical Analysis File serves as the starting point for probabilistic editing, data validation, and data analysis. Statistical methods of interest in epidemiologic studies include contingency table analysis and survival analysis procedures that can be used to evaluate potential associations between occupational radiation exposure and mortality. The purposes of this paper are to discuss (1) the adequacy of this data structure for single- and multiple-facility analysis and (2) the statistical computing problems encountered in dealing with large populations over extended periods of time.

Gengozian, N., and Ostby, D. A.
Antibodies selectively reactive to autologous and host-type platelets are obtained following interspecies immunizations in marmosets.
Clin. Exp. Immunol. 43: 128-134; 1981.

Interspecies immunization of marmosets with platelets leads to an acute thrombocytopenia which appears to be immunologically mediated. The thrombocytopenia is preceded by the formation of antibodies to the donor platelet antigen and deposition of IgG on the host's platelets. Examination of sera obtained from these animals during the course of the disease has revealed the presence of antibodies toward autologous and host-type platelets. Autologous antibody was found when thrombocytopenia was severe; upon platelet recovery, this antibody was absent or reduced in titer. This in vivo absorption of host-specific (autologous) antibody led to detection in the serum of antibodies reactive to other members of the host species. The presence of such host-type antibodies appeared to be related to the intensity of antigenic stimulation, the amount of antibody formed, and the degree of thrombocytopenia. Deposition of IgG on the host's platelets coincided with the appearance of the antihost antibodies; whether the IgG detected on the host's platelets is exclusively cross-reactive antibody or includes also an immune complex is not yet known. Finally, the data have identified potential immunogenetic differences among members of two marmoset species with respect to platelet antigens.

Hayes, R. L., and Byrd, B. L.
Transfer of ^{67}Ga from hamster dam to fetus and offspring.
In *Third International Radiopharmaceutical Dosimetry Symposium,* Oak Ridge, Tennessee, 1980, edited by E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, HHS Publication FDA 81-8166, pp. 447-453, Rockville, Maryland: U.S. Bureau of Radiological Health, 1981.

We have studied the transfer of ^{67}Ga from the hamster dam to the fetus and also to the postpartum offspring. During gestation, the concentration of ^{67}Ga in the mammary glands increased by approximately 20-fold, and ^{67}Ga was transferred across the placenta into the fetus. The concentration of ^{67}Ga in the fetus decreased during the terminal stages of gestation, partly due to a decreased transfer of ^{67}Ga itself and partly as the result of the rapid growth of the fetus. One-day-old offspring were able to absorb from the gastrointestinal tract the ^{67}Ga contained in ingested dam's milk, whereas 9-day-old offspring retained essentially all such ingested ^{67}Ga. The ^{67}Ga radiation hazards to the fetus during gestation and that resulting from the transfer of ^{67}Ga to the newborn through ingestion of the mother's milk should receive further investigation.

Hayes, R. L.; Rafter, J. J.; Byrd, B. L.; and Carlton, J. E.
Studies of the in vivo entry of ^{67}Ga into normal and malignant tissue.
J. Nucl. Med. 22: 325-332; 1981.

Previous studies of the effect of scandium on the tissue distribution of ^{67}Ga suggest that ^{67}Ga makes its initial in vivo entry into normal and malignant tissues by different routes. (Scandium blocking of plasma protein ^{67}Ga binding increased ^{67}Ga excretion, decreased its uptake in normal tissues, but had little effect on rodent tumors.) In further studies we have used other methods to alter the plasma binding of ^{67}Ga. Iron saturation of plasma produced effects on ^{67}Ga tissue distribution similar to those observed with scandium. On the other hand, increasing ^{67}Ga plasma binding through induction of anemia and administration of apotransferrin produced the reverse of the effects observed with scandium and iron. We conclude that the initial in vivo entry of ^{67}Ga into tumor tissue involves mainly an unbound or loosely bound form of ^{67}Ga, whereas its uptake by normal soft tissues is strongly promoted by its binding to transferrin.

Hübner, K. F.; King, P.; Gibbs, W. D.; Washburn, L. C.; and Hayes, R. L.
Positron emission computerized tomography: A potential tool for in vivo quantitation of the distribution of radiopharmaceuticals.
In *Third International Radiopharmaceutical Dosimetry Symposium,* Oak Ridge, Tennessee, 1980, edited by E. E. Watson, A. T. Schlafke-Stelson, J. L. Coffey, and R. J. Cloutier, HHS Publication FDA 81-8166, pp. 77-87, Rockville, Maryland: U.S. Bureau of Radiological Health, 1981.

The principles and some of the difficulties in quantitative positron emission computerized tomography have been discussed. We have shown that "randoms" and scattered events are a major cause of noise and counting errors in positron emission computerized tomography. The noise has been identified as a convoluting process and a mathematical solution has been presented. Examples of phantom studies and in vivo measurements have demonstrated that the distribution of positron-emitting radiopharmaceuticals can be quantitated with much improved accuracy using the deconvolution equation to remove undesired noise.

Hübner, K. F.; Krauss, S.; Washburn, L. C.; Gibbs, W. D.; and Holloway, E. C.
Tumor detection with 1-aminocyclopentane and 1-aminocyclobutane [^{11}C] carboxylic acid using positron emission computerized tomography.
Clin. Nucl. Med. 6: 249-252; 1981.

Eight patients with proven or suspected neoplastic lesions were examined with 1-aminocyclobutane [^{11}C] carboxylic acid and 1-aminocyclopentane [^{11}C] carboxylic acid using positron emission computed tomography. The results of this comparative study have shown that both of these unnatural amino acids have a high affinity for malignant tumors. The potential of nonmetabolizable ^{11}C-labeled amino acids as noninvasive indicators of metabolic tumor activity is demonstrated and discussed.

Huff, V.; Littlefield, L. G.; DuFrain, R. J.; and Wilmer, J. L.
Factors affecting the sensitivity of rabbit lymphoid and bone marrow cells to streptonigrin-induced chromosome aberrations.
Cytogenet. Cell Genet. 30: 15-24; 1981.

In previous cytogenetic studies of rabbits injected with the clastogen, streptonigrin, we observed a higher incidence of chromosome aberrations in lymph node cells than in marrow cells recovered 6 hr after treatment. In this study we examined several factors that may be responsible for this difference in the incidence of lesions. Results from streptonigrin pulse-treated

1028505

lymphoid and marrow cultures demonstrated that the responses were different in vitro also. In addition, at higher doses of streptonigrin, lymphoid cells displayed a greater sensitivity to the drug when treated during DNA synthesis; marrow cells showed no differences in sensitivity throughout the cell cycle. Analysis of the percentage of lymph node and marrow metaphases labeled following 2, 4, or 6 hr of culture in the presence of tritiated thymidine indicated that G_2 was less than 2 hr in lymph node cells and approximately 4 hr in marrow cells. From these data we conclude that the difference in the length of G_2 in the two cell types is partially responsible for the difference in the incidence of lesions 6 hr after an in vivo streptonigrin exposure. However, our data also indicate that lymph node cells are innately more sensitive to the induction of lesions than are marrow cells.

Khare, M., and Federer, W. T.
A simple construction procedure for resolvable incomplete block designs for any number of treatments.
Biom. J. 23: 121-132; 1981.

A simple, straightforward procedure, which requires no special tables or generators, is presented for constructing resolvable incomplete block designs for $v = pk$, $v = p^2k$, . . . , treatments, for $k \leq p$, in incomplete blocks of size k. Also, it is shown how to obtain incomplete block designs for any v in blocks of size k and $k + 1$. The procedure allows construction of balanced incomplete block designs for $p = k$ a prime number. For $p = n$ not a prime number, incomplete block designs can be obtained by the procedure but are not balanced. However, for ps being the smallest prime factor of n, $p_s + 1$ for $v = n^2$, $p^2s + ps + 1$ for $v = n^3$, . . . , arrangements can be obtained for which the occurrence of any treatment pair in the blocks is either zero or one. This is called a zero-one concurrence design. Procedures are described for obtaining additional zero-one concurrence arrangements. It is shown that the efficiency of these designs is maximum. Both intra-block and inter-block analyses are described.

King, P. H.; Hübner, K.; Gibbs, W.; and Holloway, E.
Noise identification and removal in positron imaging systems.
IEEE Trans. Nucl. Sci. NS-28: 148-151; 1981.

The noise (scattered and random) generated in two positron emission imaging systems has been studied and mathematically modeled as an exponential convolving function. Compton scattering is presumed to be a dominant factor, as opposed to random events. Deconvolution of the sensed data has enabled us to eliminate noise and to obtain images which can be quantified. Further, these images have improved diagnostic quality.

Lee, T-c., and Snyder, F.
Phase transition of an unnatural analog of phosphatidylcholine: Phosphatidyl-*N*-isopropylethanolamine.
Lipids 16: 75-76; 1981.

The phase-transition temperature of an aqueous dispersion of dipalmitoyl phosphatidyl-*N*-isopropylethanolamine was determined using *trans*-parinaric acid as a fluorescent probe. Phosphatidyl-*N*-isopropylethanolamine has a phase-transition temperature that is 3°-4° C higher than the corresponding natural analog of dipalmitoyl phosphatidylcholine.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Substrate specificity in the biocleavage of the *O*-alkyl bond: Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid) and its metabolites.
Arch. Biochem. Biophys. 208: 353-357; 1981.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine possesses both hypotensive and platelet-activating properties. Our data show that after removal of the acetyl group at the *sn*-2 position by an acetyl hydrolase, the product, 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine, can be cleaved by a microsomal tetrahydropteridine-dependent alkyl monooxygenase in the liver and spleen of rats. Results obtained for the tetrahydropteridine requirement, tissue distribution, responses to thermal inactivation and catalase, and substrate inhibition of the enzyme indicate that the ether linkage of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine is hydrolyzed by a monooxygenase that appears to be identical to the one responsible for cleavage of the *O*-alkyl moiety of alkylglycerols.

Leonard, R. B., and Ricks, R. C.
Emergency department radiation-accident protocol.
Ann. Emerg. Med. 9: 462-470; 1980.

Every emergency department faces the potential problem of handling one or more victims of a radiation accident. While emergency departments near nuclear power plants or isotope production laboratories probably have a detailed protocol for such emergencies, a similar protocol is needed for the emergency department that may have to handle an isolated event, such as a vehicular accident that spills radioactive material and contaminates passengers or bystanders. This communication attempts to answer that need, presenting a step-by-step protocol for decontamination of a radiation victim, the rationale on which each step is based, a list of needed supplies, and a short summary of decorporation procedures that should be started in the emergency department.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Physical, chemical, and biological factors affecting sister chromatid exchange induction in human lymphocytes exposed to mitomycin C prior to culture.
Mutat. Res. 81: 377-336; 1981.

In experiments to assess the effects of several biological, chemical, and physical variables on sister chromatid exchange (SCE) induction in cultured lymphocytes exposed to mitomycin C (MMC) before phytohaemagglutinin stimulation, we observed (1) high SCE frequencies in female cells and normal SCE frequencies in Y-bearing metaphases in mixed cultures containing equal numbers of MMC-treated female lymphocytes and untreated male lymphocytes, (2) small, but statistically significant decreases in SCE with increasing pH after G_0 exposure in the pH range 6.6-7.6, and (3) pronounced reductions in MMC-induced SCE in lymphocytes exposed at 4°C vs. 37°C. In other studies, SCE induction was evaluated in cultures exposed during G_0 to MMC concentrations ranging from 0.25 to 2.5 μg/ml for varying time intervals ranging from 5 min to 24 hr. For all concentrations tested, SCE induction varied as a linear function of G_0 exposure time. To compare SCE induction between cultures, we calculated the mean frequencies of SCE induced per metaphase/unit dose MMC/unit G_0 exposure time (SCE/μg/h). A mean frequency of 20.7 ± 4.8 SCE/μg/h was observed for 41 lymphocyte cultures, suggesting that a single term adequately describes the rate of SCE induction following G_0 exposure to a 10-fold range in concentration of MMC for time intervals of 30 min to 24 h.

Morrison, W. D.; Huff, V.; Colyer, S. P.; DuFrain, R. J.; and Littlefield, L. G.
Cytogenetic effects of *cis*-platinum(II)diaminedichloride in vivo.
Environ. Mutagen. 3: 265-274; 1981.

The chemotherapeutic agent, *cis*-diaminedichloride (*cis*-PDD), has been shown to be mutagenic, teratogenic, and carcinogenic. We determined the cytogenetic effects of *cis*-PDD on human and rabbit lymphocytes in vitro and on rabbit marrow cells, lymph node cells, and lymphocytes in vivo. Lymphocyte cultures from two humans and one rabbit were treated in vitro with *cis*-PDD. For in vivo studies, five New Zealand white rabbits were given i.v. injections of *cis*-PDD. Posttreatment blood samples were withdrawn for analysis and rabbits were

sacrificed at either 6 or 24 hr for cytogenetic analysis of marrow and node cells. Sister chromatid exchange (SCE) analysis of human and rabbit metaphases from lymphocytes treated in vitro showed that rabbit lymphocytes are more sensitive to SCE induction by *cis*-PDD. Significant increases in SCE were observed in lymphocyte cultures obtained as early as 1 h posttreatment from injected rabbits. Analysis of node, marrow, and lymphocyte metaphases from injected rabbits showed a high number of chromosome aberrations in these cells with bone marrow showing a delayed response to treatment. These results indicate that *cis*-PDD is clastogenic in hematopoietic tissues in vivo and that SCE methodology may be useful in monitoring patients receiving *cis*-PDD therapy.

Nickerson, D. A., and Gengozian, N.
Functional capabilities of marmoset T and B lymphocytes in primary in vitro antibody formation.
Cell. Immunol. 57: 408-419; 1981.

In vitro tests of T- and B-lymphocyte function of two marmoset species, *Saguinus fuscicollis* and *Saguinus oedipus oedipus*, were examined to explore the lower immune response profile previously reported for *S. o. oedipus*. The helper capability of *S. o. oedipus* T cells was demonstrated by their ability to restore the sheep red blood cell response of (a) T-deficient mouse spleen cell cultures and (b) *S. fuscicollis* B-cell cultures. Experiments with trinitrophenyl-lipopolysaccharide (TNP-LPS) revealed peripheral blood leukocytes (PBL) from both species capable of antibody formation. This response was both T cell and monocyte independent; indeed, removal of T cells led to an enhanced response, indicating a regulatory role for this cell in each species. Studies with the nonmitogenic form of TNP-LPS, trinitrophenyl-base-hydrolyzed-lipopolysaccharide, revealed that plaque-forming cells could be obtained from *S. fuscicollis* PBL while *S. o. oedipus* PBL were unresponsive, suggesting some differential in B-cell stimulation for the two species. This report also demonstrates that hemopoietic chimerism, a feature common to all marmosets, has a negative influence on antibody-forming capabilities.

Polednak, A. P., and Frome, E. L.
Mortality among men employed between 1943 and 1947 at a uranium-processing plant.
J. Occup. Med. 23: 169-178; 1981.

Mortality is described in a cohort of 18,869 white males who were employed between 1943 and 1947 at a uranium conversion and enrichment plant in Oak Ridge, Tennessee. Workers in certain departments (especially chemical workers) were exposed to high average air levels of uranium dust. Based on deaths reported in 1974 by the Social Security Administration and using mortality rates for U.S. white males, standardized mortality ratios (SMR) for various causes in the entire cohort were generally less than 1.00. After correction for unascertained deaths and missing death certificates, the SMR for lung cancer was 1.22 (95% confidence limits, 1.10 and 1.36). SMR for various causes, including lung cancer, did not tend to be higher in 8345 workers employed in areas where uranium dust was present or in 4008 of these 8345 workers employed for 1 year or longer at the plant. Other causes of particular interest (i.e., bone cancer, leukemia, diseases of respiratory and genitourinary systems) did not exhibit high SMR. The suggestive finding of the authors was an increased number of lung cancer deaths in a group of chemical workers hired at $\geq$ 45 years of age. Continued follow-up of the cohort is necessary for further evaluation of the long-term health effects of exposure to uranium.

Renooij, W., and Snyder, F.
Synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor and a hypotensive lipid) by choline-phosphotransferase in various rat tissues.
Biochim. Biophys. Acta. 663: 545-556; 1981.

The unique alkyl phospholipid, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, has been reported to exhibit powerful antihypertensive activity (Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks, B.; and Muirhead, E. E. (1979) *Biochem. Biophys. Res. Commun.* 90: 1194-1200) and appears to be an extremely potent platelet-activating factor (Demopoulos, C. A., Pinckard, R. N.; and Hanahan, D. J. (1979) *J. Biol. Chem.* 254: 9355-9358). In the present study, microsomal preparations from several rat tissues were found to catalyze the synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by a 1-alkyl-2-acetyl-*sn*-glycerol: CDP-choline cholinephosphotransferase reaction. Optimal conditions to measure enzyme activity were established. A subcellular survey of this cholinephosphotransferase activity showed that the enzyme was of microsomal origin. Enzyme activity was found in microsomes from several tissues; however, spleen has the highest activity of the tissues examined. Three different species of 1-alkyl-2-acetyl-*sn*-glycerol were all found to be substrates. The 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine synthesized in the microsomes could be hydrolyzed by adding the 100,000 x *g* supernatant fraction to the incubation medium. The optimum pH for formation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine was 8.0, which was different from the pH optimum of 8.5 observed for the long-chain diacylglycerol cholinephosphotransferases. Activity of cholinephosphotransferase towards 1-alkyl-2-acetyl-*sn*-glycerol was slightly enhanced and stabilized by dithiothreitol, whereas the activity towards a diacylglycerol was inhibited by dithiothreitol. The possible involvement of two different enzymes in the conversion of 1-alkyl-2-acetyl-*sn*-glycerol and diacylglycerol to their respective phospholipid products is discussed.

Richter, C. B.; Lushbaugh, C. C.; and Swartzendruber, D. C.
Cancer of the colon in cotton-topped tamarins.
In *The Comparative Pathology of Zoo Animals*, edited by R. J. Montali and G. Migaki, pp. 567-571, Proceedings of a Symposium, 1978. Washington, D.C.: Smithsonian Institution Press, 1980.

Necropsies were conducted on 114 captive adult cotton-topped tamarins (*Saguinus oedipus oedipus*) that had 5 months or more colony residence time. Fourteen of the tamarins had adenocarcinoma of the colon. These neoplasms occurred among 109 wild-caught animals and occurred in animals that also had chronic colitis. By comparison, necropsies on 517 adult white-lipped tamarins (*Saguinus fuscicollis ssp.*) yielded only one adenocarcinoma of the small intestine in a laboratory-bred animal 49 months of age. One colonic adenocarcinoma was also observed in a series of four necropsies on laboratory-bred *Callimico goeldii*. Although chronic colitis is also frequently observed in *S. fuscicollis ssp.*, it seems that this disease may be precancerous in the cotton-topped tamarin.

Sagan, L. A., and Fry, S. A.
Radiation accidents: A conference review.
Nucl. Saf. 21: 562-569; 1980.

Since 1940, 98 separate accidents in which persons have been injured by accidental exposure to ionizing radiation have been recorded worldwide. Among these were 14 criticality accidents with 5 fatalities, none of which occurred after 1965. Since 1965, the majority of accidents have been caused by exposure to such radiation devices as machines or sources, sometimes producing localized burns with no fatalities (48 accidents), but sometimes producing total-body irradiation resulting in 9 fatalities (20 accidents). This experience, including details of medical management, was reviewed at a conference convened by the Radiation Emergency Assistance Center/Training Site, a unit of the Medical and Health Sciences Division operated for the U.S. Department of Energy by Oak Ridge Associated Universities, to address the topic, "The Medical Basis for Radiation Accident Preparedness." The conference, which was held October 18-20, 1979, in Oak Ridge, Tennessee, is reviewed in this article.

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Sayer, A. M.; Littlefield, L. G.; Dufrain, R. J.; and Richter, C. B.
Analysis of mutagen-induced chromosome damage in a primate oecies (*Saguinus oedipus oedipus*) at risk for spontaneous denocarcinoma of the colon.
Cancer Genet. Cytogenet. 3: 161-169; 1981.

A high incidence of adenocarcinoma of the colon (>16%) has been observed at necropsy in the South American primate, *Saguinus oedipus oedipus (S. oedipus)*, while the disease has not been found in tamarins of the closely related species, *Saguinus fuscicollis spp.*, housed in the same research colony. Cytogenetic analysis in cultured lymphocytes from ten *S. oedipus* and ten *S. fuscicollis illigeri (S. fuscicollis)* demonstrated no differences in the average frequencies of spontaneous or mitomycin C-induced sister chromatid exchanges between animals of the two species. However, highly significant variability in mitomycin C-induced chromosome lesions was observed between the individual *S. oedipus,* with one animal exhibiting increased sensitivity for both sister chromatid exchanges and chromosome breakages. At present we do not know the relationship, if any, between increased sensitivity to mutagen-induced cytogenetic lesions in specific *S. oedipus* tamarins and the increased risk for colon cancer that has been documented in this primate species. However, our cytogenetic findings in this one *S. oedipus* are similar to data obtained in evaluations of persons with several autosomal recessive conditions in which there is a genetic predisposition for developing malignancies.

Swartzendruber, D. C., and Richter, C. B.
Mucous and argentaffin cells in colonic adenocarcinomas of tamarins and rats.
Lab. Invest. 46: 523-530; 1980.

Primary and metastatic neoplasms of four naturally occurring olonic adenocarcinomas in the cotton-topped tamarin, *Saguinus edipus oedipus,* and four chemically induced rat colonic adenocarcinomas were examined with light and electron microscopy. In each case, we observed mixtures of mucous and argentaffin cells in metastatic sites, proving that endocrine cells are an integral part of these tumors. The occurrence of mixed cell populations in metastases in both species suggests, but does not prove, that colonic tumor cells have the capacity for multidirectional differentiation and that neoplastic mucous and argentaffin cells in the colon have a common origin. We also observed mitotic figures in neoplastic argentaffin cells, confirming the observations of others that these cells can label with tritiated thymidine and divide.

Tardif, S. D., and Richter, C. B.
Competition for a desired food in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*).
Lab. Anim. Sci. 31: 52-55; 1981.

Four captive groups of common marmosets (*Callithrix jacchus*) and three groups of cotton-top tamarins (*Saguinus oedipus*) were each presented with a desired food, and their consumption was monitored. Inequalities in consumption between individuals were found for each group of each species. Generally, adult females consumed most of the desired food, followed by juveniles, and finally adult males. High consumption levels by all individuals during isolated feedings indicated that consumption inequalities within groups were most likely due to competition rather than individual preference differences. Aggressive defense of the food cup was performed most frequently by adult females in both species. In *Callithrix jacchus* groups, this aggression was most often directed against adult males, but in *Saguinus oedipus* groups, it was most often directed against juveniles. Modification of the method of food presentation, such that food was more dispersed, lessened, but did not eliminate inequalities in consumption.

Wykle, R. L.; Malone, B.; and Snyder, F.
Enzymatic synthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, a hypotensive and platelet-aggregating lipid.
J. Biol. Chem. 255: 10256-10260; 1980.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, derived chemically from choline plasmalogens of beef heart, has been shown to possess powerful antihypertensive activity (Blank, M. L.; Snyder, F.; Byers, L. W.; Brooks., B.; and Muirhead, E. E. (1979) *Biochem. Biophys. Res. Commun.* 90: 1194-1200) and to be an extremely potent platelet-activating factor (Demopoulos, C. C.; Pinckard, R. N.; and Hanahan, D. J. (1979) *J. Biol. Chem.* 254: 9355-9358). In the present study, microsomal preparations of rat spleen were shown to synthesize 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine by an acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero 3-phosphocholine acetyltransferase reaction; the acetyltransferase appears to be different from the acyltransferase responsible for the transfer of palmitate to glycerolipids. The apparent K_m for acetyl-CoA was 67 μM; the phosphocholine was 30 μM. Higher concentrations of the lyso substrate were inhibitory. When acetyl-CoA (100 μM) and 1-hexadecyl-*sn*-glycero-3-phosphocholine (30 μM) were incubated with spleen microsomes under optimal conditions; the specific activity was approximately 10 nmol of 1-alkyl-1-acetyl-*sn*-glycero-3-phosphocholine/min/mg of protein. Although the reaction was inhibited by the addition of EDTA or EGTA to the incubation mixture, the acetyltransferase did not appear to require a divalent cation. An acyl analog, 1-palmitoyl-2-lyso-*sn*-glycero-3-phosphocholine, also served as a substrate for the acetyltransferase; however, the unnatural isomer, 3-alkyl-2-lyso-*sn*-glycero-1-phosphocholine, did not. The acetyltransferase activity was found in a variety of tissues, but the spleen had the highest activity of the tissues examined; lung, lymph nodes, and thymus also had high activities. Identification of the product and the possible physiological importance of the pathway are discussed.

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Training Programs

Training at the Medical and Health Sciences Division includes programs for postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, high school vocational trainees, and participants in special short-term courses on radiation health problems.

Graduate students participating in Ph.D. training programs have been from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama, and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals. Advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by Oak Ridge National Laboratory, University Programs at ORAU (sponsored by DOE), the University of Tennessee (Hilton A. Smith Fellowship), individual research grants, and operational subcontracts.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows have come from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health and American Cancer Society grants, and divisional funds.

Summer research participation sponsored by University Programs at ORAU makes it possible for academic staff and beginning seniors from colleges and universities throughout the United States to participate in any of the division's research programs. Secretarial and laboratory aide training is also provided to high school students who participate in vocational programs at the high schools in Clinton and Oak Ridge.

Short-term courses in the management of health problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of ten REAC/TS courses were given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

The following individuals participated in the training programs at the division during the past year:

1028509

Predoctoral students
Naima Abd Elghany** (Epidemiology) (University of North Carolina)
Alex J. Brown* (Biochemistry) (University of Tennessee)
Arvind Carpenter** (Epidemiology) (University of Alabama)
Donna Cragle*** (Epidemiology) (University of North Carolina)
Diane Cookfair* (Epidemiology) (University of North Carolina)
Elizabeth Dupree Ellis* (Epidemiology) (University of North Carolina)
Claudia Moore* (Biochemistry) (University of Tennessee)
Dan Strom** (Epidemiology) (University of North Carolina)
Jefferson Surles** (Biochemistry) (University of North Carolina)
Suzette D. Tardif* (Marmoset Program) (Michigan State University)
Jerome Wilson* (Epidemiology) (University of North Carolina)



Students pursuing MPH or MS degrees
Shirley Fry* (Epidemiology) (University of North Carolina)
Alan Johnson*** (Epidemiology) (University of Alabama)
Kolam Mani** (Epidemiology) (University of North Carolina)
Bruce Parrish** (Epidemiology) (University of North Carolina)
Ravishankar Rao* (Epidemiology) (University of Alabama)

Postdoctoral fellows
Daniel H. Albert (Biochemistry)
Dennis R. Hoffman (Biochemistry)

Visiting scientists
Arthur E. Hougland (Biochemistry) (East Tennessee State University)
Akira Imahori (Nuclear Medicine) (Juntendo University, Tokyo)
Christine McDermott (Immunobiology) (University of Scranton)
Edward N. Lambremont (Biochemistry) (Louisiana State University)

College student research participants
Michael Horan (Biochemistry) (Canisius College, Buffalo, NY)
James Gray (Epidemiology) (University of Tennessee)
Glenda Talley (Epidemiology) (University of Tennessee)
Trent Wallace (Radiopharmaceuticals) (Vanderbilt University)

High school vocational participants
Alesia Cummins (Biochemistry) (Clinton Senior High School)
Sabrina Nicholson (Biochemistry) (Clinton Senior High School)
Janie Saunders (Biochemistry) (Clinton Senior High School)

*Thesis research being completed at the division.
**Portion of thesis research being done at the division.
***Nonthesis work.

Preparation of female rabbits for studies designed to detect chemically induced chromosome abnormalities in oocytes. A grant from the National Institute of Child Health and Human Development has been renewed to continue this project in Cytogenetics.

1028510

Collaborative Research

M. C. Cabot
Lipid metabolism in TPA-differentiating human leukemia
Oak Ridge National Laboratory, Biology Division, E. Huberman

Alterations in lipid metabolism during oncogenic transformation of cultured fibroblasts
University of Southern California, Los Angeles, Cancer Research Center, C. Heidelberger

R. J. DuFrain
Human cytogenetic dosimetry for neutron radiation
Oak Ridge National Laboratory, H. W. Dickson; University of California, Los Angeles, Department of Radiological Sciences and Radiation Oncology, A. Norman

Cytogenetic effect studies on chlorinated biphenol degradation products
University of Tennessee, Knoxville, Department of Microbiology, G. S. Sayler

Gene mapping the laboratory rabbit
Oak Ridge National Laboratory, Biology Division, P. A. Lalley

Statistical distributions in cytogenetic dose response
Sangamon State University, Springfield, Illinois, Department of Mathematics, K. G. Janardan; Illinois Environmental Protection Agency, Springfield, D. J. Schaffer

Alpha particle dosimetry for cytogenetic analysis
Battelle Pacific Northwest Laboratories, Richland, Washington, Radiological Sciences Department, D. R. Fischer

N. Gengozian
Fetal hemoglobin synthesis in the marmoset
University of Tennessee Center for the Health Sciences, Knoxville, Department of Medical Biology, J. E. Fuhr

Immunocontraception in the marmoset
University of Tennessee, Knoxville, Department of Zoology, C. A. Shivers

Radiation sensitivity (cytogenetic) of the marmoset
Oak Ridge National Laboratory, Biology Division, R. J. Preston

Thrombopoiesis in the marmoset
University of Tennessee Center for the Health Sciences, Knoxville, Department of Medical Biology, T. P. McDonald

R. Hayes
Synthesis of labeled compounds using organoborane techniques
University of Tennessee, Knoxville, Department of Chemistry, G. W. Kabalka

Collaborative studies in radiopharmaceutical development
Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies
University of Tennessee Center for the Health Sciences, Knoxville, Department of Medicine, I. R. Collmann; Vanderbilt University Hospital, Nashville, Department of Gastroenterology, G. R. Avant

Differentiation of glioblastomas and meningiomas with ^{11}C-labeled amino acids and positron emission computed tomography
Vanderbilt University, Nashville, Department of Nuclear Medicine, C. L. Partain;
University of Tennessee Center for the Health Sciences, Memphis, Department of Neurosurgery, J. T. Robertson; University of North Carolina, Chapel Hill, Department of Surgery, M. S. Mahaley, Jr.; Fort Sanders Presbyterian Hospital, Knoxville, Department of Surgery, J. T. Purvis

Radiation pneumonitis, a model for early detection of pulmonary disease using perfusion and ventilation techniques
University of Tennessee Center for the Health Sciences, Knoxville, Director of Radiotherapy, F. V. Comas, and Department of Medicine, T. A. Sullivan

Tumor metabolism
University of Tennessee Center for the Health Sciences, Knoxville, Department of Medicine, S. Krauss

Development of software for quantitative positron tomography
Vanderbilt University, Nashville, Department of Biomedical Engineering, P. H. King

K. F. Hübner and L. C. Washburn
Measurement of regional brain protein synthesis using ^{11}C-labeled amino acids and positron emission computed tomography
University of Michigan, Ann Arbor, Department of Neurology, S. Gilman, and Neuroscience Laboratory, B. W. Agranoff

L. G. Littlefield
Analyses of radiosensitivity in human B lymphocytes, T lymphocytes, and lymphoblastoid lines of persons with ataxia telangiectasia
Northwestern University Medical School, Chicago, Department of Pediatrics, M. M. Cohen

Cytogenetic dose response kinetics in G-trisomic lymphocytes exposed to low LET radiation
Medical College of Georgia, Augusta, Department of Anatomy, D. A. Welter

Cytogenetic follow-up studies of a survivor exposed to ^{241}Am
Hanford Environmental Health Foundation, Washington, B. D. Breitenstein

L. G. Littlefield and R. J. DuFrain
Chemical carcinogen testing in laboratory rodents
University of Tennessee-DOE Comparative Animal Research Laboratory, Oak Ridge, Cytogenetics Laboratory, A. F. McFee

1028511

C. C. Lushbaugh
Comprehensive epidemiology of DOE atomic workers
University of North Carolina, Chapel Hill, Department of Epidemiology, M. A. Ibrahim, C. M. Shy, and H. A. Tyroler

United States Transuranium Registry
Hanford Environmental Health Foundation, Washington, B. D. Breitenstein

C. C. Lushbaugh and L. S. Washburn
Diethylenetriaminepentaacetate (DTPA) project
Department of Energy contractors, 40 coinvestigators

F. Snyder
Effect of NO_2 and other toxicants on the lung surfactant system
Oak Ridge National Laboratory, Biology Division, W. E. Dalbey

Mass spectrometry of unique lipids
Oak Ridge National Laboratory, Analytical Chemistry Division, W. T. Rainey, Jr.

Nuclear magnetic resonance studies of unique lipids
Oak Ridge National Laboratory, Chemistry Division, B. M. Benjamin

Synthesis of lipid analogues and their precursors
University of North Carolina, Chapel Hill, Department of Medicinal Chemistry, C. Piantadosi and K. S. Ishaq

Effects of a new group of antihypertensive phospholipids on cardiovascular function
University of Tennessee, Knoxville, J. E. Lawler and W. Hubbard

The role of intracellular transfer proteins in the cellular movement of 1-alkly-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-aggregating phospholipid)
Western Carolina University, Cullowhee, North Carolina, Department of Biology, R. H. Lumb

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine
NIH and Uniformed Services University, Bethesda, Department of Neurology, G. Feuerstein

Selective cytoxic activity of alkyllysophospholipids in tumor cells
Technischen Universtät München, West Germany, Division of Oncology, W. E. Berdel

Pulmonary studies of an antihypertensive phospholipid
University of British Columbia, Vancouver, Pulmonary Research Laboratory, R. R. Schellenberg

Metabolism of platelet-activating factor in human blood cells
University of California, San Diego, Division of Rheumatic Diseases, S. I. Wasserman

L. S. Washburn
Pharmacological studies using ^{11}C-labeled compounds
University of Kentucky, Lexington, School of Pharmacy, A. Digenis

C-labeled amines for pulmonary function studies
University of Alberta, Edmonton, Faculty of Pharmacy and Pharmaceutical Sciences, A. A. Noujaim, and Department of Medicine, S. F. P. Man

Grant-Funded Research

N. Gengozian
"Autoimmune" thrombocytopenia
National Heart, Lung, and Blood Institute
$70,181* (July 1, 1978, to June 30, 1981)

Development of markers for assessment of damage to the immune system
Environmental Protection Agency
$328,000** (October 1, 1978, to June 30, 1981)

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies
National Pancreatic Cancer Project
National Cancer Institute
$75,250* (July 1, 1981, to June 30, 1984)

L. G. Littlefield
Evaluation of induced cytogenetic lesions in oocytes
National Institute of Child Health and Human Development
$92,915* (September 1, 1981, to August 31, 1984)

C. C. Lushbaugh
Biomedical research support grant
National Institutes of Health, Division of Research Resources
$38,310** (April 1, 1981, to March 31, 1982)

R. Ricks
Proposal to revise a document, entitled "Responder's Guidebook for Radioactive Materials Transportation Incidents"
Department of Transportation
$23,587** (September 1, 1979, to September 30, 1980)

R. Ricks
Development of written and videotape training materials
Federal Emergency Management Agency (FEMA)-DOE Interagency Agreement (Contract)
$123,340** (September 30, 1980, to September 30, 1982)

F. Snyder
Ether lipids in cancer: Enzyme mechanisms and inhibitors
National Cancer Institute
$57,404* (May 1, 1980, to April 30, 1985)

Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells
American Cancer Society
$72,688** (December, 1, 1980, to December 31, 1981)

A new class of biologically active phospholipids
National Heart, Lung, and Blood Institute
$60,700* (April 1, 1981, to March 31, 1986)

*Direct costs (current year)
**Total costs, i.e., direct plus overhead (current year)

1028512

Organization and Staff

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Fred Snyder, Associate Chairman
John Crockett, Business Officer

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D. Vola

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Staff as of October 1, 1981.

*Mellon Postdoctoral Fellowship (Executive Office)

‡Predoctoral Fellowship (NCI Training Grant, ORNL)

§Postdoctoral Fellowship (NCI Training Grant, ORNL)

1028513

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S. Holloway
A. Sipe, L.P.N.

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W. Gibbs, B.S.
S. Holloway
A. Sipe, L.P.N.
E. Weaver

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D. Davies
UNC Staff

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S. Delcour
R. Snead

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T. Newport, B.A.
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UNC = University of North Carolina.
UCC-ND = Union Carbide Corporation, Nuclear Division.

1028514

Extramural REAC/TS Faculty

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Carol Berger, Oak Ridge National Laboratory
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Howard Dickson, Oak Ridge National Laboratory
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University of Tennessee, Knoxville

Biomedical Advisory Committee 1981

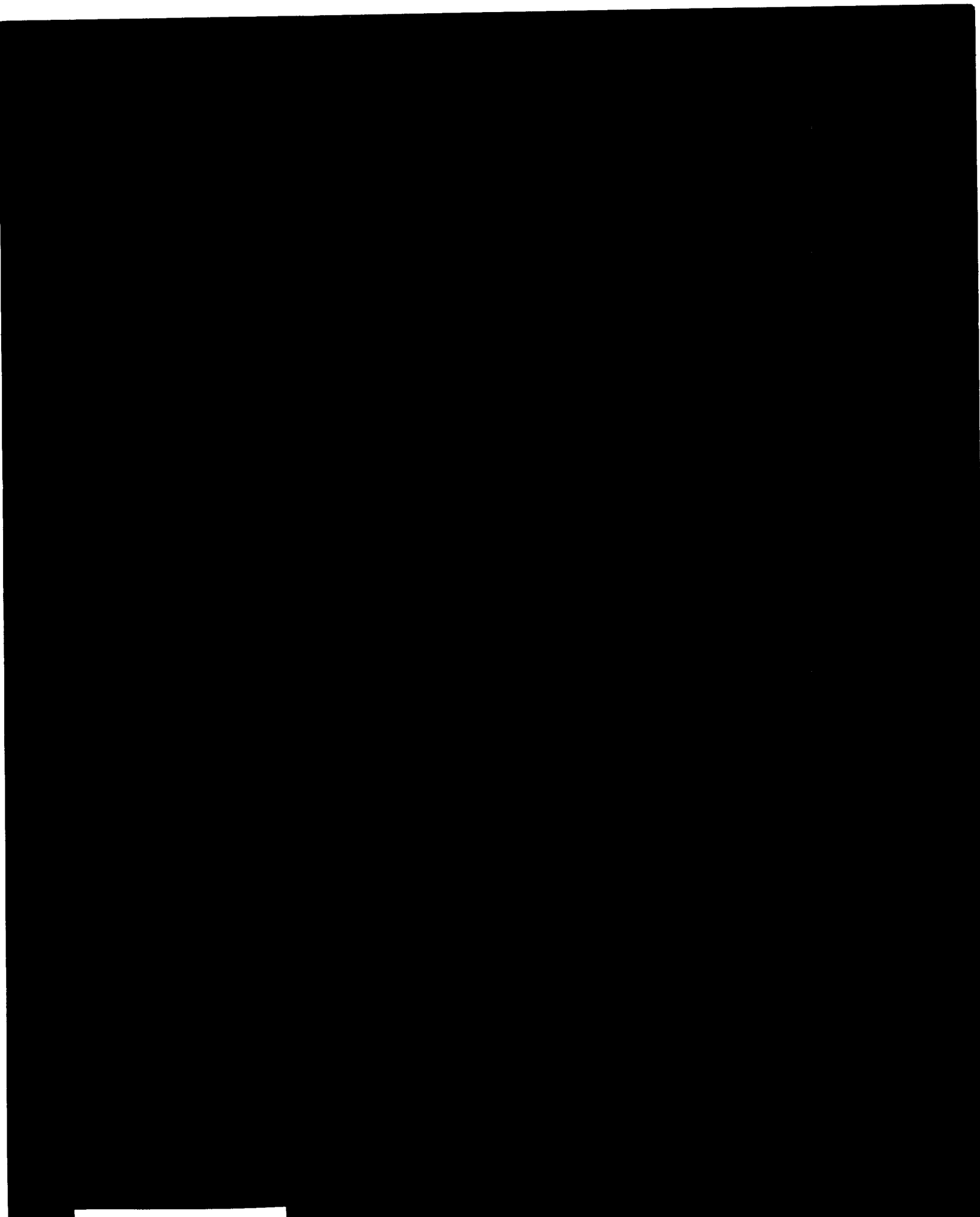
A. Bertrand Brill, Brookhaven National Laboratory
Lois T. Ellison, Medical College of Georgia (Liaison, ORAU Board of Directors)
Louis H. Hempelmann, Jr., University of Rochester School of Medicine and Dentistry
David L. Jackson, Case Western Reserve University Hospital
John Papaconstantinou, University of Texas Medical Branch, Galveston
Arthur A. Spector, University of Iowa, *Chairman*

1028515

ORAU Member Institutions

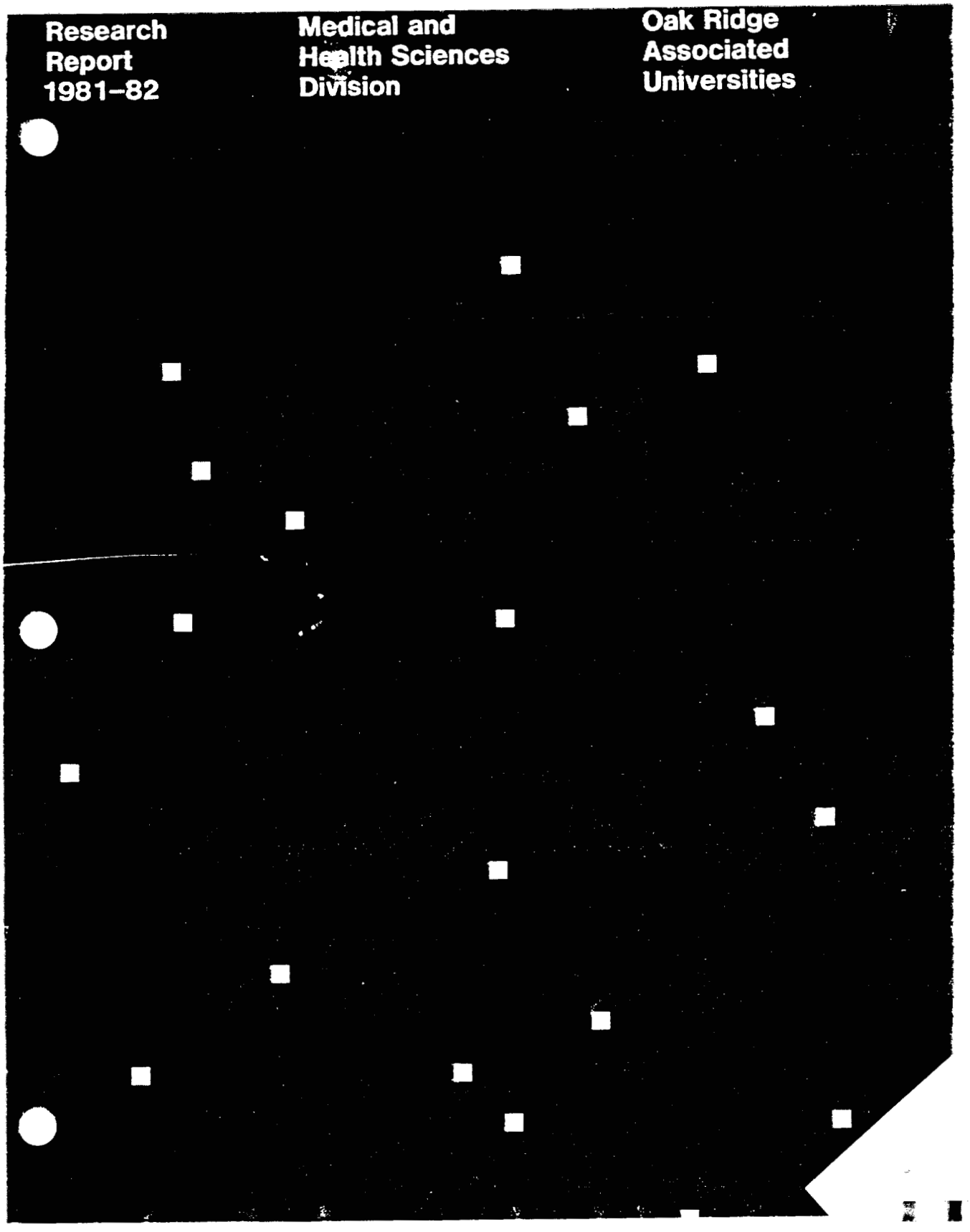
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at Carbondale
Southern Methodist University
Tennessee Technological University
University of Tennessee
Texas A&M University
University of Texas at Austin
Texas Christian University
Texas Woman's University
Tulane University
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Vanderbilt University
University of Virginia
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State University
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1028516



1981-82

1028517


Research
Report
1981–82
Medical and
Health Sciences
Division
Oak Ridge
Associated
Universities

1028518

The Medical and Health Sciences Division conducts research on biomedical problems associated with diseases of the respiratory, vascular, and gastrointestinal systems. Emphasis is on cancers related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Major programs in the division are focused on the following research areas: (a) preclinical and clinical development of new radiopharmaceutical agents and in vivo techniques for positron tomographic detection and studies of diseases in man, (b) lipid and membrane studies of biochemical problems pertaining to cancer, biologically active lipids, and the pulmonary surfactant system, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), and (e) interdisciplinary studies of the marmoset as a primate model for investigating spontaneous colon cancer and chemical toxicants. Predoctoral and postdoctoral training programs are provided in each of the programs. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a demonstration and training unit for medical preparedness in radiation accidents.

Oak Ridge Associated Universities is a private, not-for-profit association of more than 50 colleges and universities. It is a contractor of the U.S. Department of Energy, conducting research and educational programs in the areas of energy, health, and the environment for DOE, other private and governmental organizations, and ORAU's member institutions.

Information included in this report summarizes research results and related activities for the period of October 1, 1981, to September 30, 1982. Reprints of specific publications can be obtained by sending a request to one of the authors or to the chairman's office.

ORAU-208, October 1982

Distribution list: ORAU/MHSD-1982

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

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1028519

Research
Report
1981–82

Medical and
Health Sciences
Division

Oak Ridge
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Universities

1028520

Table of Contents

Preface 3

Scope of Research Programs 4

Biochemistry 4
Radiopharmaceuticals and Nuclear Medicine 5
Marmoset Research 7
Cytogenetics 8
Radiation Emergency Assistance Center/Training Site 10
Occupational Epidemiology 11

Publications 12

Full-Length Journal Articles 12
Chapters, Review Articles, and Other Publications 13
Published Abstracts of Papers Presented at Meetings 15

Summaries of Published Papers 17

Training Programs 24

Seminars and Conferences 26

Non-DOE-Funded Research 27

Collaborative Research 28

Organization and Staff 30

1028521

Preface

The Medical and Health Sciences Division publishes this report annually to review the past year's achievements, staffing, and programmatic directions. The format this year is identical to those of previous years except that a section has been added that lists the titles of lectures and the speakers who have participated in our regular or special seminars.

A reorganization of the division was made in June to make both the scientific and administrative operation more effective An organizational chart reflecting this change can be seen on pages 30–31 of this report. The division consists of seven major research programs and several research support groups. Programs in radiopharmaceutical development, nuclear medicine, biochemistry, epidemiology, cytogenetics, and marmoset research are conducted primarily for the U.S. Department of Energy, but grants and contracts from other agencies (National Institutes of Health, American Cancer Society) also support some of the projects. Highlights of each program are given in the scope section. The aim of our research efforts is to solve selected health problems related to respiratory, vascular, and gastrointestinal diseases and various cancers that originate from occupational exposures to chemicals or ionizing radiation as a result of energy production.

Recently a consortium research group was developed to seek program support for investigating the spontaneous colon cancer that occurs in a specific strain of marmoset (*S. o. oedipus*); a group of these South American monkeys is maintained at the ORAU marmoset colony. The consortium consists of ORAU intramural staff and a diverse group of expert scientists from other institutions throughout the U.S. A grant application for support of the marmoset studies is expected to be submitted soon.

Work in progress for two new facilities is expected to be completed during the early part of FY-83. A building to include the Epidemiology Program at the ORAU administration site will be occupied by the epidemiology staff before the end of 1982. Also, a new small-animal inhalation facility is expected to be ready for experimental use in 1983.

In a year of tightening federal budgets for biomedical research, some of our staff have continued to compete successfully for shrinking funds in health research. We believe this reflects the high quality of output and the excellence of scientific talents available in the division.

C. C. Lushbaugh, Chairman
Fred Snyder, Associate Chairman
October 1982

1028522

Scope of Research Programs

Biochemistry

Research in the biochemistry program addresses health problems that involve the metabolism and functional role of lipids in biological membranes. Experimental results from these investigations provide molecular interpretations of basic biochemical processes that occur at both cellular and subcellular levels.

Research in this program covers the following areas: (1) a new class of biologically active phospholipids and their mode of action, (2) enzymes of ether lipid metabolism as biochemical markers of oncogenesis, (3) the surfactant system and membrane functions in lung cells and the effects of airborne toxicants, and (4) tumor-promoting and carcinogenic agents and the involvement of lipids. The following synopsis describes the current status of each.

Biologically active phospholipids and their mode of action

Studies of a new class of phospholipids (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines, discovered at ORAU in 1979) that possesses potent biological properties have developed into a major subject of research in the biochemistry program. This bioactive lipid class can stimulate hypotensive, platelet-aggregating, inflammatory-allergic, and anaphylactic responses and thus appears to function physiologically as cellular mediators much like hormones.

Results from this project have established the enzymatic pathways responsible for the synthesis (activation) and catabolism (inactivation) of the bioactive molecules as well as pinpointing the essential chemical structural features required for expression of the biological activities. The enzymes involved in the metabolism of the active lipid appear to be distributed ubiquitously in mammalian cells. Important clues toward advancing a biochemical understanding of these mediators have come from data that demonstrate the biologically active phospholipids possess ionophoretic-like properties, as seen by their ability to stimulate calcium uptake in platelets. In turn, calcium levels also appear to affect some of the enzyme activities that metabolize the active forms. In addition, the active phospholipids appear to be important in modulating the formation of other biologically active hydroxy acid derivatives and leukotrienes derived from arachidonate metabolism via the lipoxygenase pathway.

The aim of this work is to seek the mechanisms responsible for the biological activities and to identify the factors that regulate the enzymatic pathways of this new group of cellular phospholipid mediators. Recent findings have shown that unnatural, metabolically stable, alkylphospholipid analogs (closely related to the acetylated type of lipid mentioned above) appear to be highly cytotoxic in tumor cells in comparison to normal cells; the cellular mode of action of these potentially clinically useful analogs are also under active investigation.

Enzymes of ether-lipid metabolism as early markers of oncogenesis

Significant progress has been made this past year in studies of alkyldihydroxyacetone-P synthase, the enzyme that makes the lipid ether bond. This key enzyme, which is prominent in cancer cells, is deeply embedded in the microsomal membrane matrix; it has just recently been solubilized and partially purified without significant loss of activity. The newly developed purification procedure for alkyldihydroxyacetone-P synthase has eliminated enzymes present in cruder preparations that have previously competed for the substrates.

The purified enzyme fraction derived from Ehrlich ascites cell microsomes is currently being used for kinetic and substrate mechanistic studies of the ether bond-forming reaction. Results of these efforts have provided strong evidence to support the concept that the reaction catalyzed by alkyldihydroxyacetone-P synthase occurs via a ping-pong mechanism, i.e., by forming binary complexes. Future plans are to complete mechanistic studies on molecular transitions that occur in specifically labeled compounds involved in the alkyldihydroxyacetone-P synthase catalyzed reaction and to attempt to prepare an antibody against the purified enzyme.

The pulmonary surfactant system and membranes of lung cells—mechanisms and early detection of toxicant damage (NO_2, O_3, and hydrocarbons)

Earlier investigations in this project were concerned with how pulmonary surfactant is synthesized enzymatically in alveolar type II cells derived from pulmonary

1028523

adenomas. The phospholipid surfactant system is of special importance because of its central function in the lung as a stabilizing agent at the alveolar surface and because surfactant production appears to represent a highly sensitive response for detecting lung exposures to irritant oxidant gases, e.g., NO_2 and O_3.

A new topic for study is the utilization of alveolar macrophages to investigate the metabolism of biologically active lipid molecules that are involved in inflammation. Macrophages will also be used to examine the catabolic pathways involved in the degradation and regulation of lung surfactants. A primary aim is to determine how airborne toxicants affect specific lipid pathways of the lung and how such oxidants interact with membrane lipid components. A new small-animal inhalation facility in the division will be completed during FY 83.

or-promoting and carcinogenic agents: lipid involvement

Recent results from our laboratory have documented that ether-linked lipids, which are characteristically high in tumors, also accumulate in cells that have been oncogenically transformed in vitro. A tumor promoter (12-*O*-tetradecanoylphorbol-13-acetate, i.e., TPA) that induces cell differentiation in human leukemia cells (HL-60) was found to increase the deposition of fatty acids in triacylglycerols and to decrease the desaturation of stearic acid.

The involvement of lipids in TPA-mediated responses has been further emphasized by data that show when the polar head group composition of membrane phospholipids is modified; binding of TPA to receptor sites on the cell surface so markedly influenced. The ... of this project is to develop biochemical concepts to explain how membrane lipids participate in tumor promotion and cell differentiation induced by TPA.



In 1979 we discovered a class of phospholipids that is biologically active; current studies are looking at the metabolism and mechanism of action of these lipid molecules that are involved in platelet aggregating, antihypertensive, and hypersensitivity responses. We use this thin-layer chromatographic technique to quantitate the active lipid released by stimulated alveolar macrophages.

Radiopharmaceuticals and Nuclear Medicine

These related research programs concentrate on the development of new radiopharmaceutical agents and phase I and II testing of such materials in subsequent clinical studies. Involved are identification of medical needs, design of appropriate radiopharmaceutical agents, tests of these substances in animals for effectiveness and toxicity, and efficacy trials in humans following U.S. Food and Drug Administration approval. Emphasis is placed on the use of positron-emitting radionuclides to provide tomographic imaging.

Positron decay produces annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions through various angles with a positron emission computerized tomograph, it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of such radiopharmaceuticals. The images obtained are similar to autoradiograms, but unlike autoradiography, the positron tomographic technique is noninvasive.

The images obtained by positron tomography reflect differences in tissue function rather than differences in tissue density as with X rays. Thus, they can be used specifically to detect metabolic alterations that are associated with disease processes. The clinical nuclear medicine program uses tomography to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications and seeks to improve the image and data analysis capabilities of the division's emission computerized axial tomographic scanner ($ECAT_{TM}$) for more accurate quantitative evaluation of the in vivo distribution of such radiopharmaceuticals.

Our clinical positron tomographic investigations have included studies using ^{11}C-labeled amino acids in patients with pancreatic disease, neoplasms, and nonneoplastic brain diseases. Amino acids with carbon-11 substituted for carbon-12 offer distinct advantages for studying protein synthesis and protein turnover in vivo, since ^{11}C-labeled agents are indistinguishable physiologically and chemically from their unlabeled counterparts.

1028524

Positron tomographic pancreatic studies, i.e., visualization of the normal pancreas and pancreatic cancers after injection of ^{11}C-labeled DL-valine, DL-tryptophan, or 1-aminocyclobutanecarboxylic acid, have continued with support from the National Pancreatic Cancer Project of the National Cancer Institute (present level: $75,250 per year).

Several years ago we developed a general method for the synthesis and purification of ^{11}C-carboxyl-labeled amino acid racemates (mixtures of the naturally occurring L-isomer and the unnatural D-isomer). For truly physiologic studies of the pancreas and brain, the ^{11}C-labeled L-amino acid must be separated from the mixture by a process that is compatible with the 20.4-minute half-life of carbon-11. Optical resolution of three amino acids of nuclear medical interest, DL-[^{11}C]valine, DL-[^{11}C]tryptophan, and DL-[^{11}C]leucine, has been accomplished by high-performance liquid chromatography (HPLC). A conventional reverse-phase HPLC column and a chiral mobile phase (an aqueous solution of L-proline, cupric acetate, and sodium acetate) are used. The method is based upon the formation of mixed diastereoisomeric copper complexes with L-proline and the amino acids to be separated. That is, in the case of DL-[^{11}C]valine, two diastereoisomeric complexes, L-[^{11}C]valine-copper-L-proline and D-[^{11}C]valine-copper-L-proline, are formed. These are then separated by HPLC.

With valine and leucine, the copper must be removed from the L-amino acid fraction by precipitation as the sulfide. Final purification by cation-exchange chromatography yields the ^{11}C-labeled L-amino acid in a form that is acceptable for clinical studies. This purification method does not remove the L-proline introduced in the resolution process, but the amount of L-proline involved is innocuous and does not affect the tissue distribution of the ^{11}C-labeled L-amino acid. In the case of tryptophan, however, the copper-amino acid complex is weaker, and cation-exchange chromatography alone separates L-[^{11}C]-tryptophan from both copper and L-proline.

L-[^{11}C]Tryptophan and L-[^{11}C]valine have potential for differential diagnosis of pancreatic disease, and L-[^{11}C]valine and L-[^{11}C]leucine are potentially useful for measurement of regional brain protein synthesis. Approval has been obtained from the U.S. Food and Drug Administration for the clinical use of both L-[^{11}C]valine and L-[^{11}C]tryptophan. These amino acids are being applied in clinical investigations of patients with phenylketonuria, Huntington's chorea, accidental cerebrovascular damage, and cerebral palsy. Measuring the rate of cerebral protein synthesis may provide useful prognostic information and form the basis for studying the effects of therapy on the progress of certain diseases. This work is being done in collaboration with the University of Michigan.



The use of positron-emitting radiopharmaceuticals with the ECAT scanner provides many opportunities for clinical research. In this instance, we've used carbon-11-labeled DL-tryptophan to image the pancreas; a tumor would show up as a dark spot.

Carbon-11-labeled alicyclic α-amino acids are effective tumor-localizing agents ([^{11}C]1-aminocyclobutanecarboxylic acid, [^{11}C]ACBC, and [^{11}C]1-aminocyclopentanecarboxylic acid, [^{11}C]ACPC). In addition, these types of compounds are not metabolized and therefore have potential for positron tomographic measurements of amino acid transport. Pathological conditions in the brain may influence amino acid transport, protein synthesis, or both.

In a study of the effect of chemical structure on brain uptake, 1-aminocyclohexanecarboxylic acid (ACHC) was found to have the highest affinity for brain. Therefore, [^{11}C]ACHC may be useful in conjunction with L-[^{11}C]valine or L-[^{11}C]leucine for differentiation between brain amino acid transport and protein synthesis. ACHC has been tested for toxicity, and an investigational new drug application has been prepared for submission to the U.S. Food and Drug Administration.

A major research effort is directed toward the development of radiopharmaceuticals for positron tomographic studies of pulmonary function. One of the primary metabolic activities of the lung involves regulation of various biogenic amines. Carbon-11-labeled, straight-chain primary aliphatic amines, such as [^{11}C]octylamine, are sequestered

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selectively by lung endothelial cells. However, the rapid metabolic loss of the label from these agents makes it difficult to accurately quantitate their pulmonary uptakes. We have found that branched-chain amines are metabolized much more slowly than the straight-chain analogs.

One such compound, containing a very bulky tertiary-butyl group, showed only 30% metabolic loss from rat lung between 5 and 30 minutes postinjection. We also have preliminary evidence that a long straight-chain amine, octadecylamine, has high pulmonary uptake with no apparent metabolic loss. Special media are required for solubilization of this compound, and further studies with this amine are in progress.

A collaborative arrangement with Drs. A. A. Noujaim and S. F. P. Man of the University of Alberta, Canada, involves testing the potential of ^{11}C-labeled aliphatic amines as agents for studying lung metabolism by using dog models that simulate various pathologic conditions in the lung. The first model selected for study involves the production of localized damage to lung endothelial cells by administration of oleic acid to a selected area of the lung.

In collaboration with Dr. George Digenis of the University of Kentucky, the pharmacology in the brain of [^{11}C]phenethylamine, an amphetamine analog, is being studied. Another collaborative project, with Dr. George Kabalka of the University of Tennessee, Knoxville, involves the investigation of the potential of organoboranes as precursors for incorporating radionuclides into organic and biochemical compounds of nuclear medical interest.

During this year, the last in a series of three papers on the basic pathways involved in the entry of gallium-67 into normal tissues and malignant and inflammatory lesions was published (*J. Nucl. Med.* 23:8–14; 1982). The Medical and Health Sciences Division was responsible for the initial introduction of ^{67}Ga as an agent for the detection of soft-tissue tumors, and the division, with the participation of 16 other medical institutions, was involved in follow-up studies that led to the acceptance of ^{67}Ga as an effective radiopharmaceutical agent by medical communities throughout the world. The value of the ^{67}Ga used in the United States alone is well over 5 million dollars per year.

Our findings in experimental animals have indicated that there appears to be a marked difference between the transport of ^{67}Ga through the plasma membrane of normal and malignant cells. Gallium, probably as the uncharged hydroxide [$Ga(OH)_3$], penetrates the plasma membrane of cancerous cells by passive diffusion, whereas uptake of ^{67}Ga by normal cells occurs basically by an endocytotic process. Once inside the cancerous cell, passive diffusion also occurs through lysosomal membranes. Following penetration of the lysosomal membrane, the decreased hydrogen ion concentration that is present intralysosomally (pH 4–5) causes the gallium to revert to cationic states [$Ga(OH)^{2+}$ and $Ga(OH)_2^+$]; in these forms, it is then bound more or less irreversibly to intralysosomal proteins. A similar intralysosomal protein-binding process undoubtedly occurs with the ^{67}Ga that is endocytosed by normal cells following the fusion of the resultant endocytotic vesicles with lysosomes, although the cell entry process is basically different.

Further studies with animals bearing experimental abscesses indicate that the pathway into inflammatory processes is probably similar to that occurring with normal tissues and that the macrophages that are present in such lesions are mainly responsible for the unusual ^{67}Ga uptake that occurs.

Comparison of ^{169}Yb ($T_{½}$ = 32 days) and ^{67}Ga ($T_{½}$ = 3.3 days) in rats bearing experimental abscesses has suggested that ^{169}Yb may be more effective than ^{67}Ga for the detection of inflammatory lesions, particularly at early time periods (e.g., 4 hr). Ytterbium-169 is especially attractive as a potential agent for such studies because it is produced in a reactor (^{67}Ga is cyclotron produced) and has a long shelf life. An investigational new drug application for clinical testing of ^{169}Yb has been approved by the U.S. Food and Drug Administration. We plan to collaborate on this project with Dr. Fred A. Mettler of the University of New Mexico.

A hydrous ferric oxide colloid labeled with the generator-produced positron emitter ^{68}Ga ($T_{½}$ = 68 min) has been developed and is now in clinical trials. The colloid particles have a mean diameter of ~10 nm and are prepared by heat hydrolysis of a dilute solution of ferric chloride containing ^{68}Ga chloride. The binding of ^{68}Ga to the colloid particles is essentially 100%. This preparation is expected to be very useful in positron tomographic detection of deep-seated lesions in the liver and spleen. Animal studies indicate that a similar preparation with a smaller particle size (≃ 2 nm) may also be useful for imaging the bone marrow.

Marmoset Research

The marmoset colony of Oak Ridge Associated Universities was initiated in 1961 to provide primates for studies in radiation biology. During the early years of the colony, hematologic and immunologic consequences of the natural blood chimerism were studied in these species.

Initially, *Saguinus fuscicollis* species were predominantly used; however, recently, *Saguinus oedipus oedipus* have also been imported, maintained, and bred in the colony. *S. o. oedipus* have become increasingly important because of the finding of spontaneous colon carcinomas in this tamarin. No colon tumors have been observed in several hundred *S. f. illigeri* or *Callithrix jacchus*. Animals were continually imported until 1977.

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With the recent exportation bans by several South American countries, it has become increasingly important not only to maintain the animals but also to develop a reproductive capacity. Adequate reproductive capability is much more difficult to maintain in *S. o. oedipus* than in *S. fuscicollis*. Improvements in nutritional and husbandry practices have improved both colony health and longevity. Currently, we are able not only to maintain our *S. o. oedipus* colony but also to see it increase in numbers.

Colon tumor project

In 1978, Dr. Lushbaugh and coworkers reported their observations of spontaneous color adenocarcinomas in *Saguinus oedipus oedipus*. This finding was extremely important because no other animal, either experimental or human, spontaneously develops high incidences of colon carcinomas. Whereas the initial observation of the colon tumors occurred in imported animals, tumors have also been observed in animals that have been born and reared in captivity. In 19 deaths during 1981, 16 *S. o. oedipus* died because of adenocarcinomas of the colon. The mean colony age for the 58 animals dying with colon adenocarcinoma through December 31, 1981, is approximately 55 months.

We have identified what we call "cancer family" lines from genealogies obtained from our breeding records. The genealogies include four generations of offspring from a cancer-positive animal; we are carefully observing *S. o. oedipus* that are either clinically diagnosed as cancer-positive or that are progeny and/or siblings of animals that have or had colon cancer.

Clinical studies

Recently, we developed a barium enema–air contrast radiographic procedure to detect colon cancers in living animals. This procedure enables us to follow progress of the disease, to offer a prognosis for animals bearing the colon carcinoma, and to study pathogenesis of the disease process. We are now utilizing these radiographic procedures to study not only colon cancer but also the contribution of chronic colitis and other enteropathies upon colon cancer and the general health of the colony.

The assay for fecal β-glucuronidase has been used by numerous investigators to indicate in a number of species the status and changes in the population of microbial flora and the colonic environment. We are using this assay to determine changes in both mucosal and bacterial enzyme concentrations that may indicate the colonic environment, which might help to predict either the possibility of colon cancer development or that cancer is present. We will obtain and compare data from tumor-susceptible animals with data from those species which do not develop colon cancer, *S. fuscicollis illigeri* and *Callithrix jacchus*, although the three species are housed together and fed identical diets. We also will investigate the feasibility of using the enzyme assay as an indicator of susceptibility in nontumor-bearing young animals in which tumors may be expressed at later ages.

In collaboration with scientists at other institutions, we are investigating differences between these primate species, which include bile acid profiles, colon cell kinetics, monoclonal antibody production, and DNA repair. Together with the ORAU studies, these data should provide further insight not only in detection and prognosis associated with colon cancer, but also mechanistic information toward causation and eventual control of the disease.



We recently developed a barium enema–air contrast radiographic procedure for detecting colon cancer in the cotton-top tamarin. You can see the colon restricted by a tumor in this film.

Cytogenetics

The three primary missions of the cytogenetics program are (1) to provide biological dosimetry and follow-up evaluations for persons referred to REAC/TS because of real or suspected exposures to ionizing radiation, (2) to improve the use of cytogenetic methods as a means of quantifying dose in persons for whom physical dose estimates are questionable or lacking, and (3) to conduct basic research aimed at defining the risk of human exposure to environmental mutagens, including radiation and chemical clastogens.

The analysis of radiation-induced chromosome lesions in cul-

1028527

tured lymphocytes is the most sensitive biological method available estimating absorbed dose in persons having recent whole body exposures to penetrating radiations. During the last 11 months, the cytogenetics group responded to requests for biological dosimetry or follow-up evaluations in 22 persons referred to REAC/TS because of real or suspected overexposures to ionizing radiation. Requests for dosimetry were received from individuals or agencies in seven different states, and in the majority of the referrals, cytogenetic analysis provided the only means of determining whether a significant radiation dose was actually received.

Although the methodology for conducting such analyses is well in hand for dose estimation in persons with recent radiation exposures, less information is available on the applicability of cytogenetic techniques in dose assessment in persons exposed several months or years before cytogenetic evaluation.

As a major expansion of the Radiation Dosimetry Program during FY 1982, the cytogenetics group initiated studies of persistent radiation-induced chromosome lesions in cultured lymphocytes from 40 patients who received radiation therapy for enlarged tonsils 20–30 years ago. This study is being conducted in collaboration with investigators at the Environmental Epidemiology Branch of the National Cancer Institute and is being supported by an NCI-DOE interagency agreement. During the next three years various members of the cytogenetics staff will evaluate persistent radiation-induced chromosome lesions in cultured lymphocytes from a total selected by NCI of 450 persons who received various modalities of partial body radiation 20–50 years ago. The purpose of the study will be to determine whether the incidence of chromosome lesions observed many years after exposure can be correlated with the total dose the patient received, the region of the body that was irradiated, the age at time of exposure, or the number of years since exposure.



The photographs being examined show different stages of gametogenesis preceding fertilization; our experiments are designed to determine the extent toxic chemicals affect female reproductive cells; usually such changes are detected during the first and second mitotic divisions.

In the area of basic research in radiation biology, we continued studies on the effects of radiations having differing linear energy transfer (LET) on the induction of chromosome lesions in normal lymphocytes and in lymphocytes from persons having increased sensitivity to ionizing radiation. This year we determined that lymphocytes from patients with Down's syndrome do not show increased sensitivity to chromosome lesion induction after exposure to fission neutrons, although several earlier studies have shown that Down's lymphocytes are hypersensitive to low LET radiation.

In conjunction with these studies, we are also conducting experiments aimed at further defining clastogenic factors which are presumably released as a result of radiation-induced cell-killing. Our aim will be to determine the significance of these factors in inducing chromosome damage in nonlethally irradiated cells.

Under the continuing support this year of the National Institute of Child Health and Human Development, the cytogenetics group developed the methodology to allow assessment of chemically induced cytogenetic lesions in proliferating oogonial cells and bone marrow of the neonatal rabbit. During the first six years of NIH support for studies of chemically induced chromosome lesions in female germ cells, our group determined that the model chemical, streptonigrin, induces structural chromosomal lesions in the dictyate oocyte of the mature rabbit that can be recovered as inherited lesions in the preimplantation embryo. We further observed that the incidence of lesions induced in somatic cells of similarly exposed females overestimates by a factor of 2–5 the risk of transmission of damage to the blastocyst.

The purpose of the current studies will be to determine the risk of induction by chemicals of transmissible lesions in proliferating oogonial cells. The rabbit is one of a small number of species in which the female germ cells continue proliferation after birth. Thus, the stage of germ cell development in the neonatal rabbit is comparable to that of a human fetus during the second trimester. For this reason, the newborn female rabbit is an excellent model for assessing the effects of clastogenic chemicals on the induction of chromosome

1028528

lesions in proliferating germ cells and for predicting the risk of induction of germ-cell damage in the developing fetus. Having developed the method for obtaining cytogenetic preparations of mitotically active female germ cells from the neonatal rabbit, we are currently generating dose response curves for chromosome lesion induction in somatic cells and in proliferating oogonial cells after exposure of the newborn rabbit to streptonigrin.

Radiation Emergency Assistance Center/Training Site

The REAC/TS facility, a treatment center and training unit, continued to be available for rendering assistance in the management of health problems associated with radiation accidents and for formal teaching of medical and paramedical personnel.

The major objectives of the overall REAC/TS task are to (1) assist and provide medical and health physics support in the event of radiation-related emergencies by direct participation or consultation on a 24-hour basis, (2) conduct epidemiologic studies on DOE radiation workers and radiation accident survivors, (3) conduct radiation accident management training courses, exercises, and drills for medical, paramedical, and health physics personnel to meet the need for properly trained professionals in this area of occupational and emergency medicine, (4) continue to develop and refine cytogenetic techniques for dose estimation of radiation accidents and exposure assessment from chemical toxicants in the industrial or general environment, and (5) conduct research in radiation pathology, especially in the area of high dose tissue injury.

REAC/TS has a continued commitment to be available and ready to assist U.S. government nuclear facilities and private nuclear power and nuclear fuel reprocessing plants in the event of nuclear emergencies. During the past year, letter agreements with private companies increased by 4 to a total of 52. Over 150 former REAC/TS trainees are now employed at these sites, and continued testing of the emergency response system is done through REAC/TS involvement in drills and exercises at these sites.

REAC/TS participation in seven offsite drills included emergency assistance telephone checks, simulated availability for patient transfer, and simulated consultative advice. This offsite drill assistance involved calls from the Tennessee Valley Authority and various commercial nuclear facilities utilizing REAC/TS backup as previously arranged by letter agreement through DOE/ORO.

REAC/TS onsite drills were conducted on two occasions and expanded to joint exercises with the Oak Ridge Hospital of the Methodist Church. As a part of the REAC/TS collaborative services with the World Health Organization, REAC/TS dispatched a health physicist for onsite assistance following a contamination incident at a hospital in the Caribbean.

In the last year, REAC/TS has been contacted for assistance 58 times. Calls for assistance or consultation were in a few cases related to serious localized overexposure to minor contamination, accidents involving both internally and externally deposited radionuclides. In some cases, consultation provided confirmation that no radiation accident had actually occurred.

Direct and indirect long-term follow-up of victims of earlier radiation accidents represented continued efforts involving the REAC/TS registry system. The REAC/TS central registry now contains data on 5,509 individuals involved in 488 different events. Component parts of the central registry include the DTPA Registry, the DOE $\geq$ 5 rems Registry, U.S. Radiation Accident Registry, the Foreign Registry, and the Zero Dose Non-Accident Registry. All data input into the registry systems have now been encoded on Word-11. Field testing of the $\geq$ 5 rems medical questionnaire has been completed. The DOE/ORAU FDA/IND for DTPA continued with coinvestigators reporting no unusual pattern of late effects, with continuing follow-up studies projected.

The REAC/TS courses and training programs continued to be in great demand. Participants included 79 physicians, 27 health physicists, and 84 paramedical professionals. Less formalized training and information was provided through talks, a special exhibit presented by REAC/TS staff at



Ten times a year we offer courses in how to manage health programs associated with radiation accidents in which we sometimes simulate radiation mishaps. The courses are attended by physicians, health physicists, and paramedics and are so popular, there's no need to advertise them.

1028529

professional meetings, and through publication of a quarterly sletter that has an annual distribution of 10,800.

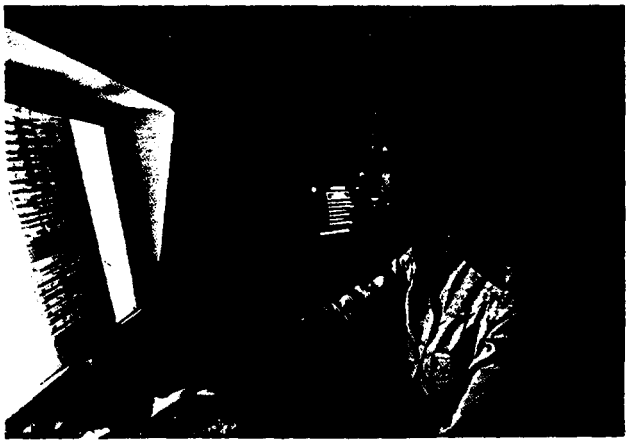
Proceedings of the international conference entitled *The Medical Basis for Radiation Accident Preparedness* continues to be in high demand with over 2,000 copies sold. Videotape training materials being developed under a special contract with the Federal Emergency Management Agency (FEMA) have now been completed. These programs, entitled *Pre-Hospital Response to Radiation Accidents* and *Hospital Emergency Department Response to Radiation Accidents* will be utilized by FEMA in a national training program and incorporated into REAC/TS training courses as well.

Occupational Epidemiology

The main objective of the epidemiology program is to examine tionships between occupational exposure to ionizing radiation and subsequent health and mortality. The health effects of chemical toxicants, especially uranium and other metals, also are being investigated both as primary and confounding (with respect to radiation) occupational stresses.

Biostatisticians and computer scientists are implementing improved methods for epidemiological analyses and data systems for merging large masses of data into analysis files. Editing and correction procedures have been developed to ensure the high quality of the data bases that extend back to the early 1940s.

The epidemiology group is continually evaluating the mortality experience of workers with respect to radiation effects. Current studies include workers at DOE facilities operated by Union Carbide Corration, Nuclear Division, in Oak dge, Tennessee, and Paducah, Kentucky, and workers at NLO in Ohio and at the Savannah River Plant operated by E. I. du Pont de Nemours & Company in Aiken, South Carolina.



Biostatisticians and computer scientists in epidemiology are developing improved methods for epidemiological and statistical analyses and systems for compiling large masses of data.

Preliminary mortality studies have been completed on two groups of workers at the Oak Ridge Y-12 Plant. A similar study of all workers at Oak Ridge National Laboratory (X-10) is in progress. The effects on health of occupational exposure to uranium and uranium compounds are being studied in former workers at the "Niagara Frontier" sites of the Manhattan Engineer District.

A completed mortality study of Oak Ridge workers potentially exposed to metallic nickel is being updated to include further follow-up data and a comparison of mortality among welders at the Union Carbide Corporation, Nuclear Division, that contains a subgroup exposed to nickel vapor. The previously reported study of mortality among workers exposed to phosgene while employed at the Tennessee Eastman Corporation facility in Oak Ridge during 1943–45 also is being updated to include deaths identified as of 1978.

Preliminary analyses are in progress of the mortality experience among the cohort of 3,100 workers identified as having equaled or exceeded the current annual occupational limit of 5 rems (set by the Nuclear Regulatory Commission and DOE) since 1947 while employed at DOE (and predecessor organization) facilities nationwide. A follow-up study of morbidity in this population (particularly nonfatal cancers and chronic diseases of major organ systems) will be initiated in 1983 on a by-site basis.

To facilitate these studies and those segments of the DOE Health and Mortality Study being conducted by epidemiology groups at Los Alamos National Laboratory (Plutonium Workers Study) and the Hanford Environmental Health Foundation with the Pacific Northwest Laboratories (Hanford Workers Study), we operate the DOE Death Certificate Retrieval Office which obtains vital status information and the causes of any deaths among former DOE employees. These data are being accumulated through the cooperation of the Social Security Administration (SSA) and the vital statistics departments of all 50 states. We also have developed several alternate data sources for following up persons whose vital status cannot be determined by the SSA. These sources include states' motor vehicle license departments, the Civil Service Commission, and the Veterans Administration.

In these studies we have the collaboration of the epidemiology departments of the schools of public health at the University of North Carolina, Chapel Hill, and at the University of Alabama, Birmingham. ■

1028530

Publications

Full-length journal articles

Albert, D. H., and Snyder, F.
Biosynthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine in rat alveolar macrophages: Phospholipase A_2 and acetyltransferase activities during phagocytosis and ionophore stimulation.
J. Biol. Chem. In press.

Berger, M. E.; Thompson, D. W.; Ricks, R. C.; and Kaufmann, D.
The medical technologist and the radiation-accident victim.
Am. J. Med. Technol. 47:829–834; 1981.

Blank, M. L.; Cress, E. A.; Lee, T-c.; Malone, B.; Surles, J. R.; Piantadosi, C.; Hajdu J.; and Snyder, F.
Structural features of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero 3-phosphocholine) required for hypotensive and platelet serotonin responses.
Res. Commun. Chem. Pathol. Pharmacol. In press.

Blank, M. L.; Dalbey, W.; Cress, E. A.; Garfinkel, S.; and Snyder, F.
Pulmonary NO_2 toxicity: Phosphatidylcholine levels and incorporation of [^{3}H]thymidine into DNA.
Environ. Res. 27:352–360; 1982.

Borman, L. S.
Modulation of mammalian cell growth by a choline analog, *N*-isopropylethanolamine.
In Vitro 18:129–140; 1981.

Borman, L. S.; Swartzendruber, D. C.; and Littlefield, L. G.
Establishment of two parental cell lines and three clonal cell strains from rat colonic carcinoma.
Cancer Res.. In press.

Brown, A. J., and Snyder, F.
Alkyldihydroxyacetone-P synthase. Solubilization, partial purification, new assay method, and evidence for a ping-pong mechanism.
J. Biol. Chem. 257:8835–8839; 1982.

Cabot, M. C.; Blank, M. L.; Welsh, C. J.; Horan, M. J.; Cress, E. A.; and Snyder, F.
Metabolism of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by cell cultures.
Life Sci. In press.

Cabot, M. C., and Welsh, C. J.
Ether lipid studies in mouse C3H/10T1/2 cells and a 3-methylcholanthrene-transformed clone.
Arch. Biochem. Biophys. 211:240–244; 1981.

Cabot, M. C., and Welsh, C. J.
Fatty acid metabolism in phorbol ester-differentiating human leukemia cells.
Cancer Res. 41:4910–4915; 1981.

Cabot, M. C.; Welsh, C. J.; and Snyder, F.
Increasing the levels of ether-linked lipids in L-M cells by glyceryl ether supplementation depresses growth and choline utilization.
Biochim. Biophys. Acta. In press.

DuFrain, R. J.; Littlefield, L. G.; Wilmer, J. L.; and Frome, E. L.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. II. A comparison of streptonigrin effects on somatic and germ cells.
Mutat. Res. 94:103–114; 1982.

Feuerstein, G.; Zukowska-Grojec, Z.; Krausz, M. M.; Blank, M. L.; Snyder, F.; and Kopin, I. J.
Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious SHR and WKY rats.
Clin. Exp. Hypertens.—Theory and Practice A4:1335–1350; 1982.

Fox, L. L.; Gengozian, N.; Shivers, C. A.; and Sieg, P.
Marmoset response to immunization with porcine zonae pellucidae: Measurement of antibody activity with an enzyme-linked immunosorbent assay.
J. Med. Primatol. 10:205–211; 1981.

Frome, E. L.
Poisson regression analysis.
Am. Stat. 35:262–263; 1981.

Frome, E. L.
Fisher's exact variance test for the Poisson distribution.
Appl. Stat. 31:67–71; 1982.

Gengozian, N., and Rice, D.
Antibody-dependent cellular cytotoxicity with platelets as the target cell: potential application to the study of immune thrombocytopenia.
Clin. Exp. Immunol. 47:431–436; 1982.

Hayes, R. L.
The interaction of gallium with biological systems.
Int. J. Nucl. Med. Biol. In press.

1028531

Hayes, P. L.; Carlton, J. E.; and Kuniyasu, Y.
A new method for labeling microspheres with ^{68}Ga.
Eur. J. Nucl. Med. 6:531–533; 1981.

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
Studies of the in vivo uptake of ^{67}Ga by an experimental abscess: concise communication.
J. Nucl. Med. 23:8–14; 1982.

Hübner, K. F.; Purvis, J. T.; Mahaley, S. M., Jr.; Robertson, J. T.; Rogers, S.; Gibbs, W. D.; King, P.; and Partain, C. L.
Brain tumor imaging by positron emission computed tomography using ^{11}C-labeled amino acids.
J. Comput. Assist. Tomogr. 6:544–550; 1982.

Huff, V.; DuFrain, R. J.; and Littlefield, L. G.
SCE frequencies in rabbit lymphocytes as a function of time after an acute dose of cyclophosphamide.
Mutat. Res. 94:349–357; 1982.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Formation of alkylacyl- and diacyl-glycerophosphocholines via diradylglycerol cholinephosphotransferase in rat liver.
Biochim. Biophys. Acta. In press.

Lee, T-c.; Malone, B.; Blank, M. L.; and Snyder, F.
1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) stimulates calcium influx in rabbit platelets.
Biochem. Biophys. Res. Commun. 102:1262–1268; 1981.

Lee, T-c.; Malone, B.; Wasserman, S. I.; Fitzgerald, V.; and Snyder, F.
Activities of enzymes that metabolize platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in neutrophils and eosinophils from humans and the effect of a calcium ionophore.
Biochem. Biophys. Res. Commun. 105:1303–1308; 1982.

Lee, T-c., and Stephens, N.
The modification of lipid composition in L-M cultured cells supplemented with elaidate: Increased formation of fatty alcohols.
Biochim. Biophys. Acta 712:299–304; 1982.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
SCE evaluations in human lymphocytes after G_o exposure to mitomycin C: Lack of expression of MMC-induced SCEs in cells that have undergone greater than two in vitro divisions.
Mutat. Res.. In press.

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; Frome, E.; DuFrain, R. J.; and Cohen, M. M.
Chromosomal radiation sensitivity in ataxia telangiectasia long-term lymphoblastoid cell lines.
Cytogenet. Cell Genet. 31:203–213; 1981.

Matthews, J. A.; Bonds, S. V.; Cianci, M. L.; Cooper, J. F.; Hanes, K.; Hodges, H. D.; Kraft, R. A.; Langan, J. K.; Porter, J. L.; Price, D. D.; and Ward, D.
Performance and responsibility standards for the nuclear medicine technologist.
J. Nucl. Med. Technol. In press.

Moore, C., and Snyder, F.
Properties of microsomal acyl coenzyme A reductase in mouse preputial glands.
Arch. Biochem. Biophys. 214:489–499; 1982.

Moore, C., and Snyder, F.
Regulation of acyl coenzyme A reductase by a heat-stable cytosolic protein during preputial gland development.
Arch. Biochem. Biophys. 214:500–504; 1982.

Morrison, W. D.; Huff, V.; Littlefield, L. G.; and DuFrain, R. J.
Chromosome preparations from rabbit preovulatory oocytes.
Mutat. Res. In press.

Polednak, A. P.
Mortality among welders, including a group exposed to nickel oxides.
Arch. Environ. Health 36:235–242; 1981.

Polednak, A. P., and Keane, A. T.
Long half-life component of uranium retention: Implications for potential health effects among early uranium workers.
Health Phys. In press.

Polednak, A. P.; Keane, A. T.; and Beck, W. L.
Estimation of radiation doses to the lungs of early uranium-processing plant workers.
Environ. Res. In press.

Renooij, W., and Snyder, F.
Transmembrane orientation of palmitoyl-CoA:lysophosphatidylcholine acyltransferase in microsomes isolated from an alveolar type II cell adenoma and rat liver.
Biochim. Biophys. Acta 666:468–474; 1981.

Sayler, G. S.; Reid, M. C.; Perkins, B. K.; Pagni, R. M.; Smith, R. L.; Rao, T. K.; Epler, J. L.; Morrison, W. D.; and DuFrain, R.
Evaluation of the mutagenic potential of bacterial polychlorinated biphenyl biodegradation products.
Arch. Environ. Contam. Toxicol. 11:577–581; 1982.

Urso, P., and Gengozian, N.
Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo(α)pyrene and X rays before or after birth.
J. Toxicol. Environ. Health. In press.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Callahan, A. P.
Production of L-[1-^{11}C]valine by HPLC resolution.
J. Nucl. Med. 23:29–33; 1982.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Kabalka, G. W.
Effect of structural alterations on the uptake and clearance of primary aliphatic amines in rat lung.
J. Labelled Compd. Radiopharm. In press.

Welsh, C. J.; Sayer, A. M.; Littlefield, L. G.; and Cabot, M. C.
Modification of lipid acyl groups by serum deprivation does not affect phorbol ester-induced differentiation of human leukemia cells.
Cancer Lett. 16:145–154; 1982.

Chapters, review articles, and other publications

Arneson, R. M.; Wander, J. D.; Cabot, M. C.; and Tan, E. L.
Resistance to lipid peroxidation by cultured neoplastic cells.
In *Protective Agents in Human and Experimental Cancers*, Proceedings of NFCR Cancer Symposium No. 2, September 1982, Uxbridge, England, National Foundation for Cancer Research. In press.

1028532

Frome, E. L. [Reply to Letter to the Editor]
ASR37: A remark on AS 73: Cross-spectrum smoothing via the finite fourier transform.
Appl. Stat. 30:354; 1981.

Fry, R. J. M., and Fry, S. A.
Effects on humans of exposure to radioactive materials: late somatic effects. In *Encyclopedia of Materials Science and Engineering.*
New York: Pergamon Press. In press.

Fry, S. A.; Rudnick, S. A.; Hollis Hudson, D. R.; Robie, D. M.; Lushbaugh, C. C.; and Shy, C. M.
Follow-up study of the health experience of U.S. Department of Energy (DOE) workers exposed to ≥50 mSv (5 rems) per year.
In *Radiological Protection—Advances in Theory and Practice*, Proceedings of Society for Radiological Protection, Third International Symposium, Inverness, Scotland, 1982, Vol. 1, pp. 346–351. Society for Radiological Protection, 1982.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; Carlton, J. E.; and Coffey, J. L.
Ytterbium-169 citrate for detection of inflammatory lesions.
In *Medical Radionuclide Imaging 1980*, proceedings of an international symposium, Heidelberg, Germany, Vol. 1, pp. 579–586. Vienna: International Atomic Energy Agency, 1981.

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
[Reply to Letter to Editor]
Re: Studies of the in vivo uptake of ^{67}Ga by an experimental abscess: concise communication.
J. Nucl. Med. 23:548; 1982.

Hübner, K. F., and Buonocore, E.
Alimentary tract radiology positron emission tomography (P.E.T.).
In *Alimentary Tract Radiology*, 3rd Edition, edited by S. E. Harshberger. St. Louis, Missouri: The C. V. Mosby Co. In press.

Hübner, K. F., and Lushbaugh, C. C.
Surgeon's approach to radiation accidents.
In *Care of the Injured—A Practical Guide*, edited by E. E. Moore, C. W. Van Way III, and B. Eiseman. St. Louis, Missouri: The C. V. Mosby Co. In press.

Hübner, K. F.; Fry, S. A.; and Lushbaugh, C. C.
Biological effects of radiation in man.
In *Funk & Wagnalls New Encyclopedia*, edited by R. S. Phillips. New York: Funk & Wagnalls. In press.

Hübner, K. F.; King, P.; Gibbs, W. D.; Partain, C. L.; Washburn, L. C.; Hayes, R. L.; and Holloway, E.
Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases.
In *Medical Radionuclide Imaging 1980*, proceedings of an international symposium. Heidelberg, Germany, Vol. 2, pp. 515–529. Vienna: International Atomic Energy Agency, 1981.

Hudson, D. H.; Frome, E. L.; and Gray, J. R.
Graphics: an important tool for communication in epidemiology. In *Proceedings of Seventh Annual SAS Users Group International Conference*, San Francisco, 1982, pp. 205–210. Cary, North Carolina: SAS Institute, 1982.

Ishioka, R.; Kuniyasu, Y.; Higashi, S.; Kakehi, H.; Hayes, R. L.; and Carlton, J. E.
Preparation for liver scanning agent labeled with positron emitters, generator-produced gallium-68.
In *Proceedings of Third World Congress of Nuclear Medicine and Biology*, Paris, France, 1982, Vol. 1, edited by C. Raynaud, pp. 662–665. Paris: Pergamon Press, 1982.

Janardan, K. G.; Schaeffer, D. J.; and DuFrain, R. J.
A stochastic model for the study of the distribution of chromosome aberrations in human and animal cells exposed to radiation or chemicals.
In *Proceedings of the International Summer School on Statistical Distributions in Scientific Work*, Trieste, Italy, 1980, edited by G. Tallie, G. P. Patil, and B. Baldessari. Boston: D. Reidel Publishing Co. In press.

Kabalka, G. W.; Gooch, E. E.; Hsu, H. C.; Washburn, L. C.; Sun, T. T.; and Hayes, R. L.
Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology.
In *Applications of Nuclear and Radiochemistry*, edited by R. M. Lambrecht and N. Morcos, pp. 197–203. New York: Pergamon Press, 1982.

Littlefield, L. G.
Current advances in cytogenetics.
In *Proceedings of the NATO RSG 5*, Panel VII meeting, Bethesda, Maryland, 1982, Armed Forces Radiobiology Research Institute Publications. In press.

Littlefield, L. G.
Effects of DNA-damaging agents on SCE.
In *Sister Chromatid Exchange*, Vol. 2 of *Progress and Topics in Cytogenetics*, series edited by A. A. Sandberg, pp. 355–394. New York: Alan R. Liss, Inc., 1982.

Lushbaugh, C. C. [Contributor]
Chapter 8. Radiation Environment. Final Report.
Report on biotechnology research requirements for aeronautical systems through the year 2000.
In *Proceedings of a Workshop*, 1982, pp. 43–45. San Antonio, Texas: Southwest Research Institute, 1982.

Lushbaugh, C. C. [Contributor]
Risks of nuclear energy and low-level ionizing radiation.
Report of an AMA advisory panel to the Council on Scientific Affairs of the AMA, 1981. Monroe, Wisconsin: American Medical Association. In press.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
The impact of estimates of human radiation tolerance upon radiation emergency management.
In *The Control of Exposure of the Public to Ionizing Radiation in the Event of Accident or Attack*, proceedings of a symposium, Reston, Virginia, 1981, pp. 46–57. Bethesda, Maryland: National Council on Radiation Protection and Measurements, 1982.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
Effects of high doses of radiation.
In *Radiation and Its Effects*, Proceedings of Eighth Life Sciences Symposium, 1980, Los Alamos, New Mexico. In press.

1028533

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Beck, W. L.
[illegible]ects on humans of exposure to radioactive materials: [illegible] effects.
In *Encyclopedia of Materials Science and Engineering.* New York: Pergamon Press. In press.

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Ricks, R. C.
Is the precision of human radiation tolerance estimates sufficient for radiation emergency management?
In *Radiological Protection—Advances in Theory and Practice*, Proceedings of Society for Radiological Protection, Third International Symposium, Inverness, Scotland, 1982, Vol. 1, pp. 56–63. Society for Radiological Protection, 1982.

Mettler, F. A., and Ricks, R. C.
Medical management of radiation accidents.
Contemp. Diagn. Rad. 5(Lesson 8):1–6; 1982.

Parker, J. C., and Richter, C. B.
Viral diseases of the respiratory system.
In *The Mouse in Biomedical Research*, Vol. 2, edited by H. L. Foster, J. D. Small, and J. G. Fox, pp. 107–157. New York: Academic Press, 1982.

Partain, C. L.; Heller, R. M.; Hübner, K. F.; Price, R. R.; Patton, J. A.; Erickson, J. J.; Pickens, D. R.; Hayes, R. L.; and James, A. E., Jr.
Functional imaging of the brain using positron emission tomography.
In *Proceedings of Copenhagen Symposium on Diagnostic Imaging*, 1982, Glostrup University Hospital, Glostrup, Denmark. In press.

Partain, C. L.; Hübner, K. F.; Price, R. R.; Patton, J. A.; Erickson, J. J.; Pickens, D. R.; Hayes, R. L.; and James, A. E., Jr.
Functional mapping of the brain: positron emission tomography and nuclear magnetic resonance imaging.
In *Functional Mapping of Organ Systems and Other Computer Topics*, 11th Annual Symposium on the Sharing of Computer Programs and Technology in Nuclear Medicine, edited by P. D. Esser, pp. 13–19. New York: Society of Nuclear Medicine, 1981.

Ricks, R. C.
Response to a spill of radioactive material.
In *Encyclopedia of Materials Science and Engineering.* New York: Pergamon Press. In press.

Snyder, F.
Platelet activating factor (PAF), a novel type of phospholipid with diverse biological properties.
Annu. Rep. Med. Chem. In press.

Stutz, D. R.; Ricks, R. C.; and Olsen, M. F.
Hazardous Materials Injuries. A Handbook for Pre-Hospital Care. Greenbelt, Maryland: Bradford Communications Corporation, 1982.

Tardiff, S. D.
[illegible]al maturation of female *Saguinus oedipus oedipus*. [illegible]D. dissertation, Michigan State University, 1982.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; Rafter, J. J.; Hayes, R. L.; Frey, K. A.; and Agranoff, B. W.
^{11}C-ACHC, a potential agent for positron tomographic measurement of brain amino acid transport.
In *Nuclear Medicine and Biology*, Proceedings of the Third World Congress of Nuclear Medicine and Biology, Paris, France, 1982, Vol. 1, edited by C. Raynaud, pp. 642–645. Paris: Pergamon Press, 1982.

Wills, J. H., and Hübner, K. F.
Pharmacologic bases for treatment of patients exposed to antipersonnel chemicals and to ionizing radiation.
In *Pharmacologic Approach to the Critically Ill Patient*, edited by C. R. Lake and B. Chernow. Baltimore: Williams and Wilkins. In press.

Published abstracts of papers presented at meetings

Abd Elghany, N.; Lushbaugh, C. C.; and Checkoway, H.
An epidemiologic study of cancer mortality among workers exposed to occupational low levels of ionizing radiation.
In *Proceedings of the XX International Congress of Occupational Health, Abstracts*, Cairo, Egypt, 1981, p. 477.

Albert, D. H., and Snyder, F.
Role of phospholipase and acetyltransferase in the release of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by rat alveolar macrophages.
Fed. Proc. 41:670; 1982.

Berdel, W. E.; Greiner, E.; Fink, U.; Stavrou, D.; Reichert, A.; Rastetter, J.; and Snyder, F.
Cytotoxic effect of alkyl-lysophospholipids (ALP) in rat brain tumor cells.
Proc. Am. Assoc. Cancer Res. 23:198; 1982.

Clapp, N. K.
Colonic adenoma incidences, pathogenesis, radiographic diagnosis, age dependence, and risk in *Saguinus oedipus oedipus*.
In *Proceedings of IXth Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Clapp, N. K.; Lushbaugh, C. C.; Gangaware, B. L.; Tankersley, W. G.; and Humason, G. L.
Investigation of spontaneous colon adenocarcinoma in a twenty-year old colony of tamarins and marmosets.
In *Proceedings of 13th International Cancer Congress*, 1982, Seattle, Washington, p. 544.

Clapp, N. K.; Lushbaugh, C. C.; Tankersley, W. G.; Gangaware, B. L.; and Humason, G. L.
Spontaneous colon adenocarcinoma in the cotton-top tamarin, *Saguinus oedipus oedipus*, as a model for human colon cancer.
Proc. Am. Assoc. Cancer Res. 23:106; 1982.

Clapp, N. K.; Lushbaugh, C. C.; Tardif, S. D.; DuFrain, R. J.; Tankersley, W. G.; Richter, C. B.; Gengozian, N. B.; and Hunt, S. R.
Spontaneous colon adenocarcinoma in the cotton-top tamarin, *Saguinus oedipus oedipus* as a model for human colon cancer.
Int. J. Primatol. 3:239; 1982.

Coffey, J. L., Rafter, J. J.; Watson, E. E.; and Hayes, R. L.
Radiation dosimetry and chemical toxicity considerations for ^{99}Tc.
In *Abstracts of Papers*, 27th Annual Meeting, Health Physics Society, Las Vegas, Nevada, 1982, p. 60. New York: Pergamon Press, 1982.

1028534

DuFrain, R. J.; Batson, J.; Colyer, S. P.; Joiner, E. E.; Littlefield, L. G.; and Hübner, K. F.
Cytogenetic analysis of mutagen stressed lymphocytes from individuals with Huntington's chorea.
Mamm. Chromosomes Newslett. 23:16; 1982.

DuFrain, R. J.; Batson, J. S.; Joiner, E. E.; Colyer, S. P.; Littlefield, L. G.; and Hübner, K. F.
Cytogenetic assessment of radiation sensitivity in lymphocytes from individuals with Huntington's chorea.
Radiat. Res. In press.

DuFrain, R. J.; Littlefield, L. G.; Batson, J. S.; Jennings, C. J.; Linkous, S.; and Morrison, W. D.
Cytogenetic studies on somatic and female germ cells of adult and neonatal rabbits (*Oryctolagus cuniculus*).
Mamm. Chromosome Newslett. In press.

DuFrain, R. J.; Littlefield, L. G.; and Sayer, A. S.
Some cytogenetic aspects of colon cancer in *Saguinus oedipus oedipus*.
In *Proceedings of IXth Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action.
Biometrics. In press.

Fry, S. A.; Lushbaugh, C. C.; and Hübner, K. F.
An overview of radiation accidents and injuries.
Int. J. Nucl. Med. Biol. 9:152-153; 1982.

Fukushi, K.; Byrd, B. L.; Sun, T. T.; Washburn, L. C.; and Hayes, R. L.
Transport of ^{14}C-labeled 1-aminocyclohexanecarboxylic acid (ACHC) and ring methylated derivatives into the brain.
J. Nucl. Med. 23:P101; 1982.

Gengozian, N.
Immunological observations in *S. fuscicollis* and *S. o. oedipus* tamarins.
In *Proceedings of IX Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Hayes, R. L.
Gallium-67: an agent for detecting tumors and inflammatory processes.
In *Information, Program, Abstract of Papers*, 33rd Annual Southeastern Regional Meeting of the American Chemical Society, Lexington Section, Lexington, Kentucky, 1981, p. 90.

Hayes, R. L., and Carlton, J. E.
The association of ^{67}Ga with a 40K molecular weight protein in tumors and normal tissues.
J. Nucl. Med. 23:P37; 1982.

Hayes, R. L.; Brown, D. H.; Palisano, J. R.; and Colyer, S. P.
The subcellular distribution of ^{67}Ga in hepatoma tissue labeled by in vitro and in vivo techniques.
Clin. Nucl. Med. 6:P146; 1981.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Studies of microsomal diacylglycerol and alkylacylglycerol cholinephosphotransferase activities in rat liver.
Fed. Proc. 41:670; 1982.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
Lack of expression of MMC-induced SCEs in human lymphocytes that have undergone greater than two in vitro cell divisions.
Environ. Mutagen. 4:359, 1982.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. S.; and DuFrain, R. J.
Lack of correlation between hematological parameters and baseline sister chromatid exchanges in lymphocyte cultures from control subjects.
Mamm. Chromosome Newslett. 23:27; 1982.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. S.; and DuFrain, R. J.
Comparisons of baseline and mutagen-induced SCEs in "early dividing" and "late dividing" lymphocyte populations.
Mamm. Chromosome Newslett. In press.

Lushbaugh, C. C.
ORAU marmoset colony history: Early colon cancer observations.
In *Proceedings of IX Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Lushbaugh, C. C.; Beck, W. L.; Dupree, E. A.; Frome, E. L.; Fry, S. A.; Hansard, M. S.; Hudson, D. R.; and Polednak, A. P.
Health and mortality of DOE workers.
Trans. Am. Nucl. Soc. 38:81; 1981.

Partain, C. L.; Price, R. R.; James, A. E., Jr.; Hübner, K. F.; Mahaley, M. S.; Scatliff, J. H.; and Robertson, J. T.
Positron emission tomography: Brain tumor imaging using carbon-11 amino acids.
In *Medical Radionuclide Imaging 1980*, proceedings of an international symposium, Heidelberg, Germany, Vol. 2, pp. 397-398. Vienna: International Atomic Energy Agency, 198[illegible]

Richter, C. R.
The effect of nutrition and husbandry on reproduction, longevity and colon carcinoma in the cotton-top tamarin.
In *Proceedings of IX Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Richter, C. B.; Tankersley, W. G.; Davis-Tardif, S.; and Carson, R. L.
Preference for high sugar diets by the common marmoset.
Am. J. Primatol. 1:313; 1981.

Rossi, R. M.; Urso, P.; and Gengozian, N.
The in vitro effect of benzo-pyrene on the immune response.
Fed. Proc. 41:446; 1982.

Tankersley, W. G.; Clapp, N. K.; Henke, M. A.; and Gangaware, B. L.
Fecal β-glucuronidase in cancer-susceptible and nonsusceptible Callitrichidae.
In *Proceedings of IX Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Tardif, S. D., and Richter, C. B.
The use of behavior studies to enhance breeding success in *Saguinus oedipus*.
In *Proceedings of IX Congress of the International Primatological Society*, 1982, Atlanta, Georgia. In press.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Hayes, R. L.
The chemistry of ^{11}C-labeled radiopharmaceuticals.
In *Information, Program, Abstracts of Papers*, 33rd Annual Southeastern Regional Meeting of the American Chemical Society, Lexington Section, Lexington, Kentucky, 1981, p. 90. ■

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Summaries of Published Papers

Berger, M. E.; Thompson, D. W.; Ricks, R. C.; and Kaufmann, D.
The medical technologist and the radiation-accident victim.
Am. Soc. Med. Technol. 47:829–834; 1981.

Radiation accidents may produce external or internal contamination with radionuclides, local or whole-body exposure to penetrating radiation, or a combination of these. Trauma and medical problems that require rapid diagnosis and treatment may accompany a radiation injury. The role of the medical technologist in the early postinjury period may include the use of precautions in handling of contaminated specimens, prompt and repeated determinations of the absolute lymphocyte count, establishment of baseline values of the cellular and chemical components of the blood, and urinalysis. Following significant whole-body exposure to penetrating radiation, laboratory tests are required to monitor the development of granulocytopenia and thrombocytopenia and to provide information necessary for the prevention, control, and treatment of infection and/or bleeding. Chromosome analysis may aid in dosimetry, and a procedure for collecting and shipping blood for analysis by experts in radiation cytogenetics is described.

Blank, M. L.; Dalbey, W.; Cress, E. A.; Garfinkel, S.; and Snyder, F.
Pulmonary NO_2 toxicity: phosphatidylcholine levels and incorporation of [^{3}H]thymidine into DNA.
Environ. Res. 27:352–360; 1982.

Lungs from rats exposed to 30 ppm NO_2 (μl/liter) for either 1 or 5 hr had higher incorporation rates of [^{3}H]thymidine into DNA and greater amounts of phosphatidylcholine than the lungs from control animals. Although there was no difference between the pulmonary phosphatidylcholine levels in rats exposed to NO_2 for 1 or 5 hr, the incorporation of [^{3}H]thymidine into DNA was considerably higher in the lungs of animals exposed to NO_2 for 5 hr than those exposed for 1 hr. Analyses of autoradiograms of lung tissue showed essentially the same labeling index for the alveoli from animals exposed to NO_2 for 1 or 5 hr; however, the animals exposed to NO_2 for 5 hr had a greater number of labeled cells in the lower airways and alveolar ducts than in the lungs from rats exposed for only 1 hr. These results suggest that the increased levels of phosphatidylcholine after exposure to NO_2 are directly related to damage of the alveolar cells, whereas the incorporation of [^{3}H]thymidine into lung DNA represents more widespread damage to areas involving the lower airways. Second and third exposures (24 hr apart) of rats to 30 ppm NO_2 for either 1 or 5 hr caused little increase in the incorporation of [^{3}H]thymidine into pulmonary DNA and no further increase in lung phosphatidylcholine levels. Decreased incorporation of thymidine into the DNA of pulmonary cells after second and third exposures to NO_2 is referred to as "adaptation" in this report. When NO_2-adapted animals were again exposed to NO_2 7 days after the previous NO_2 treatment, the lungs again exhibited increases in both [^{3}H]thymidine incorporation and levels of phosphatidylcholine.

Borman, L. S.
Modulation of mammalian cell growth by a choline analog, *N*-isopropylethanolamine.
In Vitro 18:129–140; 1982.

The choline analog, *N*-isopropylethanolamine (IPE), inhibits the growth of both Chinese hamster ovary CHO-K_1 and mouse L-M cells by two kinetically distinct mechanisms; I, a reversible and concentration-dependent reduction in the logarithmic population doubling rate and the saturation density of cultures by low IPE levels in the media; and II, an irreversible and time-dependent killing of cells by high IPE concentrations. Both types of inhibition are independent of media depletion, cell density, or the time of treatment after cell plating; however, the actual IPE concentration that is necessary to elicit Type I or Type II inhibition in each cell line is dependent on the choline level of the media. Ethanolamine, methionine, or betaine have no effect on IPE-induced growth inhibition. From a mutagenized population of CHO-K_1 cells we isolated variant cell strains that are resistant to the lethal effect of IPE. It was determined that with both the wild type and variant strains the sensitivity of cells to growth inhibition by IPE (both Type I and Type II) was proportional to the degree by which choline uptake was inhibited by the analog. Retinoic acid, which inhibits the growth of some fibroblast and epithelial cell lines by a concentration-dependent reduction in population doubling rate and saturation density, behaves synergistically with IPE to inhibit the growth of CHO-K_1 cells. Dibutyryl cyclic AMP, on the other hand, causes only an additive increase in the growth inhibition of CHO-K_1 populations that also are treated with IPE.

Brown, A. J., and Snyder, F.
Alkyldihydroxyacetone-P synthase: Solubilization, partial purification, new assay method, and evidence for a ping-pong mechanism.
J. Biol. Chem. 257:8835–8839; 1982.

Alkyldihydroxyacetone-P (alkyl-DHAP) synthase, the enzyme that forms the ether linkage of alkyl and alk-1-enyl glycerolipids, has been solubilized from Ehrlich ascites cell microsomes using Triton X-100 and acetone precipitation. The solubilized enzyme, which is stabilized by glycerol or ethylene glycol, was then purified further by chromatography on DEAE-cellulose, QAE-Sephadex, Matrex Red, and

1028536

hydroxylapatite with the aid of a new rapid assay method using DEAE-cellulose disks. Four enzymes involved in the metabolism of acyl-DHAP and alkyl-DHAP (acyl-DHAP/alkyl-DHAP oxidoreductase. DHAP acyltransferase, alkyl-DHAP phosphohydrolase, and a dinitrofluorobenzene-insensitive acyl-DHAP acylhydrolase) are removed under these conditions along with endogenous fatty acids and fatty alcohols. Two other activities copurify with the alkyl-DHAP synthase forward reaction: an acyl exchange reaction, in which [1-^{14}C]palmitic acid is incorporated into palmitoyl-DHAP, and an alkyl exchange reaction, in which [1-^{14}C]hexadecanol is incorporated into hexadecyl-DHAP. Exchange reactions of this type are characteristic properties of a ping-pong mechanism but not a sequential mechanism. This is confirmed by documentation that palmitic acid is a competitive inhibitor with respect to hexadecanol. In addition, low levels of palmitoyl-DHAP (<100 μM) show competitive inhibition with respect to hexadecanol, possibly due to palmitic acid formed from palmitoyl-DHAP by alkyl-DHAP synthase under these conditions. Based on the observations presented here and previously, a molecular mechanism for alkyl-DHAP synthase is proposed.

Cabot, M. C., and Welsh, C. J.
Ether lipid studies in mouse C3H/10T1/2 cells and a 3-methylcholanthrene-transformed clone.
Arch. Biochem. Biophys. 211:240–244; 1981.

C3H/10T1/2 mouse embryo cells and a transformed clone were used in these initial experiments to investigate the future application of this model culture system to studies of ether-linked lipids in cancer cells. Clone 8 cells are nontumorigenic, nontransformed, and maintain normal morphology to passages 15–20. Clone 16 cells were derived from morphologically transformed foci of clone 8 cells exposed to the chemical carcinogen, 3-methylcholanthrene, and are highly tumorigenic. The data presented here demonstrate that the high amounts of ether-linked lipids, characteristic of tumors, are likewise elevated in cells that have been oncogenically transformed in vitro. When incubated with labeled fatty alcohols, the transformed cells show a stimulated incorporation of radioactivity into alkyldiacylglycerols (>100% over clone 8), whereas radioactivity in the alkyl moiety of the phospholipids is not altered. Analysis of the lipids formed from [1-^{14}C]hexadecanol indicates that the nontransformed cells have a greater capacity to oxidize hexadecanol and incorporate the resulting carboxylic acid into acyl groups. Quantitative analysis of cellular lipids shows that in the oncogenically transformed cells alkyldiacylglycerols are increased (123% over clone 8).

Cabot, M. C., and Welsh, C. J.
Fatty acid metabolism in phorbol ester-differentiating human leukemia cells.
Cancer Res. 41:4910–4915; 1981.

Human promyelocytic leukemia cells (HL-60) undergo differentiation when treated with the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). As the primary target for TPA action is membranes, studies were undertaken to determine whether phorbol ester exposure would influence fatty acid metabolism in these cells. In cells incubated with labeled fatty acids for 1 hr, the percentage of distribution in lipid radioactivity is highest in the phospholipid fraction of control cultures, whereas in TPA-supplemented cells, substantially more label is associated with triacylglycerols. The specific activity of phospholipids and triacylglycerols was lower in treated than in control cells; however, the amount of cellular triacylglycerols increased 3.2-fold (lipid per mg protein). The increase in the amount of cellular phospholipids in TPA-treated cells is not as pronounced (approximately 50% above control), and this only occurs at higher concentrations of TPA. At early times after TPA exposure, there is no stimulation of the cellular uptake of labeled fatty acids; however, differentiating cells (24 to 48 hr of TPA), when incubated with label, contained more radioactivity than did control cultures. Cells treated with TPA for 48 hr show a marked decrease in the conversion of [1-^{14}C]stearic acid to monoenoic product (22% of control); this decrease is dose dependent and occurs within 24 to 48 hr of treatment. Although the phospholipid fatty acid composition of differentiating cells was similar to control cells, acyl groups of triacylglycerols isolated from treated cells showed a marked decrease in the percentage of unsaturates. These data provide evidence which demonstrates that TPA treatment of HL-60 cells has a profound effect on fatty acid metabolism. The lack of an effect of TPA on fatty acid metabolism after short-term exposure to the promoter suggests that the modifications observed may be the result of cellular differentiation rather than a direct effect exerted by the presence of TPA in the culture media.

DuFrain, R. J.; Littlefield, L. G.; Wilmer, J. L.; and Frome, E. L.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. II. A comparison of streptonigrin effects on somatic and germ cells.
Mutat. Res. 94:103–114; 1982.

The dose-response relationships for streptonigrin (NSC-45383)-induced chromosome aberrations in rabbit somatic cells are compared with dose-response data derived from the analysis of inherited structural chromosome abnormalities in preimplantation embryos from female rabbits treated with streptonigrin prior to mating. The incidence of inherited aberrations assessed in over 1000 karyotype preparations from 361 6-day blastocysts obtained from 55 female rabbits is used to derive a measure of the transmissible cytogenetic damage induced in the oocytes. The cytogenetic damage assessed in 2300 lymphoblast metaphases from 23 rabbits and 2750 marrow-derived metaphases from 27 rabbits which were collected and prepared for examination 6 hr after the initiation of streptonigrin dosing are used to obtain estimates of the somatic cell insult. A uniform maximum likelihood analysis technique is applied individually to the 3 sets of data to derive the coefficients of the dose-response relationships. The resulting equations are $Y = 0.6 \pm 28.0\ (\times 10^{-5}) + 8.2 \pm 5.1\ (\times 10^{-4})\chi$ for inherited aberrations in 6-day blastocysts, $Y = 9.7 \pm 3.3\ (\times 10^{-3}) + 1.9 \pm 0.1\ (\times 10^{-3})\chi$ for bone-marrow cells, and $Y = 2.8 \pm 0.7\ (\times 10^{-2}) + 4.8 \pm 0.2\ (\times 10^{-3})\chi$ for the lymphoblasts. In the somatic tissues Y is the percentage of cells with chromosome breakage, while in the blastocyst data Y is the percentage of 6-day blastocysts with consistent structural chromosome aberrations, and in all equations χ is the total streptonigrin dose in μg/kg. The study shows that streptonigrin injections in the range of 30–90 μg/kg when given to sexually mature female rabbits cause dose-dependent increases in chromosome aberrations in 2 types of somatic cells and in the incidence of inherited aberrations recovered in 6-day blastocysts. The coefficients of damage recovered in blastocysts

1028537

versus damaged recovered in somatic cells have the ratio of 1:2.3:5.8 (blastocysts:bone marrow:lymphoblasts). The results are discussed in terms of risk assessment and kinetics of ...rration loss during meiosis and early embryonic development. The conclusion drawn from the study is that somatic cell cytogenetic damage is in some way predictive of damage incurred by oocytes which can be passed on to preimplantation embryos, at least for agents like streptonigrin.

Feuerstein, G.; Zukowska-Grojec, Z.; Krausz, M. M.; Blank, M. L.; Snyder, F.; and Kopin, I. J.
Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious SHR and WKY rats.
Clin. Exp. Hypertens.—Theory and Practice A4:1335–1350; 1982.

Injections of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkylacetyl-GPC, 0.2–5.0 nmol/300 g body weight) induced dose-related hypotension and tachycardia in spontaneous hypertensive (SHR) and normotensive control (WKY) rats. The hypotension that developed was more pronounced in SHR than in WKY rats and was unchanged by indomethacin pretreatment. Plasma norepinephrine (NE) and epinephrine (EPI) levels were markedly increased at the time of maximal hypotension (2 min after injection of alkylacetyl-GPC); plasma EPI (but not NE) was higher in the SHR than in WKY animals. Plasma levels of TXB_2, but not 6-keto-$PGF_{1\alpha}$, increased in both groups; the increase was more pronounced in SHR than in WKY rats. In pithed SHR ..., alkylacetyl-GPC caused only short lasting hypotension ...out any effect on heart rate or circulating levels of NE or EPI. These data suggest that there is an increased vascular sensitivity to alkylacetyl-GPC in SHR rats and activation of thromboxane-generating elements in both SHR and WKY rats.

Fox, L. L.; Gengozian, N.; Shivers, C. A.; and Sieg, P.
Marmoset response to immunization with porcine zonae pellucidae: Measurement of antibody activity with an enzyme-linked immunosorbent assay.
J. Med. Primatol. 10:205–211; 1981.

An enzyme-linked immunosorbent assay (ELISA) was used to detect antibodies in sera from a marmoset inoculated with porcine zonae. Antibodies which bound to heat-solubilized zonae immobilized in microtiter plates were detected by an enzymatic reaction using goat antimarmoset IgG conjugated to alkaline phosphatase. During a 9-week immunization period, the antibody response profile as determined with the ELISA was parallel to that obtained with an indirect immunofluorescent test on intact zonae. This procedure has certain advantages over presently used methods of titrating antizona sera.

Gengozian, N., and Rice, D. T.
...body-dependent cellular cytotoxicity with platelets as the ...et cell: potential application to the study of immune thrombocytopenia.
Clin. Exp. Immunol. 47:431–436; 1982.

Antibody-dependent cellular cytotoxicity (ADCC) with platelets as the target cell is demonstrated with white blood cells, platelets and antiplatelet antibody obtained from marmosets. Greatest ADCC activity was observed with cell populations enriched for polymorphonuclear (PMN) cells and adherent cells. Nonadherent cells and lymphocytes obtained from blood pretreated with carbonyl iron showed a lower yet significant degree of activity. The virtual absence of PMNs and monocytes from these preparations indicates that a nonphagocytic-mediated destruction of platelets can occur by an ADCC process. Platelets having IgG deposited on them as a consequence of an autoimmune reaction following an interspecies platelet immunization are not necessarily susceptible to lysis by an ADCC mechanism. The ADCC reaction system as described and the results obtained suggest one can monitor platelets, effectors, and soluble factors from immune thrombocytopenic patients and animals with respect to this mode of destruction in vivo.

Hayes, R. L.; Byrd, B. L.; Rafter, J. J.; Carlton, J. E.; and Coffey, J. L.
Ytterbium-169 citrate for detection of inflammatory lesions.
In *Medical Radionuclide Imaging 1980*, proceedings of an international symposium, Heidelberg, Germany, Vol. 1, pp. 579–586. Vienna: International Atomic Energy Agency, 1981.

Like ^{67}Ga, ^{169}Yb shows an affinity for inflammatory processes. Unlike ^{67}Ga, ^{169}Yb can be reactor-produced and at a much lower cost. It has photon emissions that are also quite suitable for scanning. The authors have made a detailed comparison of the tissue distribution of ^{67}Ga and ^{169}Yb citrates at 4 and 24 hr after intravenous administration in rats bearing an experimental abscess to determine the relative worth of the two radionuclides for abscess detection. At 4 hr the abscess-to-normal tissue ratios for lung, muscle, bone marrow, blood and skin were significantly higher with ^{169}Yb, whereas with ^{67}Ga the ratios were significantly better only with kidney and bone, suggesting that ^{169}Yb may be of use for the more rapid detection of inflammatory lesions. At 24 hr the two agents were approximately equal in utility with better ratios being obtained again for kidney and bone with ^{67}Ga but only for muscle and blood with ^{169}Yb. A drawback to the possible clinical use of ^{169}Yb for detection of inflammatory processes is its long $T_{1/2}$ (32 d) which limits the ^{169}Yb dose that could be clinically administered. On the other hand, ^{169}Yb's long $T_{1/2}$ would result in a cost-effective shelf-life for this radiopharmaceutical, especially for facilities where ^{67}Ga is not used because of its cost or where it is frequently unavailable.

Hayes, R. L.; Carlton, J. E.; and Kuniyasu, Y.
A new method for labeling microspheres with ^{68}Ga.
Eur. J. Nucl. Med. 6:531–533; 1981.

We have developed a simple, highly efficient, and rapid technique for labeling a commercially available human serum albumin microsphere preparation with ^{68}Ga for use in positron tomography. An isotonic acetate buffer system is used. Maximum ^{68}Ga labeling is obtained at pH 4.8 (~97%) but deviations from that pH (e.g., 0.5 of a pH unit) produce only minor decreases in binding efficiency. The preparation is stable on standing over a period of 4 hr, and in vivo studies in the rat and dog show it to be specific for the lungs. Little change occurs in the tissue distribution of ^{68}Ga over a period of approximately 1 hr.

1028538

Hayes, R. L.; Rafter, J. J.; Carlton, J. E.; and Byrd, B. L.
Studies of the in vivo uptake of ^{67}Ga by an experimental abscess: concise communication.
J. Nucl. Med. 23:8–14; 1982.

The blocking of ^{67}Ga plasma protein-binding sites—by administration of scandium citrate, ferric citrate, and a colloidal hydrous ferric oxide preparation—reduced the uptake of ^{67}Ga in normal soft tissues and also that in the viable portion of an experimental abscess. On the other hand, enhancement of ^{67}Ga plasma protein binding by administration of rabbit apotransferrin increased ^{67}Ga uptake in both abscess and normal soft tissues. These results indicate that the pathways of ^{67}Ga from blood into inflammatory processes and normal soft tissues may be similar. However, when ^{67}Ga plasma protein binding was increased by inducing anemia, a markedly decreased ^{67}Ga uptake in the abscess resulted, whereas uptake in normal soft tissue was still elevated. It is possible that the discrepancy between the effects of apotransferrin and anemia on abscess-tissue uptake of ^{67}Ga resulted from a secondary effect produced by anemia, i.e., a decrease in the macrophage population in the abscess. Taken as a whole, the results obtained suggest that ^{67}Ga leaves the blood and enters inflammatory lesions by pathways that are probably quite different from those in a soft-tissue tumor and that the routes for abscesses may be similar to those occurring in normal soft tissues.

Hübner, K. F.; King, P.; Gibbs, W. D.; Partain, C. L.; Washburn, L. C.; Hayes, R. L.; and Holloway, E.
Clinical investigations with ^{11}C-labeled amino acids using positron emission computerized tomography in patients with neoplastic diseases.
In *Medical Radionuclide Imaging 1980*, proceedings of an international symposium, Heidelberg, Germany, Vol. 2, pp. 515–529. Vienna: International Atomic Energy Agency, 1981.

The high affinity of the DL-racemates of tryptophan and valine and the unnatural alicyclic amino acids 1-aminocyclobutane-carboxylic acid and 1-aminocyclopentane-carboxylic acid for a variety of malignant tumors in humans is demonstrated. These amino acids were labelled in the carboxyl group by using a modified Bücherer-Strecker technique, and clinical imaging studies were performed with a commercially available positron emission computed axial tomograph (ECAT-1_{TM}). Attempts to quantitate amino-acid uptake by tumors using the ECAT-1 system and the implications for metabolic in vivo studies in tumors are discussed.

Hübner, K. F.; Purvis, J. T.; Mahaley, S. M., Jr.; Robertson, J. T.; Rogers, S.; Gibbs, W. D.; King, P.; and Partain, C. L.
Brain tumor imaging by positron emission computed tomography using ^{11}C-labeled amino acids.
J. Comput. Assist. Tomogr. 6:544–550; 1982.

We report our preliminary clinical results on positron emission tomography of human cerebral tumors with ^{11}C-tagged amino acids. Carbon-11-DL-valine and ^{11}C-DL-tryptophan were used for imaging the tumors. Some attempts at quantitation of the uptake were also carried out. The implications of these observations are discussed.

Hudson, D. H.; Frome, E. L.; and Gray, J. R.
Graphics: an important tool for communication in epidemiology.
In *Proceedings of Seventh Annual SAS Users Group International Conference*, San Francisco, 1982, pp. 205–210 Cary, North Carolina: SAS Institute, 1982.

The field of occupational epidemiology requires communication between people from many varied disciplines and interests. Traditional methods of presentation, such as charts and tables of numbers, often confuse individuals unfamiliar with the statistical methods involved (i.e., corporate management, medical directors, etc.). Since interaction with these individuals is important in order to meet the goals of this kind of research project, visual aids and alternative explanations are needed. This paper demonstrates graphic methods being developed to enhance both data interpretation and presentation for an occupational health and mortality project. Data interpretation often involves the absorption and comparison of data categorized in several dimensions. It is shown that the use of three-dimensional plots assists in quickly detecting outliers, differences, and trends in population distributions and mortality ratios, and Freeman-Tukey residuals are shown to be easily displayed and interpreted using PROC CHART and GCHART. Viewing Freeman-Tukey residuals for various data groups (i.e., ordinal exposure categories, age groups, etc.) in this manner makes it possible to show the existence or nonexistence of trends without the need for lengthy explanations or the questionable use of formal parametric tests.

Huff, V.; DuFrain, R. J.; and Littlefield, L. G.
SCE frequencies in rabbit lymphocytes as a function of time after an acute dose of cyclophosphamide.
Mutat. Res. 94:349–357; 1982.

Following acute and chronic exposures to various chemicals in vivo, the average SCE frequency in human and rabbit lymphocytes has generally been shown to decrease with time posttreatment. The rate of this decline varies, however, and little data have been published pertaining to the decrease in SCEs soon after exposure. To gain more information about the immediate decline in SCEs with time, we injected rabbits with a single dose of 35 mg/kg cyclophosphamide (CP) and determined SCE levels in circulating lymphocytes at various times 5 hr to 2 weeks after treatment. We observed a rapid decline in SCE frequencies within 5 days, and by 10 days postexposure the SCE levels were back to control values. The distributions of SCEs among cells and the number of circulating lymphocytes were also analyzed at each time. Within 2–3 days posttreatment we observed a rapid loss of cells with high SCE levels concomitantly with a rapid decline in circulating lymphocytes and a decrease in the average SCE frequency. When the number of lymphocytes began to increase, the number of cells with normal SCE values also increased. By 10–11 days after CP, the lymphocyte count had recovered, the SCE frequency had returned to control levels, and the distribution of SCEs among cells was almost identical to the control distribution. These data, in addition to published information on rabbit lymphocyte lifespan suggest that the decline in SCE levels with time posttreatment is a function of lymphocyte turnover.

Kabalka, G. W.; Gooch, E. E.; Hsu, H. C.; Washburn, L. C.; Sun, T. T.; and Hayes, R. L.
Rapid and mild syntheses of radioiodinated estrogen derivatives via organoborane technology.

1028539

In *Applications of Nuclear and Radiochemistry*, edited by M. M. Lambrecht and N. Morcos, pp. 197–203. New York: Pergamon Press, 1982.

Radioiodinated estrogen derivatives of potential use as receptor-binding agents have been synthesized via the reaction of organoboranes with ^{125}ICl. The utility of organoboranes as intermediates in radiopharmaceutical syntheses is discussed.

Lee, T-c.; Malone, B.; Blank, M. L.; and Snyder, F.
1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) stimulates calcium influx in rabbit platelets.
Biochem. Biophys. Res. Commun. 102:1262–1268; 1981.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) induces an influx of Ca^{2+} in rabbit platelets that is time-, temperature-, and concentration-dependent. The ionophoretic activity of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is much higher (>2-fold at 10^{-10}M) than 3-hexadecyl-2-acetyl-*sn*-glycero-1-phosphocholine, 1-hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine, 1-acyl-2-acetyl-*sn*-glycero-3-phosphocholine, or 1-hexadecanoyl-2-lyso-*sn*-glycero-3-phosphocholine.

Lee, T-c.; Malone, B.; Wasserman, S. I.; Fitzgerald, V.; and Snyder, F.
Activities of enzymes that metabolize platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) in neutrophils and eosinophils from humans and the effect of a calcium ionophore.
Biochem. Biophys. Res. Commun. 105:1303–1308; 1982.

Enzymatic systems in human blood cells are described for the activation and inactivation of a biologically active phospholipid (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) with hypotensive, platelet-aggregating, and inflammatory properties. The results document the presence of alkyldihydroxyacetone-phosphate synthase (forms the *O*-alkyl linkage in lipids), 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase (produces the biologically active molecule), and 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine:acetylhydrolase (destroys the biological activity) in human neutrophils and eosinophils. Both the acetyltransferase and acetylhydrolase activities are increased severalfold after treatment of normal neutrophils with ionophore A23187; however, alkyldihydroxyacetone-phosphate synthase activity is not influenced by the ionophore. Eosinophils isolated from patients with eosinophilia have significantly greater activities of all the enzymes studied than the eosinophils isolated from normal individuals. Our results indicate the acetyltransferase responsible for 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine synthesis may serve an important role in human blood cells that release this biologically active phospholipid. Moreover, the acetyltransferase activity was found to be dramatically influenced by calcium flux.

, T-c., and Stephens, N.
The modification of lipid composition in L-M cultured cells supplemented with elaidate: Increased formation of fatty alcohols.
Biochim. Biophys. Acta 712:299–304; 1982.

Supplementation of culture medium with elaidic acid (40 μg/ml) resulted in the incorporation of this acid into 50% of the acyl groups of phospholipids in L-M cells; elaidate was esterified at both the *sn*-1 and the *sn*-2 positions of phosphatidylcholine and phosphatidylethanolamine. In addition, elaidate supplementation of L-M cells induces the accumulation of free fatty alcohols, alkyldiacylglycerols, and wax esters/cholesterol esters. The concentration of intracellular free fatty alcohols increased as a function of the concentration of elaidic acid in the growth medium and the duration of exposure. However, the concentration of alcohol-containing lipids, total alkyl and alk-1-enyl lipids, was only slightly decreased. This decrease was much less than the increase in the level of free fatty alcohols. Therefore, we conclude that when elaidic acid is supplemented to L-M cells in culture, a net increase in the production of free fatty alcohols occurs.

Littlefield, L. G.
Effects of DNA-damaging agents on SCE.
In *Sister Chromatid Exchange*, Vol. 2 in *Progress and Topics in Cytogenetics*, series edited by A. A. Sandberg, pp. 355–394. New York: Alan R. Liss, Inc., 1982.

Literally hundreds of studies have assessed the effects of DNA-damaging agents on the induction of sister chromatid exchanges (SCEs). In this report I have reviewed some of the published findings on SCE induction in cells exposed to agents having known mechanisms of action on DNA. Several general conclusions can be reached. First, agents such as ionizing radiation and radiomimetic chemicals are highly efficient in inducing molecular lesions that lead to chromosome aberrations but relatively inefficient in inducing the types of DNA lesions or alterations that lead to SCEs. On the other hand, UV-light and alkylating chemicals are efficient inducers of SCEs at doses or concentrations that yield only minor increases in chromosomal aberrations. Studies with several different types of DNA-damaging agents have shown that long-lived lesions that lead to SCEs may be induced at all stages of the cell cycle, but that a period of DNA synthesis is required for the expression of SCEs. Attempts to correlate induced SCEs with known repair processes in mammalian cells or with specific types of molecular lesions in DNA have failed to yield consistent results.

Littlefield, L. G.; Colyer, S. P.; Joiner, E. E.; DuFrain, R. J.; Frome, E.; and Cohen, M. M.
Chromosomal radiation sensitivity in ataxia telangiectasia long-term lymphoblastoid cell lines.
Cytogenet. Cell Genet. 31:203–213; 1981.

Elevated frequencies of spontaneous chromosome breakage and hypersensitivity to radiation-induced chromosome damage are characteristic findings in cultured lymphocytes and skin fibroblasts of patients with ataxia telangiectasia (AT). To determine whether long-term AT lymphoblastoid cells (B-lymphocytes), which do not express spontaneous chromosomal instability, exhibit increased chromosomal radiation sensitivity, four AT lymphoblastoid cell lines were exposed to various doses of 250 kVp X rays during G2. At doses of 25 and 50 rads, the AT cells exhibited a 2- to 3-fold increase in radiation damage relative to that observed in B-cell lines or PHA stimulated lymphocytes from normal donors, while at 100 rads, the AT cells demonstrated approximately 1.5 times more lesions. These findings suggest that the genetic (or other) factors responsible for increased chromosomal radiation sensitivity in AT lymphocytes and fibroblasts also operate in long-term lymphoblastoid AT cells.

1028540

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Ricks, R. C.
Is the precision of human radiation tolerance estimate sufficient for radiation emergency management?
In *Radiological Protection—Advances in Theory and Practice*, Proceedings of Society for Radiological Protection, Third International Symposium, Inverness, Scotland, 1982, Vol. 1, pp. 56–63. Society for Radiological Protection, 1982.

About 100 years of retrospective clinical evaluations of the deleterious consequences of accidental and therapeutic radiation exposures provide us working estimates of human radiation tolerance (or radiosensitivity) of admittedly variable accuracy, acceptance, and utility. Their inaccuracy results from the fact that in accidents, where normal persons have been irradiated, doses have usually been unknown, whereas in radiotherapy, where doses are known precisely, the additivity of various diseases and cellular abnormalities upon final radiosensitivity remains largely unknown. Even so, from follow-up studies of radiation-accident victims, we do know that human radiation biology is not qualitatively different from that of other animals, and so the mechanisms of human radiation lethality are sufficiently understood to dictate therapeutic measures and suggest radiation dosage limits for their effectiveness in the case of a few irradiated patients or where large populations are exposed under austere conditions. Several attempts to quantify this medical experience have been published. The best attempt to date (NCRP 42) to distill this wisdom into a 3 × 3 "Penalty Table" for civil defense personnel to use to control postattack radiation exposures within high-level radioactive fallout fields has not met with general acceptance and is criticized as not providing adequate guidelines for planning purposes. It can be shown, however, to be a conservative application of the (Strandqvist-Bateman) "cube root of dose-rate models" for human radiation-damage repair and a "best fit" to our clinical knowledge of human radiosensitivity. Although the resulting radiation dose/biologic effect/"temporal window" appears to some as "three-dimensional clinical mythology", there are no recent data to support the wishful concept, proposed by others, that man is a super radio-resistant large animal.

Moore, C., and Snyder, F.
Properties of microsomal acyl coenzyme A reductase in mouse preputial glands.
Arch. Biochem. Biophys. 214:489–499; 1982.

Synthesis of long-chain fatty alcohols in preputial glands of mice is catalyzed by an NADPH-dependent acyl coenzyme A (CoA) reductase located in microsomal membranes; sensitivity to trypsin digestion indicates that the reductase is on the cytoplasmic side of the membrane. Results with pyrazole and phenobarbital demonstrate the reaction is not catalyzed by a nonspecific alcohol dehydrogenase or an aldehyde reductase. Acyl-CoA reductase activity is sensitive to sulfhydryl and serine reagent modification, is stimulated by bovine serum albumin, and produces an aldehyde intermediate. The activity is extremely detergent sensitive and cannot be restored even after removal of the detergents. Phospholipase C or asolectin treatment does not release the acyl-CoA reductase from microsomal membranes, but causes a significant decrease in the activity recovered in the membrane pellet. Glycerol does not solubilize the reductase activity, nor does 3.0 M NaCl; however, the combination of glycerol and 3.0 M NaCl did release about 50% of the acyl-CoA reductase from the microsomal pellet. Substrate concentration curves obtained in the presence or absence of bovine serum albumin show significant differences in enzyme activities. The reductase is sensitive to the concentration of palmitoyl-CoA and is progressively inhibited at levels beyond the critical micellar concentration of the substrate. The apparent K_m for acyl-CoA reductase is 14 μM; however, the maximum velocity varies with the concentration of albumin used. Expression of enzyme activity in delipidated microsomes requires specific phospholipids, which suggests that in vivo regulation of acyl-CoA reductase activity could be achieved through modifications in membrane lipid composition.

Moore, C., and Snyder, F.
Regulation of acyl coenzyme A reductase by a heat-stable cytosolic protein during preputial gland development.
Arch. Biochem. Biophys. 214:500–504; 1982.

A low-molecular-weight protein located in the cytosol of mouse preputial glands has been shown to stimulate the activity of a microsomal acyl coenzyme A (CoA) reductase in the gland. This cytoplasmic protein was stable to heating and lyophilization, but was destroyed by trypsin digestion. It was able to bind palmitoyl-CoA, and gel elution behavior indicated it had a molecular weight of 10,000–12,000. The level of this stimulatory cytosolic protein and the activity of acyl-CoA reductase were shown to correlate with differentiation of the preputial gland during development of puberty in male mice; the acyl-CoA reductase activity first appeared at 4 weeks of age and increased dramatically up to 6 weeks of age. By 8 weeks, when sexual maturity was attained, the reductase activity decreased to that level found in mature male mice. The cytosol from the preputial glands of the youngest mice (3 weeks) contained sufficient heat-stable acyl-CoA binding protein to stimulate acyl-CoA reduction; however, the 3-week-old preputial gland microsomes had little or no acyl-CoA reductase activity. As the animal matured, the stimulatory capacity in the heat-treated cytosol increased, reaching a maximum at 6 weeks; by 8 weeks, the stimulatory capacity of the soluble fraction had decreased to that found in mature male mouse. Results of this study suggest that the concentration of acyl-CoA, cytoplasmic acyl-CoA binding protein, and acyl-CoA reductase activity regulate the level of fatty alcohols in vivo and that the reductase activity and binding protein have similar patterns of development during puberty.

Polednak, A. P.
Mortality among welders, including a group exposed to nickel oxides.
Arch. Environ. Health 36:235–242; 1981.

Mortality and causes of death were examined among 1,059 white male welders employed between 1943 and 1973 at three plants in Oak Ridge, Tennessee. Based on deaths reported as of 1974, the standardized mortality ratio (SMR) for all causes was 87 (173 deaths observed vs. 199.0 expected on the basis of death rates for U.S. white males). There were no deaths resulting from sinonasal cancer, and the SMR for lung cancer was 150 (95% confidence limits = 87 and 240). A subgroup of these welders (N = 536) was exposed to nickel oxides at the Oak Ridge Gaseous Diffusion (K-25) Plant; recent air concentrations of nickel (from personal air samplers) were greater than proposed standards. Standardized mortality ratios for lung cancer and diseases of the respiratory system were not higher among K-25 Plant

1028541

welders than among other welders (N = 523). Welders employed for ≥ 50 wk at the K-25 Plant had an SMR for lung cancer of 188 (95% confidence limits = 61 and 438) based on small numbers of deaths (5 observed vs. 2.66 expected). Further follow-up of these groups is needed.

Renooij, W., and Snyder, F.
Transmembrane orientation of palmitoyl-CoA:lysophosphatidylcholine acyltransferase in microsomes isolated from an alveolar type II cell adenoma and rat liver.
Biochim. Biophys. Acta 666:468–474; 1981.

1. The transverse localization of palmitoyl-CoA:lysophosphatidylcholine acyltransferase in the membrane of microsomal vesicles isolated from mouse lung adenomas and rat liver was studied by treating intact and deoxycholate-disrupted microsomes with trypsin and pronase. 2. The latency of mannose-6-phosphatase was preserved during protease treatment, suggesting that membrane integrity was not affected. 3. In adenoma microsomes 35–50% and in liver microsomes 35% of lysophosphatidylcholine acyltransferase activity is accessible to the action of the proteases. Our results suggest that at least a sizable portion of the active center of the enzyme that is responsible for remodeling phospholipids is embedded in the membrane interior. 4. Since enzymes involved in de novo lipid synthesis are reported to be located at the cytoplasmic surface of the microsomal membrane, our results support the notion that in lipid metabolism distinct metabolic pools might exist at opposite sides of the microsomal membrane.

Sayler, G. S.; Reid, M. C.; Perkins, B. K.; Pagni, R. M.; Smith, R. L.; Rao, T. K.; Epler, J. L.; Morrison, W. D.; and DuFrain, R.
Evaluation of the mutagenic potential of bacterial polychlorinated biphenyl biodegradation products.
Arch. Environ. Contam. Toxicol. 11:577–581; 1982.

Potential bacterial biodegradation products of polychlorobiphenyls (PCBs) were evaluated as environmental mutagens. *p*-Chlorophenylglyoxylic acid, *p*-chloromandelic acid, and three monochlorinated benzoic acids were not mutagenic in either the sister chromatid exchange (SCE) or *Salmonella typhimurium* histidine reversion mutagenesis assays. Bacterial degradation may, therefore, tend to decrease the mutagenic potential of lesser chlorinated PCBs.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Callahan, A. P.
Production of L-[1-^{11}C]valine by HPLC resolution.
J. Nucl. Med. 23:29–33; 1982.

Based on a recently developed analytical technique, preparative high-performance liquid chromatographic (HPLC) resolution of DL-[1-^{11}C]valine has been achieved. A conventional reverse-phase HPLC column and a chiral mobile phase (aqueous solution of L-proline, cupric acetate, and sodium acetate) were used. The copper can be removed from the L-valine fraction by precipitation as the sulfide, and final purification by cation-exchange chromatography yields L-[1-^{11}C]valine in a form that is acceptable for clinical positron tomographic studies. This purification method does not remove the L-proline introduced in the resolution process, but added L-proline did not affect the tissue distribution of L-[1-^{14}C]valine in rats. We have produced up to 60 mCi of L-[1-^{11}C]valine in an overall synthesis and resolution time of 50 min. This procedure should be adaptable to the rapid resolution of other ^{11}C-labeled amino acid racemates.

Welsh, C. J.; Sayer, A. M.; Littlefield, L. G.; and Cabot, M. C.
Modification of lipid acyl groups by serum deprivation does not affect phorbol ester-induced differentiation of human leukemia cells.
Cancer Lett. 16:145–154; 1982.

12-*O*-Tetradecanoylphorbol-13-acetate (TPA), a potent tumor promoter, can induce differentiation of human promyelocytic leukemia cells grown in a serum-free environment. Removal of serum produces altered acyl group composition of cellular phospholipids, most notably, marked decreases in the polyunsaturated fatty acid content. Although higher amounts of TPA are required to induce differentiation in serum-free cell populations, induction assessed by morphological and enzymatic markers is similar to the "macrophage-like" differentiated state reported for cells grown in a serum-rich medium. ■

1028542

Training Programs

Training at the division includes programs for postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, high school vocational trainees, and participants in special short-term courses on radiation health problems.

Graduate students participating in Ph.D. training programs have been from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama, and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals. Advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by Oak Ridge National Laboratory, University Programs at ORAU (sponsored by DOE), the University of Tennessee (Hilton A. Smith Fellowship), individual research grants, and operational subcontracts.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows have been from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health and American Cancer Society Grants, and divisional funds.

Summer research participation sponsored by University Programs at ORAU makes it possible for academic staff and beginning seniors from colleges and universities throughout the United States to participate in any of the division's research programs. Secretarial and laboratory aide training is also provided to high school students who participate in vocational programs at the high schools in Clinton and Oak Ridge, Tennessee.

Short-term courses in the management of health problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of ten REAC/TS courses was given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

The following individuals participated in the training programs at the division during the past year:



1028543

1. Predoctoral students

Naima Abd Elghany* (Epidemiology) (University of North Carolina)
Alex J. Brown† (Biochemistry) (University of Tennessee
Arvind Carpenter† (Epidemiology) (University of Alabama)
Donna Cragle‡ (Epidemiology) (University of North Carolina)
Diane Cookfair† (Epidemiology) (University of North Carolina)
Elizabeth Dupree-Ellis† (Epidemiology) (University of North Carolina)
Daniel Strom* (Environmental Sciences and Engineering) (University of North Carolina)
Jerson Surles* (Medicinal Chemistry) (University of North Carolina)
Suzette D. Tardif† (Marmoset Program) (Michigan State University)
Jerome Wilson* (Epidemiology) (University of North Carolina)

Purified enzymes are needed to study their regulation and the factors that control their activities. One of our graduate students succeeded in partially purifying the enzyme that makes the lipid ether bond, alkyldihydroxyacetone-P synthase. This enzyme preparation has been used in kinetic and substrate mechanistic studies of the ether bond-forming reaction.

2. Students pursuing MPH or MS degrees

Shirley Fry† (Epidemiology) (University of North Carolina)
Alan Johnson‡ (Environmental Sciences) (University of Alabama)
Kolam Mani* (Epidemiology) (University of North Carolina)
Bruce Parrish* (Environmental Sciences and Engineering) (University of North Carolina)
Ravishankar Rao† (Epidemiology) (University of Alabama)

3. Postdoctoral fellows

Daniel H. Albert (Biochemistry)
Dennis R. Hoffman (Biochemistry)
Eng-Lay Tan (Biochemistry)

4. Visiting scientists

Gary T. Bazer (Biochemistry) (Louisiana State University, Schreveport)
Kiyoshi Fukushi, (Radiopharmaceuticals) (National Institute of Radiological Sciences, Chiba-shi, Japan)

5. College-student research participants

Marvin Hall (Biochemistry) (University of Tennessee, Chattanooga)
David Kliewer (Cytogenetics) (Bethel College, North Newton, Kansas)
Lisa Roddy (Biochemistry) (Tennessee Technological University, Cookeville)
Julia Watson (Epidemiology) (Vanderbilt University, Nashville, Tennessee)

6. High school vocational participants

Sabrina Nicholson (Biochemistry) (Clinton Senior High School)
Gary Jackson (Epidemiology) (Oak Ridge High School)
Sherri Soles (Epidemiology) (Clinton Senior High School) ■

* Portion of thesis research being done at the division.
† Thesis research being completed at the division.
‡ Nonthesis work.

1028544

Seminars and Conferences

October 13, 1981
G. R. Eisele, Ph.D.
Food chain transport of organic toxicants

November 3, 1981
C. C. Lushbaugh, Ph.D., M.D.
Epidemiology program

November 12, 1981
A. B. Brill, Ph.D., M.D.
Brookhaven National Laboratory
Applications of quantitative autoradiography in nuclear medicine

December 8, 1981
Ethel Ayres
Graphics: better slides and view raphs

February 9, 1982
Russell J. DuFrain, Ph.D.
Metabolic and biophysical factors affecting sister chromatid exchange

February 23, 1982
L. Gayle Littlefield, Ph.D.
Sister chromatid exchanges in human lymphocytes: studies on SCE induction and the persistence of lesions that lead to SCE

March 9, 1982
Neal K. Clapp, D.V.M., Ph.D.
Antemortem contrast radiography for diagnosis of colon cancer in tamarins

March 23, 1982
Neal K. Clapp, D.V.M., Ph.D., and William G. Tankersley, M.S.
Fecal β-glucuronidase assay as an indicator of changes in the colon and its microbial environment

April 6, 1982
Lee Washburn, Ph.D., and Raymond L. Hayes, Ph.D.
Radiopharmaceutical development: progress and plans

April 20, 1982
J. D. Hoeschele, Ph.D.
Oak Ridge National Laboratory
Gold compounds and treatment of arthritis

April 29, 1982
Sibyl Nestor,
Computerized bibliographic retrieval services: an orientation to an expanded program

May 2, 1982
Wolfgang Berdel, M.D.
University of Munich, Federal Republic of Germany
The antineoplastic properties of alkyl-lysophospholipids: experimental results and first clinical trials

May 11, 1982
K. F. Hübner, M.D.
The potential of positron emission tomography in clinical research

May 14, 1982
L. M. Freeman, M.D.
Department of Nuclear Medicine, Montefiore Hospital, Bronx, New York
The clinical role of ^{99m}Tc-derivatives in hepatobiliary imaging

May 18, 1982
Mark M. Goodman, Ph.D.
Oak Ridge National Laboratory
Development of new radioiodinated fatty acids for the evaluation of heart

June 8, 1982
Donna Cragle, Ph.D.
The DOE Health and Mortality Study

June 22, 1982
Donna Hudson and **Jim Gray**
Computer graphics in epidemiology

July 22, 1982
Dr. Johanna Pohl-rueling
Department of Biophysics, University of Salzburg, Austria
Chromosome aberrations in human lymphocytes induced by low doses of radiation

August 10, 1982
Student Research Participants
Julia Watson
Vanderbilt University, Nashville
An assessment of socioeconomic status among four Oak Ridge facilities

Lisa Roddy
Tennessee Technological University, Cookeville
Mechanism responsible for the synthesis of platelet-activating factor in human eosinophils

Marvin Hall
University of Tennessee, Chattanooga
A partial characterization of plasma acetylhydrolase and comparison of its activity in normotensive versus spontaneously hypertensive rats

David Kliewer
Bethel College, North Newton, Kansas
The effects of various halogenated pyrimidines on cell cycle kinetics in lymphocytes

1028545

August 13, 1982
Student Research Participants

Trent Wallace
Vanderbilt University, Nashville
Survey of potential radiopharmaceutical agents for positron tomographic studies of the heart and lung

Steven Hill
Duke University, Durham, North Carolina
Partial purification and characterization of a specific acetylhydrolase for platelet-activation lipid in rat kidney cortex

M[illegible] Clapp
V[illegible]erbilt University, Nashville
Chemical hazard assessment for the uranium enrichment process at Y-12

August 24, 1982
Suzette Tardif, Ph.D.
Social influences on sexual maturation of female *Saguinus oedipus*

September 7, 1982
Daniel Albert, Ph.D.
Metabolism of a biologically active phospholipid in alveolar macrophages

September 24, 1982
Dennis Hoffman, Ph.D.
Studies of the selective tumor cytotoxicity of ether-linked phospholipid analogs ■

Non-DOE-Funded Research

D. Cragle
Minority high school student research apprentice program
National Institutes of Health, Division of Research Resources
$3,000* (1 March 1982 to 28 February 1983)

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies
National Pancreatic Cancer Project, National Cancer Institute
$75,250* (1 July 1981 to 30 June 1984)

L. G. Littlefield
Evaluation of induced cytogenetic lesions in oocytes
National Institute of Child Health and Human Development
$92,915* (1 August 1981 to 31 July 1984)

Studies of radiation-induced chromosome damage in humans
National Cancer Institute–U.S. Department of Energy Interagency Agreement
$107,047† (1 September 1981 to 30 September 1984)

C. C. Lushbaugh
Biomedical research support grant
National Institutes of Health, Division of Research Resources
$24,827† (1 April 1982 to 31 March 1983)

R. Ricks
Development of written and videotape training materials
Federal Emergency Management Agency (FEMA)–DOE Interagency Agreement (Contract)
$155,783† (30 September 1980 to 30 September 1982)

F. Snyder
Ether lipids in cancer: enzyme mechanisms and inhibitors
National Cancer Institute
$58,370* (1 May 1980 to 30 April 1985)

Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells
American Cancer Society
$73,375† (1 January 1982 to 31 December 1982)

A new class of biologically active phospholipids
National Heart, Lung, and Blood Institute
$58,491* (1 April 1981 to 31 March 1986) ■

* Direct costs (current year)
† Total costs, i.e., direct plus overhead (current year)

1028546

Collaborative Research

M. C. Cabot
Alterations in lipid metabolism during oncogenic transformation of cultured fibroblasts
University of Southern California, Cancer Research Center, Los Angeles, C. Heidelberger

The effect of lauricidin, a monoglyceride analog, on normal, transformed, and neoplastic cells
Michigan State University, East Lansing, J. J. Kabara

Synthesis of phorbol ester analogs and their potency as tumor promoters
Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina, Robert L. Wykle

N. K. Clapp
DNA repair in spontaneous colon tumors from the cotton-top tamarin
Oak Ridge National Laboratory, Biology Division, J. Regan, I. Kusano

Colon crypt cell kinetics in tamarins
Memorial Sloan-Kettering Cancer Center, New York, E. Deschner

Fecal bile acid analysis in marmosets
Beth Israel Medical Center, New York, B. Cohen

Monoclonal antibodies to human colon cancer cell lines in spontaneous primate colon tumors
Wistar Institute, Philadelphia, A. Steplewski

R. J. DuFrain
Human cytogenetic dosimetry for neutron radiation
Oak Ridge National Laboratory, H. W. Dickson; University of California, Department of Radiological Sciences and Radiation Oncology, Los Angeles, A. Norman

Cytogenetic effect studies on chlorinated biphenol degradation products
University of Tennessee, Department of Microbiology, Knoxville, G. S. Sayler

Gene mapping the laboratory rabbit
Oak Ridge National Laboratory, Biology Division, P. A. Lalley

Statistical distributions in cytogenetic dose response
Sangamon State University, Department of Mathematics, Springfield, Illinois, K. G. Janardan; Illinois Environmental Protection Agency, Springfield, D. J. Schaffer

Alpha particle dosimetry for cytogenetic analysis
Battelle Pacific Northwest Laboratories, Radiological Sciences Department, Richland, Washington, D. R. Fischer

Statistical analysis and Poisson regression studies in biological dosimetry
Oak Ridge National Laboratory, Computer Science Division, E. L. Frome

Studies of silver staining chromosome cores in multicentric chromosomes from irradiated blood
Samford University, Department of Biology, Birmingham, Alabama, W. M. Howell

R. L. Hayes
Synthesis of labeled compounds using organoborane techniques
University of Tennessee, Department of Chemistry, Knoxville, G. W. Kabalka

Collaborative studies in radiopharmaceutical development
Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies
The University of Tennessee Center for the Health Sciences, Department of Medicine, Knoxville, I. R. Collmann; Vanderbilt University Hospital, Department of Gastroenterology, Nashville, G. R. Avant

Differentiation of glioblastomas and meningiomas with ^{11}C-labeled amino acids and positron emission computed tomography
Vanderbilt University, Department of Nuclear Medicine, Nashville, C. L. Partain; The University of Tennessee Center for the Health Sciences, Department of Neurosurgery, Memphis, J. T. Robertson; University of North Carolina, Department of Surgery, Chapel Hill, M. S. Mahaley, Jr.; Fort Sanders Presbyterian Hospital, Department of Surgery, Knoxville, J. T. Purvis

1028547

Tumor metabolism
e University of Tennessee Center for the Health Sciences, Department of Medicine, Knoxville, S. Krauss

Development of software for quantitative positron tomography
Vanderbilt University, Department of Biomedical Engineering, Nashville, P. H. King

K. F. Hübner and L. C. Washburn
Measurement of regional brain protein synthesis using ^{11}C-labeled amino acids and positron emission computed tomography
University of Michigan, Department of Neurology, Ann Arbor, S. Gilman, and Neuroscience Laboratory, B. W. Agranoff

L. G. Littlefield
Studies on radiation-induced chromosome damage in humans
National Cancer Institute, Environment Epidemiology Branch, Bethesda, C. Land and R. Kleinerman

Analyses of radiosensitivity in human B lymphocytes, T lymphocytes, and lymphoblastoid cell lines of persons with ataxia telangiectasia
Northwestern University Medical School, Department of Pediatrics, Chicago, M. M. Cohen

Cytogenetic dose response kinetics in G-trisomic lymphocytes exposed to low LET radiation
Oak Ridge National Laboratory, Health and Safety Research Division, R. E. Swaja; Medical College of Georgia, Department of Anatomy, Augusta, D. A. Welter

Cytogenetic follow-up studies of a survivor exposed to ^{241}Am
Hanford Environmental Health Foundation, Washington, B. D. Breitenstein

C. C. Lushbaugh
Epidemiology of human carcinogenesis
University of Alabama in Birmingham, Department of Epidemiology, P. Cole

Comprehensive epidemiology of DOE atomic workers
University of North Carolina, Department of Epidemiology, Chapel Hill, C. M. Shy, M. A. Ibrahim, and H. A. Tyroler

United States Transuranium Registry
Hanford Environmental Health Foundation, Washington, B. D. Breitenstein

C. C. Lushbaugh and L. C. Washburn
Diethylenetriaminepentaacetate (DTPA) project
Department of Energy contractors, 40 coinvestigators

F. Snyder
Biological effects of unique phospholipid analogs
Boston College, Department of Chemistry, Chestnut Hill, Massachusetts, J. Hajdu

Effect of NO_2 and other toxicants on the lung surfactant system
Oak Ridge National Laboratory, Biology Division, W. E. Dalbey

Mass spectrometry of unique lipids
Oak Ridge National Laboratory, Analytical Chemistry Division, Gary Glish

Nuclear magnetic resonance studies of unique lipids
Oak Ridge National Laboratory, B. M. Benjamin

Synthesis of lipid analogs and their precursors
University of North Carolina, Department of Medicinal Chemistry, Chapel Hill, C. Piantadosi and K. S. Ishaq

Effects of a new group of antihypertensive phospholipids on cardiovascular function
University of Tennessee, Department of Psychology, Knoxville, J. E. Lawler and J. W. Hubbard

The role of intracellular transfer proteins in the cellular movement of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet-agregating phospholipid)
Western Carolina University, Department of Biology, Cullowhee, R. H. Lumb

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine
NIH and Uniformed Services University, Department of Neurology, Bethesda, G. Feuerstein

Selective cytotoxic activity of alkyllysophospholipids in tumor cells
Technischen Universitat Munchen, Division of Oncology, West Germany, W. E. Berdel

Pulmonary studies of an antihypertensive phospholipid
University of British Columbia, Pulmonary Research Laboratory, Vancouver, R. R. Schellenberg

Metabolism of platelet-activating factor in human blood cells
University of California, Division of Rheumatic Diseases, San Diego, S. I. Wasserman

L. C. Washburn
Pharmacological studies using ^{11}C-labeled compounds
University of Kentucky, School of Pharmacy, Lexington, G. A. Digenis

^{11}C-Labeled amines for pulmonary function studies
University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton, A. A. Noujaim, and Department of Medicine, S. F. P. Man ■

1028548

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[1]Predoctoral Fellowship (NCI Training Grant, ORNL)
[2]Postdoctoral Fellowship (NCI Training Grant, ORNL)
[3]Mellon Postdoctoral Fellowship (Executive Office)
[4]Visiting Scientist
[5]Educational Leave
[6]Consultant
[7]Student Research Participant (University Programs)
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[10]Part-time Continuing
[11]DOE Fellow

1028550

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1028551

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1028552

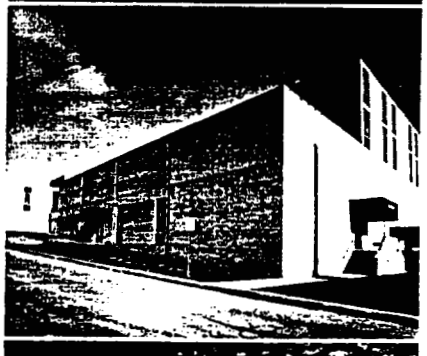
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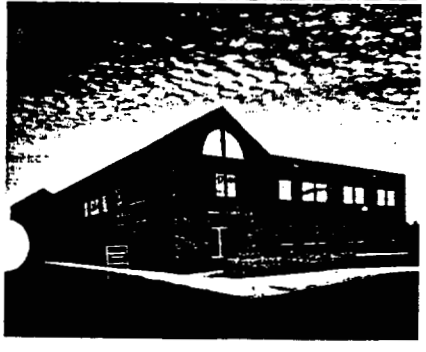
1028553











Oak Ridge
Associated Universities

Medical and Health Sciences Division

Research Report
1982–83

1028554

The Medical and Health Sciences Division conducts research on biomedical problems involving the respiratory, vascular, pancreatic, and gastrointestinal systems. Emphasis is on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Research programs in the division are focused on the following areas: (a) preclinical and clinical development of new positron radiopharmaceutical agents for in vivo diagnosis of diseases in man, (b) biochemical aspects of cellular lipid mediators and membrane lipids in disease processes, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), (e) food chain transport studies of synfuels and actinides, and (f) interdisciplinary investigations of the marmoset as a primate model for spontaneous colon cancer and other human diseases, particularly those associated with energy-related toxicants. Predoctoral and postdoctoral training is provided in each research program area. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a treatment and decontamination facility and a demonstration and training site.

Oak Ridge Associated Universities is a private, not-for-profit association of more than 50 colleges and universities. It is a contractor of the U.S. Department of Energy, conducting research and educational programs in the areas of energy, health, and the environment for DOE, other private and governmental organizations, and ORAU's member institutions.

ORAU-221, October 1983

Distribution list: ORAU/MHSD-1983

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

NOTICES

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Printed in the United States of America
Available from
National Technical Information Service
U. S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes
Printed copy: A03
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1028555

Oak Ridge
Associated Universities

Medical and Health Sciences Division

Research Report
1982–83

1028556

Preface

The cover of this report illustrates the five facilities that now constitute the Medical and Health Sciences Division. The division's central headquarters and REAC/TS are located on Vance Road. The Center for Epidemiologic Research and the Marmoset Research Center are on the ORAU campus, and the Field Site operation is at the Scarboro Facility on Bethel Valley Road.

The primary site for the division on Vance Road is the home for programs in biochemistry, preclinical and clinical nuclear medicine, and human cytogenetics. Services provided there include the library, an electronics shop, animal care facilities for small animals, special rooms for inhalation exposures, and tissue culture laboratories for cellular biology and oncology.

REAC/TS occupies a significant portion of the ground floor of the emergency wing of the Oak Ridge Hospital. This facility has treatment and decontamination rooms for handling patients involved in radiation accidents and also serves as a consultation, training, and demonstration center for training medical and paramedical personnel in management of clinical problems associated with radiation accidents.

In November 1982 the epidemiology program was relocated in the new Office and Auditorium Building on the ORAU campus. And last March the division was assigned the responsibility for managing the Marmoset Research Center and for the ICI breeding colony of marmosets and tamarins.

The food chain transport program (synfuels and actinides), reproductive toxicology, and beginning in FY 1984, the experimental animal portion of the cytogenetics program, are located at the division's Field Site. The Field Site provides unique facilities for investigations involving large domestic animals and hazardous or radioactive chemicals.

Research programs at the various sites are unified by the focus on occupational medicine in relation to human diseases associated with chemical toxicants and radiation exposure. Problems under investigation are those involved with cancer and with pulmonary, cardiovascular, and pancreatic diseases. The experimental studies utilize molecular and cellular systems, small experimental and large domestic animals, primates as disease models for extrapolation to humans, patients in nuclear medicine, and human populations for epidemiologic evaluation of mortality in occupations where the potential exposure to radiation and/or chemical hazards existed.

In addition to the primary financial support of the division by the U.S. Department of Energy, a number of our programs are funded by other agencies. Current grant and interagency contract work involves various National Institutes of Health, the American Cancer Society, the Environmental Protection Agency, and the Electric Power Research Institute, Inc. A listing of projects supported by non-DOE agencies is located on page 33.

This report covers our research accomplishments during FY 1983. Detailed descriptions of these efforts are available in the published papers listed on pages 18–24. Specific requests for reprints of published papers should be addressed to one of the authors or the Office of the Chairman.

C. C. Lushbaugh, Chairman
Fred Snyder, Associate Chairman
Karl F. Hübner, Assistant Chairman
October 1, 1983

Table of Contents

Scope of Research Programs 5
Vance Road Complex 5
Lipid Biochemistry 5
Preclinical and Clinical Nuclear Medicine 7
Cytogenetics 10
Field Site 12
Cytogenetics 12
Food Chain Transport 12
Marmoset Research Center 14
Radiation Emergency Assistance Center/Training Site 15
Center for Epidemiologic Research 16
Publications 18
Full-Length Journal Articles 18
Chapters, Review Articles, and Other Publications 20
Published Abstracts of Papers Presented at Meetings 22
Summaries of Published Papers 25
Training Programs 31
Non-DOE-Funded Research 33
Collaborative Research 34
Administration and Committees 36

1028557

Lipid Biochemistry

The program in lipid biochemistry concerns three main areas of research: (1) biologically active phospholipids and their role as cell mediators in cardiovascular and inflammatory responses, (2) basic membrane studies focusing on enzymes in lipid metabolism and their regulation, and (3) biochemical mechanisms associated with toxicant responses. Emphasis is on biochemical problems associated with cancer, cardiovascular, and pulmonary diseases.

Major efforts in the project on biologically active phospholipids center on a novel group of potent cellular mediators (alkylacetyl phospholipids) that are involved in hypotensive and hypersensitivity responses. These phospholipid mediators, released from cells exposed to foreign proteins, can also trigger anaphylaxis. The term "platelet activating factor" or "PAF," which refers to this general group of bioactive phospholipid mediators, gained popular use before the chemical structure responsible for the biological responses (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) was identified.

We are also investigating a structurally similar group of unnatural non-metabolizable phospholipid analogs because of their highly selective cytotoxic actions toward cancer cells. The aim of this work is to obtain an understanding of the mode of action of these diverse and potent phospholipid mediators, including the antineoplastic analogs.

A study of PAF biosynthesis in alveolar macrophages demonstrated that the cellullar precusor of PAF was an inactive form with a long-chain acyl group at the *sn*-2 position of the glycerol moiety of PAF and that both phospholipase A_2 and acetyltransferase activities were stimulated when phagocytic or ionophoretic agents were used to induce PAF formation. The reaction sequence that comprises the activation portion of the pathway in PAF metabolism is "*sn*-2 acyl"-PAF $\xrightarrow{\text{phospholipase A}_2}$ "lyso"-PAF $\xrightarrow{\text{acetyltransferase}}$ PAF.

The inactivation of PAF involves a highly specific acetylhydrolase coupled with an acyltransferase to produce the inactive cellular form of "acyl"-PAF. These enzymes have been shown to exist in a number of systems and are known to catalyze the following integrated reaction sequence: PAF $\xrightarrow{\text{acetylhydrolase}}$ "lyso"-PAF $\xrightarrow{\text{acyltransferase}}$ "acyl"-PAF. Acetylhydrolase activity is also present in rat plasma, where it appears to be only slightly different from the molecular form that exists intracellularly. The significant higher activities of acetylhydrolase in the plasma of hypertensive rats hint that this enzyme might be involved in the pathogenesis of hypertension. A wide variability in acetylhydrolase activities has been observed in serum from different species.

The possible involvement of an intracellular carrier protein for PAF stems from experiments that demonstrated the transfer of PAF between phospholipid bilayers can be catalyzed by an exchange protein fraction isolated from rat lung. However, a physiological role for specific proteins in mediating the translocation of PAF in cells or between cells requires further research.

Of particular interest have been our results that show PAF is a potent stimulator of calcium influx in rabbit platelets. The conclusion reached from



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Explanatory notes on page 17.

I028558

a series of kinetic experiments with PAF and arachidonic acid and the use of several inhibitors that block the lipoxygenase and/or cyclooxygenase pathway for arachidonic acid metabolism is that the stimulation of calcium uptake by PAF appears to be mediated through a direct effect of PAF on the production of an arachidonic acid metabolite formed via the lipoxygenase pathway.

We recently discovered a new class of antihypertensive neutral lipids, 1-alkyl-2-acetyl-*sn*-glycerols, that appears to be enzymatically converted to PAF by a cholinephosphotransferase after the neutral lipid is administered intravenously to either genetic-hypertensive SHR rats or normotensive animals. The hypotensive effect of 1-alkyl-2-acetyl-*sn*-glycerol is dose dependent in relation to the initial drop in blood pressure; administration of increased amounts of the lipid had little influence on the duration of the response.

Two new HPLC (high-performance liquid chromatography) methods were developed that facilitate the analysis of PAF and related ether lipids. These techniques are extremely important in metabolic studies of PAF since specific molecular species of phospholipids can now be resolved. One of the new HPLC methods that uses dibenzoate derivatives of alkyl- and alk-1-enyl-glycerols permits the quantitative measurement of the composition of the ether-linked moieties according to their chain length and degree of unsaturation.

During 1983, several of our collaborative investigations dealt with physiological responses of PAF in intact animals. Renin and norepinephrine as well as the heart rate were elevated during the antihypertensive response produced by PAF.

An investigation of eleven phospholipid analogs closely related to PAF revealed that the acetamide and methoxy analogs exhibited the most potent and selective cytotoxicity toward human leukemia cells when compared to neutrophils or kidney cells from healthy humans. The D-enantiomer was also effective in producing an anti-tumor cell response. Future experiments will be directed toward seeking a biochemical explanation for the antineoplastic action of these novel PAF analogs. One feature of lipid metabolism in neoplastic cells that could be at least partially related to the selectivity of the cytotoxic action of the PAF analogs is the low tetrahydropteridene-dependent alkyl cleavage enzyme activities in tumor cells. Such a relationship between the cleavage enzyme activity and the cytotoxic response obtained with the unnatural PAF analogs was observed in a study of several lines of rat brain tumor cells.

Emphasis in the membrane project has been the purification and characterization of the properties of several of the key enzyme systems in ether-lipid metabolism. Further detailed experiments were completed on the reaction mechanism of alkyldihydroxyacetone-P synthase, the enzyme that catalyzes the first step in the formation of ether linkages in lipids. These results demonstrated that highly purified preparations of this enzyme are devoid of any acylhydrolase activity. Also the acyl and alkyl exchange reactions were shown to quantitatively match the *pro-R*-^{3}H exchange that occurs when

1028559

alkyldihydroxyacetone-P synthase catalyzes the formation of the ether bond from palmitoyl-[1-*R*-^{3}H]dihydroxyacetone-P and [^{14}C]hexadecanol.

Studies of toxicant responses in pulmonary cells are designed to probe the biochemical mechanisms that explain how energy-related toxicants affect cellular functions, especially those associated with the respiratory tract and lung. The relationship between methanol toxicity (methanol is a major industrial solvent) and formate metabolism was investigated in marmosets. Results with this primate model indicated that the ingestion of methanol resulted in elevated blood levels of formate but produced no signs of a metabolic acidosis. The catabolic pathways for surfactant lipids and the role of lipid mediators in relation to toxic pulmonary responses are expected to be investigated once the division's new inhalation facility for small animals is completed in FY 1984.

An investigation was also completed on the use of liposomes to improve the effectiveness of chelation agents in the removal of heavy metals (actinides) from tissues. In experiments with phorbol esters (TPA), a potent tumor promoter, receptor binding of the TPA was shown to be modified by changes in the polar head group composition of phospholipids; also a serum lipase was found to be able to catalyze the hydrolysis of phorbol esters to an inactive form.

Preclinical and Clinical Nuclear Medicine

The aim of this research is to develop new radiopharmaceutical agents and to carry out phase I and II testing of such materials in clinical studies. The work involves identification of medical needs, designing of appropriate imaging agents, testing of these substances in animals for effectiveness and toxicity, and efficacy trials in humans according to U.S. Food and Drug Administration rules and guidelines. Emphasis is placed on the use of positron-emitting radionuclides to permit tomographic imaging.

Positron decay produces annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions through various angles with a positron-emission computerized tomograph, it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of radiopharmaceuticals labeled with positron emitters. The images obtained are similar to autoradiograms, but unlike autoradiography, the positron tomographic technique is noninvasive. The images obtained by positron tomography reflect differences in tissue function rather than differences in tissue density as detected with X rays. Thus, the images can be used specifically to detect metabolic alterations that are associated with disease processes.

The clinical nuclear medicine program uses tomography to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications in neoplastic, and brain diseases with emphasis on improving quantitative evaluations of the in vivo distribution of such radiopharmaceuticals.

Amino acids with positron-emitting carbon-11 substituted for stable carbon-12 offer distinct advantages for studying protein synthesis and protein turnover in vivo, since ^{11}C-labeled agents are chemically indistinguishable from their unlabeled counterparts in physiological processes. Positron tomo-

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1028560

graphic studies of the pancreas, i.e., visualization of the normal pancreas and pancreatic cancers after injection of ^{11}C-labeled natural and unnatural amino acids, have continued with support from the National Pancreatic Cancer Project of the National Cancer Institute.

Several years ago we developed a general method for the synthesis and purification of the naturally occurring L-isomer and the unnatural D-isomer from racemates of ^{11}C-carboxyl-labeled amino acids. For truly physiologic studies of the pancreas and brain, the ^{11}C-labeled L-amino acid must be separated from the mixture by a process that is compatible with the 20.4-minute half-life of ^{11}C. Optical resolution of DL-[^{11}C]tryptophan and DL-[^{11}C]valine has been accomplished by high-performance liquid chromatography using a conventional reverse-phase column and a chiral mobile phase.

The L-isomers of tryptophan and valine are most suitable for the evaluation of pancreatic function. In addition, the unnatural D-form of these amino acids when compared to results obtained with the L-forms appears to be of value in the differential diagnosis of pancreatic cancer. The Investigational New Drug (IND) applications for L-[^{11}C]tryptophan and L-[^{11}C]valine have been approved by the U.S. Food and Drug Administration for phase I and phase II testing in clinical studies. Preliminary clinical investigations have shown that L-[^{11}C]tryptophan is the most useful pancreatic scanning agent and that L-[^{11}C]valine and L-[^{11}C]tryptophan have a lower affinity for pancreatic tumors than their D-isomeric counterparts.

Two unnatural ^{11}C-labeled alicyclic amino acids, [^{11}C]1-aminocyclobutanecarboxylic acid ([^{11}C]ACBC) and [^{11}C]1-aminocyclopentane carboxylic acid ([^{11}C]ACPC), have excellent tumor localizing properties and thus have potential as unique agents for measuring differences in amino acid transport in tumors due to the metabolic and proliferative properties of tumors.

The L-forms of valine and leucine, both essential amino acids, are also useful in vivo agents for measuring the rate of protein synthesis in the brain. This has been demonstrated in our laboratory as well as at other research institutions; in a study of the effect of chemical structural modifications of amino acids on brain uptake, 1-aminocyclohexanecarboxylic acid (ACHC) was found to have the highest affinity. Pathological conditions in the brain may influence amino acid transport, protein synthesis, or both. Therefore, [^{11}C]ACHC may be useful in conjunction with L-[^{11}C]valine or L-[^{11}C]leucine for differentiating between brain amino acid transport and protein synthesis. ACHC has been tested for toxicity and an IND application has been prepared for submission to the U.S. Food and Drug Administration.

We have previously described a striking structure-activity relationship within the series of alicyclic α-amino acids; compounds with 4- and 5-membered ring systems showed good tumor specificities, whereas those with 3- and 6-membered rings did not. This observation, coupled with the selective uptake of α-aminoisobutyric acid (AIB) by tumor tissue, suggested that analogs of AIB, such as α-aminoisovaleric acid, α-amino-α-ethylbutyric acid, and

1028561

α-amino-α-methylpentanoic acid might exhibit even better tumor specificities. These amino acids were radiolabeled with carbon-14, and their uptakes by tumors were measured. Unfortunately the tumor specificity was maximum for the parent amino acid, AIB. Since the size of the fluoromethyl or trifluoromethyl group is approximately the same as that of a methyl group, an ^{18}F-labeled fluoromethyl or trifluoromethyl analog of AIB (α-fluoromethylalanine or α-trifluoromethylalanine, respectively) would be expected to show a similar affinity for tumor tissue. A tumor-imaging agent labeled with ^{11}F, a positron emitter which has a longer half-life than ^{11}C (110 min vs. 20.4 min), could be produced in a reactor; synthesis of these compounds is in progress.

All of the ^{11}C work depends on the availability of a cyclotron. Difficulties with the unexpected downtime of the cyclotron have in part stimulated efforts to develop radiopharmaceuticals labeled with gallium-68, also a positron emitter, which is available from a generator (germanium-68). The most recently developed compound of this sort is ^{68}Ga-hydrous ferric oxide colloid for the evaluation of metastatic liver disease. An IND has been obtained from FDA for this new liver scanning agent, and limited clinical trials using positron tomography as an imaging technique have demonstrated the advantages of this approach over conventional liver scanning.

A major research effort is directed toward the development of radiopharmaceuticals for positron tomographic studies of pulmonary function. An important metabolic activity of the lung involves the regulation of the levels of various biogenic amines. ^{11}C-Labeled primary aliphatic amines (straight-chain) are sequestered very selectively by lung endothelial cells. However, the rapid metabolic loss of the label from the smaller amines in this series makes it difficult to accurately quantitate their pulmonary uptake. We have found, however, that a long straight-chain amine (octadecylamine) has a high pulmonary uptake with no metabolic loss from the lung during the first 30 minutes after injection. In studies of radiation pneumonitis as a lung disease model, we found that rats receiving a unilateral lung radiation dose of 3000 or 5000 rads showed a significantly lower uptake of ^{14}C-labeled octadecylamine in the exposed lung than in the nonexposed lung. Studies with other lung disease models are planned.

We have recently initiated an investigation of radiolabeled monoclonal antibodies for use in the diagnosis and treatment of cancer. Antibodies to tumor-associated antigens have been considered leading candidates for the long-sought "magic bullet" in targeting cancer cells. However, only since the discovery and refinement of the monoclonal antibody technique (production of antibodies in tissue culture by lymphocyte-myeloma cell hybridomas) have homogeneous and high-purity antibodies been available in sufficient quantity for applications in nuclear medicine.

Monoclonal antibodies labeled with radioiodine have been investigated by others with some success for cancer diagnosis and therapy; however, since loss of the iodine from the protein occurs in vivo, there is a considerable drawback in using iodine as the labeling agent. In collaboration with the Wistar Institute (Philadelphia), we are studying the feasibility of producing

1028562

chelate-conjugated monoclonal antibodies to facilitate their radiolabeling with metal ions. Techniques for covalent attachment of chelating agents, such as diethylenetriaminepentaacetic acid (DTPA), to monoclonal antibodies are being developed, and chromatographic techniques are also being designed for purification of the radiolabeled antibodies. We plan to study monoclonal antibodies labeled with several positron-emitting radionuclides (^{64}Cu, ^{44}Sc, ^{68}Ga, and ^{140}Nd\$^{140}$Pr) for the diagnosis of cancer by positron tomography. Monoclonal antibodies labeled with alpha-emitting ^{255}Fm and beta-emitting ^{90}Y will also be investigated as possible radioimmunotherapeutic agents.

Cytogenetics

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The cytogenetics program conducts both applied and basic research aimed at defining the genetic risk of human exposure to environmental mutagens, including radiation and chemical toxicants. The long-term goals of these research efforts are (1) to define and quantify the immediate and long-term cytogenetic sequelae of human exposure to genotoxic agents, (2) to determine the utility of cytogenetic studies in predicting the risk of late effects in populations exposed to mutagenic or carcinogenic agents, and (3) to determine the degree to which mutagen-induced chromosome lesions in somatic cells can be used to estimate the risk of untoward effects in human reproductive cells. To accomplish these goals, we employ systematic collection and evaluation of cytogenetic data from defined human populations, in vitro experiments using human cells, and evaluations of radiation and chemical toxicity in experimental animals as surrogates for man.

The cytogenetics group responded to requests for biological dose estimation in 15 persons having real or suspected overexposure to ionizing radiation. Seven of these individuals had unexplained "black badges," while eight persons were not wearing dosimeters at the time of the suspected exposure. In all instances, the analysis of radiation-induced chromosome lesions in cultured lymphocytes provided the only means of determining whether a significant whole-body dose was actually received. Since 1976, evaluations have been made in cultured lymphocytes from over 150 persons having suspected external exposures to low linear energy transfer (LET) radiations or internal contamination with alpha particle-emitting radionuclides. Efforts are currently in progress to enter into computer files cytogenetic data and all information regarding each patient's exposure. When completed, files will be accessed on the basis of specific factors to determine the degree of correlation between physical and biological dose estimates, the effects of LET on chromosome lesion induction, and the degree of limitation of cytogenetic dose estimates in instances of known partial-body exposure.

In conjunction with our efforts to more precisely define the immediate cytogenetic consequences of human exposure to radiations of differing LET, we have continued long-term follow-up studies in specific groups of persons exposed many years previously. The populations being investigated include selected patients having partial body radiotherapy and persons having accidental whole body exposures to high or low-LET radiations. In collaboration with investigators at the Environmental Epidemiology Branch of the National

1028563

Cancer Institute (NCI), we are collecting data on the incidence of persistent radiation-induced chromosome lesions in cultured lymphocytes from a total of 300 persons who received radiation therapy for enlarged tonsils or for carcinoma of the cervix 20–50 years ago. This study, which is supported by an NCI-DOE Interagency Agreement, is currently in its second year of funding. To date, in excess of 38,000 lymphocyte metaphases from cultures from 180 persons have been evaluated for chromosome aberrations.

In parallel studies, we are collecting cytogenetic data from six persons having whole-body exposures to mixed fission spectrum neutron/gamma radiation in 1958, from one patient having a whole-body exposure to 144 rad cobalt-60 gamma radiation in 1971, and from a patient heavily contaminated in 1976 with americium-241, an alpha-emitting radionuclide. At the conclusion of these evaluations, data will be analyzed to determine whether the incidence of chromosome lesions observed many years after exposure can be correlated with radiation quality, the total dose the patient received, the region of the body that was irradiated, age at time of exposure, or the number of years since exposure. Such cytogenetic evaluations in selected persons exposed to radiation many years previously will eventually provide information on the utility of somatic cell data for predicting the risk of late effects in exposed populations.

In addition to these studies, we have continued basic research in reproductive toxicology. The research group handling this project will relocate to the Field Site at the beginning of FY 1984 and will join with other cytogenetics projects already there to form an informal reproductive toxicology and mammalian cytogenetics group within the cytogenetics program. One goal of the reproductive toxicology studies is to determine whether chemically induced chromosome lesions in somatic cells can be used to predict the risk of induction of transmissible chromosome lesions in female germ cells. A second aim is to determine the extent to which mammalian species differ in their reproductive and cytogenetic responses to toxicants. This information is expected to provide criteria for the extrapolation of such results obtained with experimental animals to man.

We have continued research aimed at determining whether proliferating oogonia are hypersensitive to cytogenetic insult relative to the mature dictyate oocyte at the graafian follicle stage of development. These studies, supported by the National Institute of Child Health and Human Development, employ the immature ovary of the neonatal rabbit as a model for the human fetal ovary during the first trimester. We determined the $LD_{50/30}$ in newborn rabbits injected with the potent clastogenic agent streptonigrin (SN) and evaluated streptonigrin-induced chromosome lesions in marrow cells from treated newborns. Relative to our earlier findings in adult female rabbits, 30-day survival was severely diminished in the newborn. However, we observed no detectable differences in the sensitivity of adult and newborn marrow cells to SN-induced chromosome aberrations. Studies are currently in progress to determine the sensitivity of mitotic oogonial cells to the clastogenic activity of SN.

1028564



Field Site

Cytogenetics

Studies in the cytogenetics program are also being carried out at the Field Site, located in ORAU's Scarboro Facility, to determine whether induced chromosome aberrations or sister chromatid exchanges can be used to detect and quantify exposure levels in mice treated with selected mutagenic or carcinogenic chemicals. This work is sponsored by the National Toxicology Program and is directed toward the development and quantitation of standardized techniques for the in vivo assessment of potentially genotoxic chemicals using a mouse bone marrow assay. During 1983, several technique variables were quantitatively evaluated and a standardized protocol for the assay was established. The chemical testing currently in progress will not only evaluate the chemicals but will also provide data for comparing the relative sensitivity of chromosome aberrations and sister chromatid exchanges for mutagenicity detection. In other parts of the cytogenetics program, evaluations of in vivo chemical toxicity in somatic cells are in progress in adult and neonatal rabbits and in primates of the genus *Saguinus*. Comparisons of cytogenetic data from these animal surrogates will help define the relevance of data from different animal species in predicting untoward cytogenetic effects in humans.

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In studies related to the reproductive toxicology research, we employed the boar as a large animal surrogate for investigating reproductive and cytogenetic responses of germ cells exposed to chronic radiation throughout the entire gestational period. Reciprocal translocations were evaluated in male porcine spermatogonia at maturity, and reproductive performance and dominant lethal effects were tabulated. Survival and recovery of the male stem-germ cell was compared to the number of reciprocal translocations after several acute radiation exposures; the porcine demonstrated an entirely different response to gamma radiation than that previously observed in the mouse. The differences in the reproductive and cytogenetic responses observed between the boar vs. the mouse dramatize interspecies variability and dictate the need for cytogenetic evaluations in more than one animal model.

Food Chain Transport

The food chain transport program is involved in tracing the transport of radionuclides and toxic organic compounds and their metabolites through the food chain. Emphasis is on actinide metabolism and the products of synfuel generation.

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Coal liquefaction and shale oil production produce hydrocarbon mixtures of great chemical complexity and wastes of uncertain composition. Pilot studies using coal liquefaction and shale oil products indicate that synfuel processes produce synthetic crude preparations much more carcinogenic than crude fractions of petroleum. The levels of the carcinogenic substances that will reach human populations through the food chain as synfuels become a major energy source are unknown.

Much of the body burden of toxic chemicals in food-producing animals will come from ingested feed and water that are contaminated. The primary route of human exposure to the toxic chemicals will be from consumption of

1028565

contaminated meat, milk, eggs, and plants. Research on synfuels in the food chain transport program addresses questions concerning the presence of chemical toxicants in the food chain, to the end that adequate emission standards for coal liquefaction and shale oil plants can be developed to safeguard human foods.

The specific goals of the synfuel studies are (1) to determine the biological retention of representative organic compounds (risk analysis unit, or RAU) following acute and chronic oral administration to dairy cattle, swine, and poultry, and (2) to isolate and quantitate the parent compounds in the various components of animals used as food products, i.e., meat, liver, fat, eggs, and milk.

^{14}C-Labeled naphthalene (mono/diaromatic hydrocarbon RAU), α-naphthol (phenol RAU), and 7-methylbenz(*c*)acridine (alkaline nitrogen heterocyclic RAU) were administered orally to dairy cattle, swine, and laying pullets as a single dose (acute) or daily for 31 days (chronic). All three compounds were found in the various animal products (i.e., milk and eggs) and meat, liver, and fat. The data from these experiments demonstrate the retention of variable but significant amounts of the test compounds and/or their metabolites in tissues and products of animals consumed by humans. Species differences and exposure times (single vs. chronic) to the chemical affected both the distribution and retention of the three organic compounds tested.

Future efforts in the synfuel project will continue to evaluate the uptake, metabolism, and retention of additional RAUs in consumable animal products.

The long-term study of the metabolism of actinide elements continued with the Nevada Applied Ecology Group of the Nevada Operations Office. The actinide studies seek to trace through the food chain radionuclides from weapons testing and radioactive fallout.

Society has become increasingly concerned about protection from radionuclides released into the environment. To develop realistic risk benefit decisions, accurate and precise data are required to assess the impact of radionuclide contaminants on the environment and humans. Unfortunately, the metabolism of actinides in domestic animals has not been adequately evaluated to determine the long-term implications and importance of the food chain in the exposure of humans to radionuclides. Knowledge of the absorption, tissue distribution, and movement of radionuclides in domestic animals allows one to better evaluate the potential radiation hazards of food sources for humans.

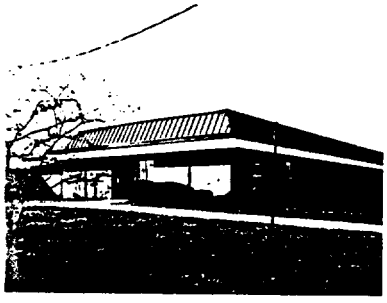
In food chain transport studies last year, ^{241}Am-citrate (5 mCi) was given orally to swine (miniature and domestic) that were killed 1, 2, 4, 8, 16, 24, 48, 72, and 96 hr and 30 days later to determine the tissue distribution of the radioactivity. Differences were observed between miniature and domestic swine, which suggests a relationship between surface area and the extent to which americium-241 is absorbed in the gastrointestinal tract. Tissue distribution data showed the bulk of the radioactivity was in bone (50%), liver (20%), and other soft tissues (30%). Cytogenetic investigations of the exposed

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animals revealed no significant increases in chromosomal anomalies over background at any time after exposure. In gametogenetic studies, alpha tracks were seen most frequently in the medullary portion of the ovary, but they occurred only randomly in the testis. Enhanced necrosis was observed in both the oocytes and gonocytes, but the total number of germ cells were unaffected.

The effects of circadian rhythm and gastrointestinal versus intravenous administrations on the body distribution of the isotope and the influence of chronic and acute exposures are currently being evaluated in ^{241}Am-treated swine.

Little data exist on the metabolism, tissue distribution, localization, and excretion of europium in large animals. Because of the lack of scientific data in large animal models and the interest in this element by the Nevada Test Group, experiments are under way to determine the fate of ingested europium in swine. These data will be utilized to better predict the potential body burden and the radiation hazard that humans could receive from the food chain.



Marmoset Research Center

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Marmoset Research Center

In March 1983, the Medical and Health Sciences Division assumed responsibility for operation of an ongoing National Cancer Institute contract which was awarded to ORAU in July 1982. The contract's objectives are (1) to maintain a breeding colony of *Saguinus oedipus oedipus* and to produce a maximum number of healthy animals at minimum cost, (2) to maintain experimental tamarins injected with oncogenic or potentially oncogenic substances in a biohazard containment area sufficient to protect breeding stock animals, personnel, and technical support from contamination, and (3) to provide professional and technical support for safely and accurately performing experimental protocols approved and requested by the National Cancer Institute.

A new consortium research proposal, "Colon Cancer Expression in Tamarins," is being prepared for submission to NIH. The proposed research projects cover the following topics: sterol metabolism and bile acid profiles, in vitro tetraploidy in skin fibroblasts, colon crypt cell kinetics, coronaviruses as a cause of colitis, antigenicity of colon cancers, genetic segregation and familial relationships, determining fecal mutagens in the three species and in colon cancer animals, induction of sister chromatid exchanges and cancer susceptibility, and species differences in fecal β-glucuronidase activities and their relationship to cancer and colitis.

Considerable progress has been made in the β-glucuronidase project. Using the modified fecal β-glucuronidase assay, we have identified and separated the bacterial-derived enzyme from that formed by the colonic cells. By use of a sonication technique, we have further identified intracellular,

1028567

extracellular, and total amounts of the enzyme activity. Activity levels increase with age and vary among the three tamarin species; however, the highest activities were found in *Saguinus oedipus oedipus*, the tamarin with the greatest incidences of colon cancer. In adult *S. o. oedipus* with histologically confirmed colon cancer, the activity of the intracellular form of β-glucuronidase in colonic cells was 25 times higher than that of age-matched noncancer tamarins. This finding suggests that fecal β-glucuronidase may be a useful marker to indicate the presence of early colon cancer in tamarins and possibly in humans.

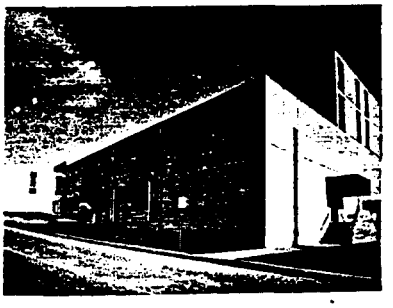
REAC/TS

The staff of the REAC/TS facility, a treatment center and training unit, continued to provide assistance as needed in the management of health problems associated with radiation accidents as needed and to teach medical and paramedical personnel on a regular schedule. REAC/TS assists and renders medical and health physics support by direct participation or consultation on a 24-hour basis and conducts training courses, exercises, and drills for medical, paramedical, and health physics personnel to meet the need for trained professionals in this area of occupational and emergency medicine. In addition, the staff conducts epidemiologic studies on DOE radiation workers and radiation accident survivors and continues to develop and refine cytogenetic techniques for dose estimation of radiation accidents and exposure assessment from chemical toxicants in the industrial or general environment.

REAC/TS staff and resources are available and ready for assisting the U.S. government and private nuclear facilities in the event of radiological emergencies. DOE-REAC/TS now has letter agreements with 52 nuclear facilities. Over 150 former REAC/TS trainees are now employed at these sites, and continued testing of the emergency response system is done through REAC/TS involvement in drills and exercises at these sites. REAC/TS participation in 12 offsite drills involved calls from the Tennessee Valley Authority and various commercial nuclear facilities, utilizing REAC/TS backup as previously arranged by letter agreement through DOE Oak Ridge Operations. REAC/TS staff members also participated through onsite and offsite medical consultation in the NUWAX-83 nuclear weapon accident exercise. REAC/TS onsite drills were conducted on two occasions and were expanded to joint exercises with the Oak Ridge Hospital of the United Methodist Church.

During the past year, REAC/TS has been contacted for assistance 37 times. In a few cases calls for assistance involved serious localized overexposure, minor contamination, or internally deposited radionuclides, and in some cases the REAC/TS staff confirmed that no radiation accident had occurred.

Long-term follow-up of victims involved in earlier radiation accidents continued through the REAC/TS Radiation Accident Registry. The central



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1028568

registry now contains data on 5654 individuals from 556 different sites. The registry consists of the DTPA Registry, the DOE $\geq$ 5 rem Registry, U.S. Radiation Accident Registry, the Foreign Registry, and the Zero Dose Nonaccident Registry. Field testing of the $\geq$ 5 rem medical follow-up questionnaire has been completed, and a program is being initiated to systematically follow up persons included in the U.S. Accident, DOE $\geq$ 5 rem, and DTPA Registries. The DOE/ORAU Food and Drug Administration–Investigational New Drug study for DTPA continued with coinvestigators reporting no unusual pattern of late effects.

The REAC/TS courses and training programs continued to be in great demand during the past year. Participants included 74 physicians, 29 health physicists, and 93 paramedical professionals. Less formalized training and information was provided through talks, a special exhibit shown at various professional meetings, and through publication of a quarterly newsletter (3000 circulation).

Videotape training materials have been developed under a special contract with the Federal Emergency Management Agency. These programs entitled *Pre-Hospital Response to Radiation Accidents* and *Hospital Emergency Department Response to Radiation Accidents* will be utilized by FEMA in a national training program and incorporated into REAC/TS training courses as well.



Center for Epidemiologic Research

Epidemiology

The main objective of the epidemiology program is to examine relationships between occupational exposure to ionizing radiation and subsequent health and mortality of radiation workers. The health effects of chemical toxicants, especially uranium and other metals, also are being investigated both as primary and confounding (with respect to radiation) occupational stresses. Biostatisticians and computer scientists are implementing improved methods for merging and managing large data files for subsequent epidemiological analyses. Editing and correction procedures have been developed to ensure the high quality of the data bases that extend back to the early 1940s.

The epidemiology group continues to evaluate the mortality experience of workers within the Oak Ridge Operations Office area with respect to radiation effects. This area includes workers at DOE facilities operated by Union Carbide Corporation, Nuclear Division, in Oak Ridge, Tennessee, and Paducah, Kentucky, and workers at NLO in Ohio and at the Savannah River Plant operated by E. I. du Pont de Nemours & Company in Aiken, South Carolina.

A preliminary mortality study has been completed for workers at Oak Ridge National Laboratory; final analyses of the data are in progress. Previously reported studies of mortality among workers exposed to metallic nickel while employed between 1948 and 1953 at the Oak Ridge Gaseous Diffusion Plant and workers exposed to phosgene while employed between 1943 and

1028569

1945 at Tennessee Eastman Corporation's Oak Ridge facility (TEC) have been updated. A case control study has been completed of 330 lung cancer deaths occurring through 1974 among white males employed between 1943 and 1947 at TEC; this study is being updated to include approximately 260 more recent deaths. A study is in progress of mortality among workers exposed to elemental mercury between 1953 and 1972 at the Oak Ridge Y-12 plant. Also in progress are case control studies of brain cancer and hematologic malignancies among workers employed at any of DOE's Oak Ridge facilities.

Studies of the health effects of occupational exposure to uranium and uranium compounds are in progress at several sites including the "Niagara Frontier" sites of the Manhattan Engineer District (MED). A study of mortality among former workers at MED sites at Linde Coporation and the Electro Metallurgical Laboratory is being conducted in collaboration with Union Carbide Corporation's corporate epidemiology group. Also in progress is a similar study of former MED site workers at Mallinckrodt Chemical Works, St. Louis. A study of nonmalignant respiratory disease among workers employed between 1952 and 1977 at NLO has been completed; a study of mortality among this cohort is in progress.

Other factors, such as socioeconomic status, that may confound evaluation of mortality experience of the Oak Ridge worker populations are being evaluated and compared with the attributes of persons living in Oak Ridge and the surrounding counties but who have never worked at the Oak Ridge facilities.

Preliminary analyses are in progress of the mortality experience among the cohort of 3,100 workers identified as having equaled or exceeded the current annual occupational limit of 5 rems (set by the Nuclear Regulatory Commission and DOE) since 1947 while employed at DOE (and predecessor organization) facilities nationwide. A follow-up study of morbidity in this population is being initiated on a by-site basis.

To facilitate these studies and those segments of the DOE Health and Mortality Study being conducted by epidemiology groups at Los Alamos National Laboratory (Plutonium Workers Study) and the Hanford Environmental Health Foundation with the Pacific Northwest Laboratories (Hanford Workers Study), we operate the DOE Death Certificate Retrieval Office which obtains information on the vital status and the causes of any deaths among former DOE employees. These data are being accumulated through the cooperation of the Social Security Administration (SSA) and the vital statistics departments of all 50 states. We also have developed several alternate data sources for following up persons whose vital status cannot be determined by the SSA. These sources include states' death indexes, the National Death Index, states' Motor Vehicle License departments, the Civil Service Commission, and the Veterans Administration.

In these studies we have the collaboration of the Epidemiology Departments of the Schools of Public Health of the University of North Carolina, Chapel Hill, and of the University of Alabama in Birmingham.

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1028570

Publications

Full-length journal articles

Albert, D. H., and Snyder, F.
Biosynthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine in rat alveolar macrophages.
Phospholipase A_2 and acetyltransferase activities during phagocytosis and ionophore stimulation.
J. Biol. Chem. 258:97–102; 1983.

Barnes, D. S.; Clapp, N. K.; Scott, D. A.; Oberst, D. L.; and Berry, S. G.
Effects of wheat, rice, corn, and soybean bran on 1,2-dimethylhydrazine-induced large bowel tumorigenesis in F344 rats.
Nutr. Cancer 5:1–9; 1983.

del, W. E.; Greiner, E.; Fink, U.; Sta··rou, D.; Reichert, ·· Rastetter, J.; Hoffman, D. R.; and Snyder, F.
toxicity of alkyl-lysophospholipid derivatives and low alkyl ···vage enzyme activities in rat brain tumor cells.
Cancer Res. 43:541–545; 1983.

Berger, M. E., and Hübner, K. F.
Excessive exposure to radiation in the health environment: Diagnostic testing.
Am. J. Nurs. 83:1155–1159; 1983.

Blank, M. L.; Cress, E. A.; Lee, T-c.; Malone, B.; Surles, J. R.; Piantadosi, C.; and Snyder, F.
Structural features of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) required for hypotensive and platelet serotonin responses.
Res. Commun. Chem. Pathol. Pharmacol. 38:3–20; 1982.

Blank, M. L.; Cress, E. A.; Lee, T-c.; Stephens, N.; Piantadosi, C.; and Snyder, F.
Quantitative analysis of ether-linked lipids as alkyl- and alk-1-enyl-glycerol benzoates by high-performance liquid chromatography.
Anal. Biochem. In press.

Blank, M. L.; Cress, E. A.; and Snyder, F.
Ethanol toxicity and elevated formate levels in the marmoset.
Res. Commun. Subst. Abuse. In press.

Blank, M. L.; Hall, M. N.; Cress, E. A.; and Snyder, F.
Inactivation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by a plasma acetylhydrolase: Higher activities in hypertensive rats.
Biochem. Biophys. Res. Commun. 113:666–671; 1983.

Blank, M. L., and Snyder, F.
High-performance liquid chromatographic separation of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) and other phospholipids.
J. Chromatogr. 273: 415–420, 1983.

Borman, L. S.; Swartzendruber, D. C.; and Littlefield, L. G.
Establishment of two parental cell lines and three clonal cell strains from rat colonic carcinoma.
Cancer Res. 42:5074–5083; 1982.

Brown, A. J., and Snyder, F.
The mechanism of alkyldihydroxyacetone-P synthase.
Formation of $[^3H]H_2O$ from acyl[1-*R*-^{3}H]dihydroxyacetone-P by purified alkyldihydroxyacetone-P synthase in the absence of acylhydrolase activity.
J. Biol. Chem. 258:4184–4189; 1983.

Cabot, M. C.
Effect of cellular phospholipid modification on phorbol diester binding.
Cancer Res. 43:4233–4238; 1983.

Cabot, M. C.; Blank, M. L.; Welsh, C. J.; Horan, M. J.; Cress, E. A.; and Snyder, F.
Metabolism of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by cell cultures.
Life Sci 31:2891–2898; 1982.

Cabot, M. C.; Welsh, C. J.; and Snyder, F.
Increasing the levels of ether-linked lipids in L-M cells by glyceryl ether supplementation depresses growth and choline utilization.
Biochim. Biophys. Acta 713:16–22; 1982.

Clapp, N. K.; Henke, M. A.; Holloway, E. C.; and Tankersley, W. G.
Spontaneous colon carcinoma in the cotton-top tamarin, *Saguinus oedipus oedipus*.
J. Am. Vet. Med. Assoc. In press.

Coffey, J. L.; Hayes, R. L.; Rafter, J. J.; Watson, E. E.; and Carlton, J. E.
Radiation dosimetry and chemical toxicity considerations for ^{99}Tc.
Health Phys. In press.

1028571

DuFrain, R. J.
Sister chromatid exchange distributions in rabbit lymphocytes treated with streptonigrin.
Environ. Mutagen. In press.

Erickson, B. H., and Hall, G. G.
Stem-spermatogonal renewal and mitotic activity following γ-irradiation in mouse and rat.
Mutat. Res. 108:317–335; 1983.

Frome, E. L.
The analysis of rates using Poisson regression models.
Biometrics. In press.

Hayes, R. L.
The interaction of gallium with biological systems.
Int. J. Nucl. Med. Biol. In press.

Hoffman, D. R.; Hajdu, J.; and Snyder, F.
Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts.
Blood. In press.

Hubbard, J. W.; Cox, R. H.; Lawler, J. E.; Blank, M. L.; and Snyder, F.
Antihypertensive effects of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine on plasma renin activity and catecholamine responses in spontaneously hypertensive rats.
Life Sci. 32:221–232; 1983.

Lackey, R. J., and Cabot, M. C.
Acetate utilization and fatty acid metabolism in phorbol ester and dimethyl sulfoxide-differentiated human leukemia cells.
Cancer Lett. In press.

Lackey, R. J., and Cabot, M. C.
Serum lipase active in the hydrolysis of the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate.
Cancer Lett. 19:165–172; 1983.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Formation of alkylacyl- and diacylglycerophosphocholines via diradylglycerol cholinephosphotransferase in rat liver.
Biochim. Biophys. Acta 713:479–483; 1982.

Lee, T-c.; Malone, B.; and Snyder, F.
Stimulation of calcium uptake by 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) in rabbit platelets: Possible involvement of the lipoxygenase pathway.
Arch. Biochem. Biophys. 223:33–39; 1983.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
SCE evaluations in human lymphocytes after G_0 exposure to mitomycin C: Lack of expression of MMC-induced SCEs in cells that have undergone greater than two in vitro divisions.
Mutat. Res. 107:119–130; 1983.

Lushbaugh, C. C.; Humason, G. L.; and Clapp, N. K.
Colonic adenocarcinoma.
Comp. Pathol. Bull. In press.

Lumb, R. H.; Pool, G. L.; Bubacz, D. G.; Blank, M. L.; and Snyder, F.
Spontaneous and protein-catalyzed transfer of 1-alkyl-2 1-*sn*-glycero-3-phosphocholine (platelet-activating factor) b en phospholipid bilayers.
Biochim. Biophys. Acta 750:217–222; 1983.

Matthews, J. A.; Vann Bonds, S.; Cianci, M. L.; Cooper, J. F.; Hanes, K.; Hodges, H. D.; Kraft, R. A.; Langan, J. K.; Porter, J. L.; Price, D. D.; and Ward, D.
Performance and responsibility standards for the nuclear medicine technologist.
J. Nucl. Med. Technol. 10:163–169; 1982.

McFee, A. F., and Long, S. E.
Sister chromatid exchanges in the active and inactive X chromosomes.
Chromosoma (Berl.) 87:359–362; 1982.

McFee, A. F.; Lowe, K. W.; and San Sebastian, J. R.
Improved sister chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice.
Mutat. Res. 119:83–88; 1983.

McFee, A. F., and Sherrill, M. N.
Sister chromatid exchanges induced in swine lymphocytes by chronic oral doses of dimethylbenzanthracene.
Mutat. Res. 116:349–359; 1983.

Morrison, W. D.; Huff, V.; Littlefield, L. G.; and Du a, R. J.
Chromosome preparations from rabbit preovulatory oocytes.
Mutat. Res. 119:169–175; 1983.

Polednak, A. P., and Keane, A. T.
Retention of uranium in the chest: Implications of findings in vivo and postmortem.
Health Phys. In press.

Polednak, A. P.; Keane, A. T.; and Beck, W. L.
Estimation of radiation doses to the lungs of early uranium-processing plant workers
Environ. Res. 28:313–328; 1982.

Stathis, V. J.; Miller, C. M.; Doerr, G. F.; Coffey, J. L.; and Hladik, W. B. III.
Effect of technetium Tc 99m pertechnetate on bacterial survival in solution.
Am. J. Hosp. Pharm. 40:634–637; 1983.

Tardif, S. D.
The relationship between social interactions and sexual maturation in female *Saguinus oedipus oedipus.*
Folia Primatol. In press.

Tardif, S. D.
Social influences on sexual maturation of female *Saguinus oedipus oedipus.*
Am. J. Primatol. In press.

1028572

Urso, P., and Gengozian, N.
Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[α]pyrene and X-rays before or after birth.
J. Toxicol. Environ. Health 10:817–835; 1982.

...rth, N.; Swanson, D.; Shapiro, B.; Nakajo, M.; Coffey, J. L.; Eckhauser, F.; and Owyang, C.
A conveniently prepared ^{99m}Tc resin for semi-solid gastric emptying studies.
J. Nucl. Med. In press.

Chapters, review articles, and other publications

Arneson, R. M.; Wander, J. D.; Cabot, M. C.; Tan, E.-L.; Shenley, R. L.; and Hsie, A. W.
Resistance to lipid peroxidation by cultured neoplastic cells.
In *Protective Agents in Cancer*, edited by D. H. C. McBrien and T. F. Slater, N.F.C.R. Cancer Association Symposia No. 2, Uxbridge, England, 1982, pp. 101–124. New York: Academic Press, 1983.

Beck, W. L.; Cookfair, D. L.; Sowder, C. L.; and Gissel, K.
Radiation dosimetry for an epidemiologic lung cancer study.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 436–443, Albuquerque, New Mexico, 1983.

Brown, A. J.
Mechanism of ether bond formation by alkyldihydroxy...one-P synthase.
... D. Dissertation, The University of Tennessee, Knoxville, 1982.

Checkoway, H.; Mathew, R. M.; Wolf, S. H.; Shy, C. M.; Muller, S. M.; Beck, J.; Watson, J. E., Jr.; Wray, M. L.; and Fry, S. A.
Mortality among workers at the Oak Ridge National Laboratory.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 90–104, Albuquerque, New Mexico, 1983.

Clapp, N. K.; Littlefield, L. G.; and Lushbaugh, C. C. [Letter]
Colon carcinoma in subhuman primates.
Gastroenterology 83:519; 1982.

Cookfair, D. L.
A case control study of lung cancer among workers at a uranium processing plant.
Ph.D. Dissertation, University of North Carolina, Chapel Hill, 1982.

...fair, D.; Beck, W. L.; Shy, C. M.; Lushbaugh, C. C.; ... Sowder, C. L.
Lung cancer among workers at a uranium processing plant.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 398–406, Albuquerque, New Mexico, 1983.

Cragle, D. L.; Hollis, D. R.; Shy, C. M.; and Newport, T. H.
A retrospective cohort mortality study among workers occupationally exposed to metallic nickel powder at the Oak Ridge Gaseous Diffusion Plant.
In *Symposium on Nickel in the Human Environment*, International Agency for Research on Cancer, Lyon, France, 1983. In press.

DuFrain, R. J.
Probing sister chromatid exchange mechanisms with halogenated pyrimidines.
In Proceedings of the International Symposium on Sister Chromatid Exchange, Brookhaven, New York, 1983. In press.

Erickson, B. H.
Effects of ionizing radiation on mammalian spermatogenesis: A model for chemical effects.
In *Target Organ Toxicity Gonads*, edited by R. L. Dixon. New York: Raven Press. In press.

Fry, R. J. M., and Fry, S. A.
Effects on humans of exposure to radioactive materials: Late somatic effects.
In *Encyclopedia of Materials Science and Engineering*. New York: Pergamon Press. In press.

Gissel, K. C.; Hansard, M. S.; and Wray, M. L.
HMS error detection, reporting, and resolution system.
In *Improvement of the Quality of Data Collected by Data Collection Systems*, proceedings of a conference, Oak Ridge, Tennessee, 1982. New York: Academic Press. In press.

Hammond, B. F., and Martin, P. G.
Calcium-cadmium effects on bone density and strength of the femur from multiparous rats.
In Proceedings of the Symposium on Health Effects and Interactions of Essential and Toxic Elements, Lund, Sweden, 1983. In press.

Hayes, R. L. Detection of malignancies with radionuclides.
In *Recent Advances in Nuclear Medicine*, Vol. 6. New York: Grune & Stratton. In press.

Hayes, R. L., and Hübner, K. F.
Basis for the clinical use of gallium and indium radionuclides.
In *Metal Ions in Biological Systems*, Vol. 16, *Methods Involving Metal Ions and Complexes in Clinical Chemistry*, edited by H. Segel, pp. 279–315. New York: Marcel Dekker, Inc., 1983.

1028573

Hayes, R. L.; Rafter, J. J.; Byrd, B. L.; and Carlton, J. E.
Studies of the in vivo entry of ^{67}Ga into normal and malignant tissue.
Original publication: *J. Nucl. Med.* 22:325–332; 1981. *Year Book Cancer*, 1982, 221–222.

Hodges, H. D. [Book Review]
Medical Radiation Biology, Second Edition, edited by D. J. Pizzarello, and R. L. Witcoski, 164 pp. Philadelphia: Lea & Febiger, 1982.
J. Nucl. Med. Technol. In press.

Hodges, H. D. [Commentary]
New specialty for nuclear medicine technologists.
J. Nucl. Med. Technol. In press.

Hübner, K. F.
Decontamination procedures and risks to health care personnel.
Bull. N.Y. Acad. Med. In press.

Hübner, K. F., and Buonocore, E.
Alimentary tract radiology positron emission tomography (PET).
In *Alimentary Tract Radiology*, 3rd Edition, edited by S. E. Harshberger, pp. 2486–2494. St. Louis, Missouri: C. V. Mosby Co., 1982.

Hübner, K. F., and Lushbaugh, C.
Radiation injury.
In *Care of the Injured—A Practical Guide*, edited by E. E. Moore, C. W. Van Way, III, and B. Eiseman. St. Louis, Missouri: C. V. Mosby Co. In press.

Hudson, D. H.; Beck, W. L.; Fry, S. A.; Lushbaugh, C. C.; and Watson, J.
Considerations in using radiation dosimetry data in epidemiologic studies.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 199–207, Albuquerque, New Mexico, 1983.

Janardan, K. G.; Schaeffer, D. J.; and DuFrain, R. J.
A stochastic model for the study of the distribution of chromosome aberrations in human and animal cells exposed to radiation or chemicals.
In *Proceedings of the International Summer School on Statistical Distributions in Scientific Work*, edited by G. Tallie, G. P. Patil, and B. Baldessari, Trieste, Italy, 1980. Boston: D. Reidel Publishing Co. In press.

Littlefield, L. G.
The analysis of radiation-induced chromosome lesions in lymphocytes as a biological method for dose estimation.
In *Assessment of Ionizing Radiation Injury in Nuclear Warfare*, Proceedings of the NATO RSG 5, Panel VII Meeting, Bethesda, Maryland, 1982, Armed Forces Radiobiology Research Institute Publications. In press.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. M.; and DuFrain, R. J.
Persistence of SCE-inducing lesions after G_0 exposure of human lymphocytes to differing classes of DNA-damaging chemicals.
In Proceedings of the International Symposium on Sister Chromatid Exchange. Brookhaven, New York, 1983. In press.

Littlefield, G.; Joiner, E.; DuFrain, R. J.; Colyer, S.; and Breitenstein, B.
Six year cytogenetic follow-up study of an individual heavily contaminated with americium-241.
In *Dosimetry, Radionuclides and Technology*, edited by J. J. Broerse, G. W. Barendsen, H. B. Kal, and A. J. van der Kogel. Proceedings of the 7th International Congress of Radiation Research, Amsterdam, 1983. The Netherlands: Martinus Nijhoff, Rijswijk. In press.

Lushbaugh, C. C.
Accidental radiation exposure [A. Questions and Answers].
J. Am. Med. Assoc. 9:1207; 1983.

Lushbaugh, C. C.
Panel discussion: Section IV care of the radiation-exposed and injured.
Bull. N.Y. Acad. Med. In press.

Lushbaugh, C. C.
Chairman's concluding comments to Panel discussion: Section IV care of the radiation-exposed and injured.
Bull. N.Y. Acad. Med. In press.

Lushbaugh, C. C. [Book Review]
Fajardo, L. F. *Pathology of Radiation Injury*. New York: Masson Publishing, Inc., 1982, 285 pp.
J. Nucl. Med. 24:276; 1983.

Lushbaugh, C. C.; Brown, D. G.; and Frome, E. L.
Longevity of irradiated burros.
In Proceedings of the Twenty-Second Hanford Life Sciences Symposium, 1983, Richland, Washington. In press.

Lushbaugh, C. C.; Fry, S. A.; Shy, C. M.; and Frome, E. L.
DOE health and mortality study at Oak Ridge.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 105–114, Albuquerque, New Mexico, 1983.

1028574

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
Radiation, biological effects.
In *Funk & Wagnalls New Encyclopedia*, Revised Edition, edited by R. S. Phillips, pp 69–70. New York: Funk & Wag- , 1983.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
Effects of high doses of radiation.
In Proceedings of the Eighth Life Sciences Symposium, Radiation and Its Effects, Los Alamos, New Mexico, 1980. In press.

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Beck, W. L.
Effects on humans of exposure to radioactive materials: Acute effects.
In *Encyclopedia of Materials Science and Engineering*. New York: Pergamon Press. In press.

Mani, K.
Assessment of Oak Ridge National Laboratory internal dosimetry data for DOE health and mortality study.
M.S. Dissertation, University of North Carolina, Chapel Hill, 1983.

Partain, C. L.; Heller, R. M.; Hübner, K. F.; Price, R. R.; Patton, J. A.; Erickson, J. H.; Pickens, D. R.; Hayes, R. L.; and James, A. E., Jr.
`nctional imaging of the brain using positron emission tomog- ıy.
In Proceedings of the Copenhagen Symposium on Diagnostic Imaging, Glostrup University Hospital, Glostrup, Denmark, 1982. In press.

Ricks, R. C.
Response to a spill of radioactive material.
In *Encyclopedia of Materials Science and Engineering*. New York: Pergamon Press. In press.

Ricks, R. C.
Transport of irradiated or radioactively contaminated patients to the hospital.
Bull. N. Y. Acad. Med. In press.

Robie, D. M., and Fry, S. A.
Computerized micrographics in processing hard-copy records for an epidemiologic study.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 240–243, Albuquerque, New Mexico, 1983.

`ıyder, F.
:elet activating factor (PAF), a novel type of phospholipid ...ıh diverse biological properties.
Annu. Rep. Med Chem. 17:243–252; 1982.

Snyder, F.
Chemical and biochemical aspects of "platelet activating factor," a novel class of acetylated ether-linked choline-phospholipids.
Med. Res. Rev. In press.

Snyder, F.; Lee, T-c.; Blank, M. L.; Cabot, M. C.; Malone, B.; and Albert, D. H.
Enzymatic pathways for platelet activating factor.
In Proceedings of the 1st International Symposium on Platelet-Activating Factor, Paris, 1983, Elsevier Biomedical Press, Amsterdam. INSERM (Inst. Nat. Sante Rech. Med.) Symp. In press.

Snyder, F.; Lee, T-c.; and Wykle, R. L.
Ether-linked glycerolipids and their bioactive species: Enzymes and metabolic regulation.
In *The Enzymes of Biological Membranes*, Second Edition, edited by A. Martonosi. New York: Plenum Publishing Co. In press.

Strom, D. J.; Beck, W. L.; Stansbury, P. S.; Tankersley, W. G.; and Watson, J. E., Jr.
Standard procedures for pooling health physics data for epidemiologic studies.
In *Health Physics Society: Epidemiology Applied to Health Physics*, Proceedings of the Sixteenth Midyear Topical Symposium, U. S. Department of Energy Report CONF-830101, pp. 221–230, Albuquerque, New Mexico, 1983.

Wills, J. H., and Hübner, K. F.
Pharmacologic bases for treatment of patients exposed to antipersonnel chemicals and to ionizing radiation.
In *Pharmacologic Approach to the Critically Ill Patient*, edited by C. R. Lake, and B. Chernow, pp. 707–714. Baltimore: Williams and Wilkins, 1983.

Wilson, J.
An epidemiologic investigation of nonmalignant respiratory disease among workers at a uranium mill.
Ph.D. Dissertation, University of North Carolina, Chapel Hill, 1983.

Worrel, M., and Hudson, D. H.
An automated data base to SAS/graph system.
In *Proceedings of the Eighth Annual Statistical Analysis: System Users Group, International Conference*, New Orleans, 1983, pp. 221–226. Cary, North Carolina: SAS Institute, 1983.

Published abstracts of papers presented at meetings

Albert, D. H., and Snyder, F.
Ionophore-mediated release of arachidonic acid (20:4) and platelet activating factor (PAF) from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC) in rat alveolar macrophages (AM).
Fed. Proc. 42:710; 1983.

1028575

Boyd, L. M.; Ezra, D.; Snyder, F.; Feuerstein, G.; and Goldstein, R. E.
Coronary effects of platelet-activating factor.
Circulation. In press.

Brown, A. J., and Snyder, F.
Reactions catalyzed by purified alkyldihydroxyacetone-P synthase.
Fed. Proc. 42:1868; 1983.

Cabot, M. C.
Leukemia cell phospholipid modification influences phorbol diester binding.
Fed. Proc. 42:1988; 1983.

Clapp, N. K.; Lushbaugh, C. C.; Humason, G. L.; Gangaware, B. L.; and Henke, M. A.
A study of early changes in colonic environment associated with spontaneous colon adenocarcinoma in the cotton-top tamarin, *Saguinus oedipus oedipus*.
Proc. Am. Assoc. Cancer Res. 24:114; 1983.

Coffey, J. L.; Holloway, E. C.; and Hübner, K. F.
Advantages and disadvantages of three different ring source designs for use in positron tomography.
J. Nucl. Med. 24:P102; 1983.

DuFrain, R. J.
Halogenated pyrimidines and conformation hypothesis for sister chromatid exchange.
Environ. Mutagen. 4:360; 1982.

DuFrain, R. J.; Batson, J. S.; Joiner, E. E.; Colyer, S. P.; Littlefield, L. G.; and Hübner, K. F.
Cytogenetic assessment of radiation sensitivity in lymphocytes from individuals with Huntington's chorea.
Radiat. Res. 91:302–303; 1982.

DuFrain, R. J.; Littlefield, L. G.; Batson, J. S.; Jennings, C. J.; Linkous, S.; and Morrison, W. D.
Cytogenetic studies on somatic and female germ cells of adult and neonatal rabbits (*Oryctolagus cuniculus* L.).
Mamm. Chromosome Newslett. In press.

DuFrain, R. J.; Littlefield, L. G.; Joiner, E. E.; and Colyer, S. P.
No increased chromosomal radiosensitivity in Down's syndrome lymphocytes exposed to fission spectrum neutrons.
Radiat. Res. 94:571–572; 1983.

DuFrain, R. J.; McFee, A. F.; Littlefield, L. G.; Jennings, C. J.; Linkous, S.; and Lowe, K. W.
In vivo chromosome aberration and SCE analysis in somatic and female germ cells of neonatal rabbits.
Environ. Mutagen. 5:452; 1983.

French, J. A.; Snowdon, C. T.; Abbott, D. H.; Kleiman, D.; Epple, G.; Katz, Y.; Tardif, S.; and Hearn, J. P.
Reproduction and behavior in marmosets and tamarins.
Int. J. Primatol. 3:242; 1982.

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action.
Biometrics. In press.

Hammond, B. F., and Martin, P. G.
Dietary calcium-cadmium effects on bone strength, density, and minerals in the femur of adolescent pregnant rats.
In Proceedings of the Western Hemisphere Nutrition Congress VII, Miami Beach, Florida, 1983. In press.

Hollis, D. R.; Beck, W. L.; Fry, S. A.; Lushbaugh, C. C.; and Watson, J. E.
Considerations in using exposure data in epidemiologic studies.
Am. J. Epidemiol. 118:447; 1983.

Hubbard, J. W.; Cox, R. H.; Lawler, J. E.; Blank, M. L.; and Snyder, F.
Hemodynamic responses during intravenous infusion of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious normotensive and spontaneously hypertensive rats.
Fed. Proc. 42:872; 1983.

Hübner, K. F.; Washburn, L. C.; and Hayes, R. L.
The potential of positron emission tomography (PET) in the diagnosis of pancreatic disease.
In *Proceedings, American Pancreatic Association, Inc, and National Pancreatic Cancer Project Joint Meeting*, p. 25, Chicago, Illinois, 1982.

Ishioka, K.; Kuniyasu, Y.; Higashi, S.; Kakehi, H.; Hayes, R. L.; and Carlton, J. E.
Preparation for liver scanning agent labeled with positron emitters, generator produced gallium-68.
In *Proceedings, Third World Congress of Nuclear Medicine and Biology*, Paris, France, 1982, Vol. 1, edited by C. Raynaud, pp. 662–665. Paris: Pergamon Press, 1982.

Lee, T-c.; Malone, B.; and Snyder, F.
Stimulation of calcium uptake by 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor, PAF) in rabbit platelets: Possible involvement of the lipoxygenase pathway.
Fed. Proc. 42:1793; 1983.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. S.; and DuFrain, R. J.
Comparisons of baseline and mutagen-induced SCEs in "early dividing" and "late dividing" lymphocyte populations.
Mamm. Chromosome Newslett. In press.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. S.; and DuFrain, R. J.
Persistence of SCE-inducing lesions after G_0 exposure of human lymphocytes to chemicals having differing modes of action on DNA.
Environ. Mutagen. 5:409; 1983.

1028576

Littlefield, L. G.; Joiner, E. E.; Colyer, S. P.; DuFrain, R. J.; and Washburn, L. C.
Evaluation of cellular proliferation kinetics and chromosome breakages in human lymphocytes exposed to the chelating agents calcium or zinc DTPA.
Mutat. Res. 94:571; 1983.

McFee, A. F.; Lowe, K. W.; and San Sebastian, J. R.
Delivery of BrdUrd for mouse bone marrow assay is improved by partial coating of tablets.
Environ. Mutagen. 5:454–455; 1983.

McFee, A. F., and Sherrill, M. N.
Sister chromatid exchanges induced in lymphocytes of sheep and swine by chronic feeding of a mutagen.

[redacted]

Primate "wasting" syndrome—a behavioral approach.
Am. J. Primatol. 4:331; 1983.

Som, P.; Oster, Z. H.; Srivastava, S. C.; Yamamoto, K.; Meinken, G. E.; Yonekura, Y.; Sacker, D. F.; Richards, P.; Brill, A. B.; Coffey, J.; Carlton, E.; and Hübner, K. F.
The effect of carrier amphetamine and KI on the biodistribution of iodoamphetamine.
J. Nucl. Med. 24:P118; 1983.

Tice, R. R.; Luke, C.; Miller, V.; McFee, A. F.; and San Sebastian, J. R.
The in vivo mouse bone marrow cytogenetic bioassay: 1. Evaluation of acute exposure protocols and sister chromatid exchange (SCE) response.
Environ. Mutagen. 5:378; 1983.

Washburn, L. C.; Hübner, K. F.; Sun, T. T.; Byrd, B. L.; Coffey, J. L.; and Callahan, A. P.
C-11-L-Tryptophan, a potential agent for diagnosis of pancreatic disease using PET.
J. Nucl. Med. 24:P121; 1983.

Washburn, L. C.; Wallace, R. T.; Byrd, B. L.; and Sun, T. T.
Factors affecting the lung uptake and retention of primary aliphatic amines.
Clin. Nucl. Med. In press.

Summaries of Published Papers

Albert, D. H., and Snyder, F.
Biosynthesis of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine by rat alveolar macrophages.
Phospholipase A_2 and acetyltransferase activities during phagocytosis and ionophore stimulation.
J. Biol. Chem. 258:97–102; 1983.

1-Alkyl-2-acyl-*sn*-glycero-3-phosphocholine (alkylacyl-GPC) comprises 11% of the total phospholipids of rat alveolar macrophages. This endogenous pool of alkylacyl-GPC was prelabeled by incubating the macrophages with [1,2-^{3}H]-alkyllyso-GPC (54 Ci/mmol), which enters the cells and is acylated. The effect of various stimuli on the synthesis and release into the media of labeled alkylacetyl-GPC (platelet-activating factor) from the cells was used to establish the role of inactive alkylacyl-GPC as a precursor of the biologically active derivative. A phagocytic agent (zymosan, 100 μg/ml) and an ionophore (A23187, 2 μM) stimulated the release of both alkylacetyl-GPC and alkyllyso-GPC into the media at the expense of cellular alkylacyl-GPC. Phospholipase A_2 activity (at pH 4.5 and in 1 mM EDTA) was also increased in the media. The stimulatory effect of zymosan and the ionophore on alkylacetyl-GPC release was prevented by mepacrine (0.1 mM), an agent that inhibits the release of fatty acids from phospholipids. These data indicate that phospholipase activity is required for the biosynthesis of alkylacetyl-GPC. However, since the inhibitory effect of mepacrine was not apparent when acetate was present, it appears that the acetylation step is rate limiting. Exposure of alveolar macrophages in culture to zymosan or A23187 stimulated acetyltransferase activity 250–300%. In contrast, phorbol myristate acetate (1.6 μM), which stimulated the accumulation of lysophospholipids but not the level of alkylacetyl-GPC in the media, did not substantially increase acetyltransferase activity. We conclude that alkylacyl-GPC serves as a precursor of alkylacetyl-GPC and that the production of this potent mediator by rat alveolar macrophages can be stimulated by agents that affect phospholipase A_2 and acetyltransferase activities. The latter enzyme appears to have a regulatory function in the biosynthesis of alkylacetyl-GPC.

Barnes, D. S.; Clapp, N. K.; Scott, D. A.; Oberst, D. L.; and Berry, S. G.
Effects of wheat, rice, corn, and soybean bran on 1,2-dimethylhydrazine-induced large bowel tumorigenesis in F344 rats.
Nutr. Cancer 5:1–9; 1983.

This study was designed to determine the relative effects of four dietary brans on large bowel tumorigenesis in rats treated with 1,2-dimethylhydrazine (DMH). Four-week-old F344 rats were fed a 20% bran (either wheat, rice, corn, or soybean) semisynthetic diet or a no-fiber-added control diet for life. All rats except for one control group were injected with DMH (2SC doses; —150 mg/kg body weight) at 8 and 10 weeks of age. Two additional groups were fed the control diet and then at 4 and 18 weeks, respectively, after the second DMH dose, were fed the wheat bran diet. All surviving rats were killed 9 months following the first DMH dose. Survival was increased in all groups receiving bran diets. Large bowel tumor incidences in the DMH group were as follows: control, 95%; wheat, 75%; rice, 86%; corn, 100%; soybean, 84%; wheat after 4 weeks, 62% (lower than control group, $P < 0.05$); wheat after 18 weeks, 84%. All but one of the DMH groups had approximately two tumors/tumor-positive rat; the corn group had four. The effect of bran on large bowel carcinogenesis appears to depend both on the source of bran and when it is fed in relation to tumor initiation.

Berdel, W. E.; Greiner, E.; Fink, U.; Stavrou, D.; Reichert, A.; Rastetter, J.; Hotfman, D. R.; and Snyder, F.
Cytotoxicity of alkyl-lysophospholipid derivatives and low-alkyl-cleavage enzyme activities in rat brain tumor cells.
Cancer Res. 43:541–545, 1983.

Alkyl-lysophospholipids (ALP) and related derivatives inhibited the in vitro incorporation of [^{3}H]thymidine into seven different permanent cell lines derived from rat brain tumors. The cytostatic effect of ALP was dependent on dosage and incubation time. Naturally occurring 2-lysophosphatidylcholine did not exhibit cytostatic effects; under these conditions, the incorporation rates of [^{3}H]thymidine were generally more than 100% of the controls. The trypan blue dye exclusion test, which was used to assess severe cell damage, correlated with the extent that [^{3}H]thymidine incorporation was inhibited by ALP.

Preincubation of ALP (*rac*-1-octadecyl-lyso-glycero-3-phosphocholine) for more than 8 min with a tetrahydropteridine-dependent *O*-alkyl cleavage enzyme preparation from rat liver microsomes destroyed almost all of the cytotoxic properties of ALP when tested at a concentration that previously inhibited tumor growth by more than 50%. [^{3}H]Thymidine incorporation rates were greater than 100% for astrocytoma cells incubated with ALP after exposure to the alkyl cleavage enzyme.

Comparison of the microsomal activities of the tetrahydropteridine-dependent alkyl-cleavage enzyme present in astrocytoma 78-FR-G-299 cells and the pleomorphic glioma 78-FR-G-219/S4 cells to that found in normal skin fibroblasts and rat livers revealed a markedly reduced activity in the neoplastic cell lines. Moreover, those tumor cells that were more resistant to ALP cytotoxicity (pleomorphic glioma, 78-FR-G-219/S4) had a 3-fold higher tetrahydropteridine-dependent

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cleavage activity than a more cytotoxic sensitive line (astrocytoma cells, 78-FR-G-299). Our results indicate that the low-alkyl-cleavage enzyme activities in these neoplastic cells in comparison to normal cells might be a factor in explaining the relatively high cytotoxicity of ALP in tumor cells.

...k, M. L.; Cress, E. A.; Lee, T-c.; Malone, B.; Surles, J. R.; Piantadosi, C.; Hajdu, J.; and Snyder, F.
Structural features of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) required for hypotensive and platelet serotonin responses.
Res. Commun. Chem. Pathol. Pharmacol. 38:3–20; 1982.

A number of analogs similar in structure to biologically active 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor) were tested for their relative effectiveness in lowering the blood pressure of spontaneous hypertensive rats and in releasing [^{3}H]serotonin from rabbit platelets. Except for some minor variations, the two test systems gave the same pattern of relative responses for a given analog. Both biological activities (the antihypertensive response and release of platelet serotonin) appear to be stereospecific since the unnatural isomer exhibited no detectable effects. Other structural modifications that greatly reduced (>500-fold) or eliminated the two biological activities were (1) removal of the acetate group, (2) substitution of the acetate group with long-chain acyl, methoxy, benzoxy, *N*-formyl, *N*-trifluoroacetyl, *N*-hexadecanoyl, or deoxy groups such as dimethyl, propyl, isopropyl, or isobutyl, and (3) replacement of the *sn*-1 *O*-alkyl group with an acyl moiety. Biological activities were reduced to a lesser extent (100- to 500-fold) when the *sn*-2 carbon contained butyrate or hexanoate groups or if ethanolamine was substituted for the choline base. Replacing the *sn*-2 acetate group with an ethoxy ...up lowered the activity 48- and 120-fold for the blood pres... and serotonin release, respectively. Substitution of pro... ...ate for acetate at the *sn*-2 carbon gave a compound that was at least, if not more, biologically active than the parent structure; although an *N*-acetyl analog possessed biologic activities, the responses were only 0.27 to 1.0% of those obtained with the *O*-acetyl lipid. Maximum biological activity for both hypotensive effects and platelet serotonin release required a glycerolipid having an alkyl ether at the *sn*-1, acetate or propionate groups at the *sn*-2, and phosphocholine at the *sn*-3 positions.

Blank, M. L.; Hall, M. N.; Cress, E. A.; and Snyder, F.
Inactivation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by a plasma acetylhydrolase: Higher activities in hypertensive rats.
Biochem. Biophys. Res. Commun. 113:666–671; 1983.

We have partially characterized the properties of a specific acetylhydrolase in plasma from spontaneous hypertensive rats. This enzyme inactivates 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a lipid involved in platelet aggregating, hypotensive, and allergic responses) by removal of the acetate group. The extent of acetate hydrolysis was linear with both time and protein concentration, and the enzyme had an apparent K_m of 2.5 μM and a V_{max} of 2.6 nmol/min/mg protein. As with an intracellular acetylhydrolase previously characterized by us, the plasma activity was not affected by addition of phosphatidylcholine, EDTA, or Ca^{2+}. However, in contrast to the acetylhydrolase activity in the rat kidney soluble fraction, the plasma ...ity was associated with a higher molecular weight protein ...ed on a Sepharose 6B column and the plasma acetylhydrolase was not inhibited by treatment with trypsin, pronase, or subtilisin. We also compared the acetylhydrolase activity in plasma of age-matched spontaneous hypertensive rats and their normotensive controls and found approximately 20% higher levels of activity in plasma from the hypertensive animals ($P < 0.01$).

Borman, L. S.; Swartzendruber, D. C.; and Littlefield, L. G.
Establishment of two parental cell lines and three clonal cell strains from rat colonic carcinoma.
Cancer Res. 42:5074–5083; 1982.

Two epithelial cell lines were established from separate pools of cells that were harvested by a sequential proteolytic treatment of a single specimen of rat transplantable colonic carcinoma. The cells were small (10-μm diameter) and cuboidal and displayed desmosomes and tight junctions. Both cell lines grew rapidly with population-doubling times of 20 to 22 hr, were near diploid in chromosome number, contained A-2 and B-7 marker chromosomes, and were tumorigenic in rats. Each was distinguishable from the other by the shape of its cell clusters in monolayer culture, its serum requirement for growth, its modal chromosome number, and its karyotypic alterations. Undifferentiated cells, cystic cells, and vacuolated cells, but not mature mucous cells, were observed in monolayer cultures. Clonal cell strains mimic the morphological and growth properties of their respective parental cell line but display unique karyotypic alterations in addition to the A-2 and B-7 marker chromosomes.

Brown, A. J., and Snyder, F.
The mechanism of alkyldihydroxyacetone-P synthase.
Formation of [^{3}H]H_2O from acyl[1-*R*-^{3}H]dihydroxyacetone-P by purified alkyldihydroxyacetone-P synthase in the absence of acylhydrolase activity.
J. Biol. Chem. 258:4184–4189; 1983.

Alkyldihydroxyacetone-P (alkyl-DHAP) synthase catalyzes the exchange of the fatty acid esterified to C-1 of the DHAP portion of acyl-DHAP for a fatty alcohol to form 1-*O*-alkyl-DHAP, the first ether-linked intermediate in ether lipid biosynthesis. Another characteristic of the reaction is the exchange of the *pro-R* hydrogen at C-1. We have investigated this hydrogen exchange using palmitoyl-[1-*R*-^{3}H]DHAP and a 1000-fold purified preparation of alkyl-DHAP synthase. We found a small but significant *pro-R* hydrogen exchange in the absence of the co-substrate, fatty alcohol. When [^{14}C] hexadecanol was added, the increase in *pro-R* ^{3}H exchange was equal to the [^{14}C]hexadecyl-DHAP formed. Addition of [^{14}C]palmitic acid resulted in an increase in *pro-R* ^{3}H exchange that matched the formation of [^{14}C]palmitoyl-DHAP by the acyl exchange activity of alkyl-DHAP synthase. Furthermore, although whole microsomes contain at least two acyl hydrolases for acyl-DHAP, purified preparations of alkyl-DHAP synthase do not form DHAP from acyl-DHAP. These results are discussed with respect to data obtained from other laboratories using whole microsomes and in support of our proposed ping-pong mechanism for alkyl-DHAP synthase.

Cabot, M. C.
Effect of cellular phospholipid modification on phorbol diester binding.
Cancer Res. 43:4233–4238; 1983.

The influence of cellular lipid composition on the specific binding of [20-^{3}H]phorbol-12,13-dibutyrate to intact human

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promyelocytic leukemia cells was investigated. Cellular phospholipid composition could be manipulated by culturing cells in serum-free, chemically defined media containing base analogs of phospholipid polar head groups. Human promyelocytic leukemia cells grown in the presence of dimethylethanolamine, monomethylethanolamine, 3-aminopropanol, or isopropylethanolamine assimilated these natural and unnatural base moieties into endogenous phospholipids to the extent that 22 to 52% of the cell glycerophospholipids contained the base analog. The formation of the phospholipid analogs was accompanied by a pronounced reduction in the levels of intracellular choline and ethanolamine glycerophospholipids. Analog-supplemented cultures exhibited a reduced growth rate compared to control cells maintained in choline-containing medium. Specific [20-^{3}H]phorbol-12,13-dibutyrate binding was examined in lipid-altered cells and shown to be markedly higher (approximately 200% of control) in cells grown with dimethyl- or monomethylethanolamine. In contrast, exposure of cells to 3-aminopropanol or isopropylethanolamine resulted in a major reduction in [20-^{3}H]phorbol-12,13-dibutyrate binding. Only minimal changes in nonspecific binding occurred between control and experimental cells. Because phorbol esters are highly membrane targeted, it is possible that phospholipid modification or the resulting changes in membrane organization influence receptor dynamics.

Cabot, M. C.; Blank, M. L.; Welsh, C. J.; Horan, M. J.; Cress, E. A.; and Snyder, F.
Metabolism of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by cell cultures.
Life Sci. 31:2891–2892; 1982.

The metabolism of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) was studied using various cultured cell lines. All incubations were done in the presence of bovine serum albumin and serum-free media, since albumin eliminates the adsorption of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine to cultureware and serum enzymes interfere. Human leukemia (HL-60) cells, MDCK canine kidney cells, and transformed and nontransformed clones of mouse C3H10T1/2 cells display varying rates of uptake, degradation, and capacities for reacylation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. HL-60 cells displayed the highest uptake rate (24.6 pmol/mg cell protein/15 min). Whereas C3H10T1/2 cells in culture showed uptake rates comparable to other cells tested, they displayed a relative metabolic inertness towards 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine.

Cabot, M. C.; Welsh, C. J.; and Snyder, F.
Increasing the levels of ether-linked lipids in L-M cells by glyceryl ether supplementation depresses growth and choline utilization.
Biochim. Biophys. Acta 713:16–22; 1982.

To gain insight into the role of alkyl-linked lipids in biological systems, we added hexadecylglycerol (a precursor of complex ether-linked lipids) to medium required for the growth of L-M cells in culture. L-M fibroblasts cultured through several generations in the presence of hexadecylglycerol grow at a reduced rate. Experimental cells at their sixth passage, with 2 μg supplement/ml, double at 50% the rate of control cell populations. Hexadecylglycerol (10 μg/ml) added 1 day after cell passage does not retard growth; however, within 1 h it decreases the incorporation of choline into the choline glycerophosphatide fraction. Inhibition is specific for choline; ethanolamine incorporation is not affected. The inhibition of choline utilization by hexadecylglycerol-treated cells is dose-dependent and reaches a maximum 12 h after supplementation. Cellular uptake of choline is reduced (approx. 17%) but not as much as the incorporation of choline into the phospholipids (approx. 60% at 12 h). The assimilation of ether lipid precursor into cellular phospholipids was followed by incubating cells with [1-^{14}C]hexadecylglycerol. Incorporation of radioactivity into cellular phospholipids begins to plateau after 24 h, whereas the interference of hexadecylglycerol with choline metabolism could be detected as early as 1 h. The majority of the radioactivity recovered from cells incubated with labeled hexadecylglycerol is localized in the microsomal fraction (56%), where the label was distributed as free hexadecylglycerol, alkylacyl-phospholipids, and alkyldiacylglycerols. These results show that the supplementation of a glyceryl ether to L-M fibroblast growth media selectively inhibits the utilization of choline for choline glycerophospholipid biosynthesis and causes a reduction in cell growth rate when cells are continually passaged in the presence of the glyceryl ether.

Erickson, B. H., and Hall, G. G.
Comparison of stem-spermatogonial renewal and mitotic activity in the γ-irradiated mouse and rat.
Mutat. Res. 108:317–335; 1983.

The Sprague-Dawley rat exceeds the CRF_1 mouse in number of isolated or purported stem cells per unit area of seminiferous tubule by a factor of 2.1, yet the mouse exceeds the rat in number of first-generation spermatogonia (A_1) per unit of tubule by a factor of 1.8. This difference could lead to an interspecies difference in irradiation response and help clarify the identity of spermatogonial stem cell. To test this thesis, mice and rats were irradiated with 600 R of ^{60}Co γ-radiation and killed at postirradiation intervals of 1–16 weeks. Both tubular whole mounts and cross sections were evaluated. The species did not differ in percent survival of isolated spermatogonia, but the mouse exceeded the rat in survival and/or production of A_1 or clonal spermatogonia by a factor of 5. By week 16 after irradiation, the mouse testis had regained 67% of its preirradiation weight, in contrast to the rat's 52%. Clonal renewal was in progress by the end of the 2 (mouse) and 3 (rat) weeks and was substantially complete by the 8 week. In both species the isolated population, however, diminished throughout the first 8 weeks and was renewed between 8 and 26 weeks. Clonal renewal was not accompanied by an increase in the number of isolated spermatogonial mitoses but was accompanied by increases in the mitosis of pairs and clones and the apparent splitting of existing clones to form additional clones. Thus, the mouse's superiority in the rate and completeness of spermatogonial renewal was apparently due to the attributes of the undifferentiated A_1 spermatogonial clone.

Hubbard, J. W.; Cox, R. H.; Lawler, J. E.; Blank, M. L.; and Snyder, F.
Antihypertensive effects of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine on plasma renin activity and catecholamine responses in spontaneously hypertensive rats.
Life Sci. 32:221–232; 1983.

Fourteen 23-week-old male spontaneously hypertensive rats (SHR) were randomly divided into saline control or phospholipid (1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) treatment groups. Four weeks of baseline systolic blood pressure (SBP) and heart rate (HR) measurements were

1028580

determined via tail plethysmography. On week 25 of the baseline period a 1.5-ml blood sample was taken by tail clip for analysis of norepinephrine (NE), epinephrine (E), and plasma renin activity (PRA). On the following week, a single injection of phospholipid (11 μg/kg, s.c.) was given to the experimental animals following baseline SBP and HR determinations. A similar procedure was employed for control subjects, except they received an injection of normal saline (0.5 ml, s.c.). Systolic BP and HR responses were monitored for 24 minutes following the injection. A 1.5-ml blood sample was taken at the end of the 4th minute for NE, E, and PRA assays. A significant drop in SBP (202 ± 5 mmHg to 124 ± 6 mmHg) and an increase in HR (431 ± 17 bpm to 519 ± 21 bpm) were observed for experimental animals, but not for control subjects. Plasma NE increased significantly (446 ± 42 pg/ml to 1099 ± 77 pg/ml), but E remained unchanged following treatment with the phospholipid. Plasma renin activity increased for both groups, but this change was only significant for the experimental group (18.1 ± 5.7 ng Al/ml/hr to 34.3 ± 3.6 ng Al/ml/hr). Thus, it appears that 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine is a potent antihypertensive vasodilating agent which stimulates baroreceptor-mediated sympathetic discharge to the heart and kidneys of the SHR.

Lackey, R. J., and Cabot, M. C.
Serum lipase active in the hydrolysis of the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate.
Cancer Lett. 19:165–172; 1983.

Sera from numerous animal sources contain enzymes that degrade the potent tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). The activity was present in rat, mouse, guinea pig, rabbit, and goat and absent in fetal bovine, bovine, porcine, horse, chicken, and human sera. The enzyme shows a marked specificity for hydrolysis of the long-chain 12-O acyl group, which results in the formation of phorbol-13-monoacetate as the major product. Enzymatic catalysis displayed two pH optima (5.5 and 8.0) and was completely destroyed by heat inactivation (56°C, 30 min) of the serum. *N*-Ethylmaleimide and *p*-chloromercuribenzoate were relatively weak inhibitors, whereas NaF (5.0 mM) produced an approximate 80% reduction of hydrolysis. Esterase-lipase substrates, such as cholesterol oleate, α-naphthylacetate, α-naphthylpalmitate, and monotetradecanoylglycerol, were not inhibitory when added to the reaction mixture. Porcine pancreatic lipase does not catalyze the removal of the acyl or the acetyl groups from TPA.

Lee, T-c.; Blank, M. L.; Fitzgerald, V.; and Snyder, F.
Formation of alkylacyl- and diacylglycerophosphocholines via diradylglycerol cholinephosphotransferase in rat liver.
Biochim. Biophys. Acta 713:479–483; 1982.

We found that diacylglycerol and alkylacylglycerol choline phosphotransferase activities exhibited similar pH optima, thermolabilities, and inhibitions by Mn^{2+} and dithiothreitol. The V_{max} of diacylglycerol cholinephosphotransferase was higher (approx. 1–2-fold) than the V_{max} of alkylacylglycerol cholinephosphotransferase. The K_m value for diacylglycerol was somewhat greater than for the alkylacylglycerol, but no differences were found for the K_m of CDPcholine with either type of diradylglycerol as the other substrate. Endogenous levels of diacylglycerols in microsomes were 24.3 nmol/mg protein, whereas no detectable amount of alkylacylglycerols was observed. Results from this study indicate that a similar or identical enzyme catalyzes the formation of 1,2-diacyl- and 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine and that the availability of diradylglycerols and the turnover rate of ether-linked lipids would appear to be important factors in controlling the level (under 1%) of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine in rat liver.

Lee, T-c.; Malone, B.; and Snyder, F.
Stimulation of calcium uptake by 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) in rabbit platelets: Possible involvement of the lipoxygenase pathway.
Arch. Biochem. Biophys. 223:33–39; 1983.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) induces an increase of Ca^{2+} uptake in rabbit platelets. This process depends upon the extracellular concentration of Ca^{2+} with the maximum stimulation occurring at 1–3 mM; uptake under these conditions is blocked by verapamil, a calcium-entry blocker. Increase of calcium uptake by the bioactive phospholipid was independent of ADP-induced platelet responses and of metabolites of arachidonic acid metabolism formed through the cyclooxygenase pathway. However, mepacrine, *p*-bromophenacyl bromide, eicosatetraynoic acid, and nordihydroguaiaretic acid significantly or totally inhibited the stimulation of Ca^{2+} uptake by 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine. When arachidonic acid was given sufficient time to be metabolized to other products by the platelets, stimulation of Ca^{2+} uptake also occurred. Arachidonic acid and platelet-activating factor did not produce an additive or synergistic effect. Our data suggest that a metabolite(s) generated from arachidonic acid through the lipoxygenase pathway may be the mediator(s) responsible for the action of platelet-activating factor in the induction of increased Ca^{2+} uptake in rabbit platelets.

Littlefield, L. G.; Colyer, S. P.; and DuFrain, R. J.
SCE evaluations in human lymphocytes after G_0 exposure to mitomycin C: Lack of expression of MMC-induced SCEs in cells that have undergone greater than two in vitro divisions.
Mutat. Res. 107:119–130; 1983.

We conducted a series of experiments designed to determine whether DNA damage induced in G_0 lymphocytes by mitomycin C (MMC) would be expressed as sister-chromatid exchanges during the second and third post-treatment cell cycles. Lymphocytes from normal donors were exposed to MMC for 2 h prior to culture in the presence of phytohemagglutinin. MMC-treated and control cells were subsequently exposed to bromodeoxyuridine (BrdUrd) for the entire culture period (i.e., 48 h or 72 h) or for the terminal 24-h of 72-h cultures. We observed a 3–4-fold increase in SCEs in MII metaphases from lymphocytes treated with MMC and cultured in the presence of BrdUrd for the entire culture period. In contrast, in replicate cultures of MMC-treated lymphocytes that were exposed to BrdUrd for the terminal 24 h only, the SCE frequency in uniformly harlequinized metaphases was not significantly different from that observed in control cultures. We interpret these data as providing evidence that MMC-induced lesions (or alterations) in the DNA of G_0 lymphocytes are probably expressed as SCEs during the first period of mitogen-induced DNA synthesis and that these lesions do not persist and give rise to SCEs in subsequent cell divisions.

1028581

Lumb, R. H.; Pool, G. L.; Bubacz, D. G.; Blank, M. L.; and Snyder, F.
Spontaneous and protein-catalyzed transfer of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor) between phospholipid bilayers.
Biochim. Biophys. Acta 750:217–222; 1983.

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor or alkylacetyl-GPC), a bioactive phospholipid that possesses hypotensive, platelet-aggregating, and inflammatory properties, is known to be secreted by a variety of cell types. The biological activity of alkylacetyl-GPC is related to a precise chemical structure that implicates interaction with proteins. We have studied the spontaneous and protein-catalyzed transfer of alkylacetyl-GPC between phospholipid vesicles and have demonstrated the following: 1. There are at least two transferable pools of alkylacetyl-GPC in sonicated phospholipid vesicles. 2. These two pools differ in the rate at which they dissociate from the vesicles; one pool equilibrates between donor and acceptor vesicles instantaneously while the other pool is transferred much more slowly. 3. Dialysis of alkylacetyl-GPC between phospholipid vesicles through the aqueous phase is slow. 4. A protein fraction derived from rat lung cytosol catalyzes the transfer of the nonequilibrating pool of alkylacetyl-GPC between phospholipid vesicles; this transfer is superimposed on the spontaneous transfer and is unchanged in experiments using vesicles from which the rapidly equilibrating pool has been removed.

Matthews, J. A.; Vann Bonds, S.; Cianci, M. L.; Cooper, J. F.; Hanes, K.; Hodges, H. D.; Kraft, R. A.; Langan, J. K.; Porter, J. L.; Price, D. D.; and Ward, D.
Performance and responsibility standards for the nuclear medicine technologist.
J. Nucl. Med. Technol. 10:163–169; 1982.

The spectrum of nuclear medicine technology skills and responsibilities varies widely across the country. The broad descriptions of this document will provide a basis for determining the areas of knowledge and of performance for the nuclear medicine technologist. The documents used in the development of these standards were the NMTCB Task Analysis of Nuclear Medicine Technology (1), the Society of Nuclear Medicine Position Paper on Licensure, and the Job Description for the Nuclear Medicine Technologist (2). These standards should be considered a helpful checklist of those tasks that need to be performed in the various major subdivisions of nuclear medicine technology. While the editors tried to be complete, nuclear medicine technology is a dynamic, developing, and constantly changing field; therefore, any list is likely to be partially obsolete as soon as it is issued. In addition, the analysis is not designed to be a "how to" description for any of the listed activities, nor is it intended to modify or alter existing tort law. Activities listed may, at times, be legitimately omitted and, at other times, supplemented by unlisted activities.

McFee, A. F., and Long, S. E.
Sister chromatid exchanges in the active and inactive X chromosomes.
Chromosoma (Berl.) 87:359–362; 1982.

Lymphocyte cultures from female cattle in two geographic locations were used to determine the frequency of occurrence of sister chromatid exchanges on the active and inactive X chromosomes. The distribution of 129 exchanges among the two X chromosomes was not significantly different from a 1:1 ratio.

McFee, A. F.; Lowe, K. W.; and San Sebastian, J. R.
Improved sister-chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice.
Mutat. Res. 119:83–88; 1983.

A quick and easy method for partially coating 5-bromo-2'-deoxyuridine tablets with paraffin before they were subcutaneously implanted in mice increased availability of the chemical from 8 to more than 24 h and significantly reduced the baseline level of sister-chromatid exchanges. Accurate identification of first-, second-, and third-division metaphases was possible for 30 h postimplantation.

McFee, A. F., and Sherrill, M. N.
Sister-chromatid exchanges induced in swine lymphocytes by chronic oral doses of dimethylbenzanthracene.
Mutat. Res. 116:349–359; 1983.

Sister-chromatid exchanges were scored at 3-week intervals in lymphocytes of female swine ingesting daily doses of 1.25 or 2.50 mg/kg of 7,12-dimethylbenzanthracene (DMBA) for 160 days. Exchanges increased with time for about 120 days then reached a plateau at approximately 2.5 times their pretreatment level. No increase in chromosome aberrations could be identified as resulting from ingestion of the chemical. Week-old progeny of animals that had ingested the chemical throughout pregnancy showed no increase in exchanges in their peripheral lymphocytes.

Polednak, A. P.; Keane, A. T.; and Beck, W. L.
Estimation of radiation doses to the lungs of early uranium processing plant workers.
Environ. Res. 28:313–328; 1982.

Methods are described for the crude estimation of radiation doses to the lungs of workers in early uranium processing plants. Using data on selected plants, we estimated lung doses for groups of workers exposed to uranium dust alone, or in association with γ-rays and radon gas derived from radium in uranium ores. Estimated 30-year lung doses for groups of chemical workers at a large uranium enrichment plant (in operation from 1943 to 1947) varied considerably, depending upon such factors as length of employment, types of uranium compounds, average air concentrations of uranium, and isotopic composition of the uranium. Some workers may have received lung doses as high as several hundred rem. The uncertainties in these crude dose estimates are described, along with the possible effects of host factors. Implications of these dose estimates for assessment of long-term health effects (i.e., lung cancer) are discussed, with reference to preliminary findings of mortality studies on workers at uranium processing plants.

Stathis, V. J.; Miller, C. M.; Doerr, G. F.; Coffey, J. L.; and Hladik, W. B. III.
Effect of technetium Tc 99m pertechnetate on bacterial survival in solution.
Am. J. Hosp. Pharm. 40:634–637; 1983.

Survival of *Staphylococcus epidermidis* (10^2 organisms/ml) in solutions containing various levels of radioactivity was assessed.

Six test preparations contained nonbacteriostatic 0.9% sodium chloride solution; four of these contained technetium Tc 99*m* pertechnetate ($^{99m}TcO_4^-$) in various quantities (80, 250, 500, and 750 mCi). A fifth contained technetium that had decayed to an essentially nonradioactive form, and a sixth con-

1028582

:ained 0.9% sodium chloride solution only. Each of the six 20-ml solutions was inoculated with 2 ml of single-strength trypticase soy broth (TSB) containing 10^3 organisms/ml. At various times up to 12 hours after inoculation, 1-ml aliquots of 'est solution were withdrawn and passed through 0.22-μm , thereby preventing further irradiation of the filtered organisms. The filters were incubated in single-strength TSB at 37°C, and samples were examined for turbidity at 24, 48, and 72 hours.

After 24 hours, 25 of the 36 sample tubes showed turbidity; after 48 hours, the turbid samples totaled 28. Bacteria in the two nonradioactive solutions remained viable throughout the 12-hour sampling period. Accumulated doses of radiation obtained in the 250-, 500-, and 750-mCi samples inhibited bacterial growth.

To be a valid quality-control measure, sterility monitoring of prepared radiopharmaceutical dosage forms may need to be performed concurrently with their preparation.

Urso, P., and Gengozian, N.
Alterations in the humoral immune response and tumor frequencies in mice exposed to benzo[α]pyrene and X-rays before or after birth.
J. Toxicol. Environ. Health 10:817–835; 1982.

Pregnant mice were given one of three treatment regimens during mid (11 to 13 d) or late (16 to 18 d) gestation: benzol[α]pyrene (BaP) (150 μg/g body weight), 150 R X-irradiation, or X-irradiation + BaP. A group of virgin mice were injected with BaP after birth (at 1 wk or 16 wk) with doses similar to or higher than those to which progeny of pregnant mice were exposed in utero. The offspring exposed during fetal life showed a marked suppression in anti-SRBC plaque-forming cells (PFC) 1 wk after birth (pups), followed by an apparent recovery at 4 wk. Return to the immuno-suppressed state seen in pups was most noticeable in adults encountering BaP alone during gestation. In this group, increased tumor incidence was associated with a sustained reduction in PFC response. In contrast, after fetal exposure to X-rays or injection with BaP postnatally, immune competence was not appreciably different from normal in late life, and tumor incidence was only slightly higher than controls with X-rays or a high dose (150 μg/g body weight) of BaP. When X-rays were given in conjunction with BaP, it appeared that immune suppression was abated and tumor incidence reduced. The effect of BaP on PFC and tumor incidence varied according to the time in gestation when the carcinogen was given. After mid-gestation insult, immune suppression was most pronounced in 19S PFC, while after late gestation, suppression was more severe in 7S antibody formation. The liver was the primary target for tumor growths in males. In females, a high incidence of ovarian tumors was seen, but only after mid-gestation exposure. The frequency of lung tumors increased following late gestation insult in both sexes. These data show that (1) susceptibility to injury of different components of the developing humoral immune system and the disposition of the sexes for tumor induction are functions of the gestational time of exposure to BaP and (2) animals exposed prenatally to the carcinogen are more sensitive to immune suppression and increased tumor incidence than those exposed postnatally. The results support the contention that immune deficiency (suppression) influences the increase in neoplastic expression.

1028583

Training Programs

Training programs at the division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. Special short-term courses dealing with radiation health problems are available for biomedical personnel.

Graduate students participating in Ph.D. training programs come from University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama in Birmingham, and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals. Advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by Oak Ridge National Laboratory, University Programs at ORAU (sponsored by DOE), University of Tennessee (Hilton A. Smith Fellowship), individual research grants, and operational subcontracts with ORAU.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows are available from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, The Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health and American Cancer Society Grants, and divisional funds.

Summer research opportunities make it possible for academic staff and advanced undergraduate students from colleges and universities throughout the United States to participate in any of the division's research programs. The DOE-sponsored summer program is administered through the University Programs Division at ORAU. Secretarial and laboratory aide training is also provided to high school students who participate in vocational programs at the high schools in Clinton and Oak Ridge.

Short-term courses in the management of medical problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of ten REAC/TS courses were given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

The following individuals participated in the training programs at the division during the past year:

1. Predoctoral students

Naima Abd Elghany* (Epidemiology)
University of North Carolina
Arvind Carpenter* (Epidemiology)
University of Alabama
Donna Cragle† (Epidemiology)
University of North Carolina
Elizabeth Dupree Ellis‡ (Epidemiology)
University of North Carolina
Daniel Strom* (Epidemiology)
Environmental Sciences and Engineering,
University of North Carolina
Jefferson Surles* (Medicinal Chemistry)
University of North Carolina
Donna Woodard‡ (Biochemistry)
University of Tennessee

2. Students pursuing MPH or MS degrees

Shirley Fry‡ (Epidemiology)
University of North Carolina
Alan Johnson† (Environmental Sciences)
University of Alabama
Lela Nottingham‡ (Biochemistry)
University of Tennessee
Ravishankar Rao‡ (Epidemiology)
University of Alabama
Teresa Rusk (Toxicology)
University of Tennessee

3. Postdoctoral fellows

Daniel H. Albert (Biochemistry)
Alex J. Brown (Biochemistry)

1028584

Dennis R. Hoffman (Biochemistry)
Mitchell E. Robinson (Biochemistry)
Eng-Lay Tan (Biochemistry)
Jerome Wilson (Epidemiology)

4. *Visiting scientists*

Kiyoshi Fukushi (Radiopharmaceuticals)
National Institute of Radiological Sciences,
Chiba-shi, Japan
Robert T. Swindell (Biochemistry)
Tennessee Technological University,
Cookeville, Tennessee

5. *College-student research participants*

W. Michael Barret (Epidemiology)
Middle Tennessee State University, Murfreesboro
Daniel J. Lenihan (Biochemistry)
University of Tennessee, Knoxville
Joan K. Lingen (Radiopharmaceuticals)
Clarke College, Dubuque, Iowa
J. Daniel Stanley (Biochemistry)
Indiana University, Bloomington
Mike F. Ziegler (Epidemiology)
University of Tennessee, Knoxville

6. *High school vocational education students*

Cheryl Brantly (Biochemistry)
Oak Ridge High School
Cheryl Fox (Biochemistry)
Anderson County High School
Lisa Freeman (Biochemistry)
Clinton Senior High School
Gary Jackson (Epidemiology)
Oak Ridge High School
Linda Manning (Biochemistry)
Anderson County High School
Sherri Soles (Epidemiology)
Clinton Senior High School

on of thesis research being done at the division.
.ithesis work.
‡Thesis research being completed at the division.

1028585

Non-DOE-Funded Research

D. Cragle
Minority high school student research apprentice program.
National Institutes of Health, Division of Research Resources
$3,000* (1 March 1983 to 28 February 1984)

G. Eisele
Actinide metabolism studies.
Nevada Operations Office/U.S. Department of Energy‡
$228,000† (1 October 1981 to 30 September 1984)

G. Eisele
Food chain transport of synfuels.
U.S. Environmental Protection Agency–U.S. Department of Energy Interagency Agreement
$300,000† (1 October 1981 to 30 September 1983)

S. Fry
Feasibility of a mortality study of workers in nuclear power plants.
Electric Power Research Institute, Inc.
$73,000† (1 December 1982 to 31 October 1983)

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies.
National Pancreatic Cancer Project, National Cancer Institute
$80,734* (1 July 1981 to 30 June 1984)

L. G. Littlefield
Evaluation of induced cytogenetic lesions in oocytes.
National Institute of Child Health and Human Development
$101,331* (1 August 1981 to 31 July 1984)

L. G. Littlefield
Studies of radiation-induced chromosome damage in humans.
National Cancer Institute-U.S. Department of Energy Interagency Agreement
$112,000† (1 September 1981 to 30 September 1984)

C. C. Lushbaugh
Biomedical research support grant.
National Institutes of Health, Division of Research Resources
$20,684* (1 April 1983 to 31 March 1984)

A. McFee
Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations.
National Cancer Institute–U.S. Department of Energy Interagency Agreement
$185,212† (30 September 1981 to 29 September 1984)

F. Snyder
Ether lipids in cancer: Enzyme mechanisms and inhibitors.
National Cancer Institute
$61,828* (1 May 1980 to 30 April 1985)

F. Snyder
Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells.
American Cancer Society
$79,221† (1 January 1983 to 31 December 1983)

F. Snyder
A new class of biologically active phospholipids.
National Heart, Lung, and Blood Institute
$62,000* (1 April 1981 to 31 March 1986)

*Direct costs (current year).
†Total costs, i.e., direct plus overhead (current year).
‡Not under DOE prime contract.

1028586

Collaborative Research

M. C. Cabot
Lipids as effectors of tumor promoter binding.
Louisiana State University Medical Center, Department of Anatomy (Shreveport), G. T. Bazer.

N. K. Clapp
Colon crypt cell kinetics in tamarins.
Memorial Sloan-Kettering Cancer Center (New York), E. Deschner.

Fecal bile acid analysis in marmosets.
Beth Israel Medical Center (New York), B. Cohen.

Monoclonal antibodies to human colon cancer cell lines in spontaneous primate colon tumors.
Wistar Institute (Philadelphia), A. Steplewski.

In vitro tetraploidy in skin fibroblasts of colon cancer tamarins.
Cornell Medical College (New York), B. S. Danes.

Genetic segregation and familial cancer relationships in colon cancer tamarins.
Creighton University (Omaha), W. Kimberling and H. Lynch.

Coronaviruses in colitis and colon cancer.
University of Tennessee (Knoxville), College of Veterinary Medicine, R. Russell and D. Brian.

Carcinoembryonic antigens in colon tumors.
Harvard Medical School (Boston), P. Thomas.

R. J. DuFrain
Human cytogenetic dosimetry for neutron radiation.
Oak Ridge National Laboratory, H. W. Dickson; University of California (Los Angeles), Department of Radiological Sciences and Radiation Oncology, A. Norman.

Cytogenetic effect studies on chlorinated biphenol degradation products.
University of Tennessee (Knoxville), Department of Microbiology, G. S. Sayler.

Gene mapping the laboratory rabbit.
Oak Ridge National Laboratory, Biology Division, P. A. L[illegible].

St[illegible]al distributions in cytogenetic dose response.
Sangamon State University (Springfield, Illinois), Department of Mathematics, K. G. Janardan; Illinois Environmental Protection Agency (Springfield), D. J. Schaffer.

Alpha particle dosimetry for cytogenetic analysis.
Battelle Pacific Northwest Laboratories (Richland, Washington), Radiological Sciences Department, D. R. Fischer.

Statistical analysis and Poisson regression studies in biological dosimetry.
Oak Ridge National Laboratory, Computer Science Division, E. L. Frome.

Studies of silver staining chromosome cores in multicentric chromosomes from irradiated blood.
Samford University (Birmingham), Department of Biology, W. M. Howell.

R. L. Hayes
Synthesis of labeled compounds using organoborane techniques.
University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka.

Collaborative studies in radiopharmaceutical development.
Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp.

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies.
The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, I. R. Collmann; Vanderbilt University Hospital (Nashville), Department of Gastroenterology, G. R. Avant.

Differentiation of glioblastomas and meningiomas with ^{11}C-labeled amino acids and positron emission computed tomography.
Vanderbilt University (Nashville), Department of Nuclear Medicine, C. L. Partain; The University of Tennessee Center for the Health Sciences (Memphis), Department of Neurosurgery, J. T. Robertson; University of North Carolina (Chapel Hill), Department of Surgery, M. S. Mahaley, Jr.; Fort Sanders Presbyterian Hospital (Knoxville), Department of Surgery, J. T. Purvis.

Tumor metabolism.
The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, S. Krauss.

1028587

Development of software for quantitative positron tomography.
Vanderbilt University (Nashville), Department of Biomedical Engineering, P. H. King.

T-c. Lee
Effect of stress on the metabolism of platelet activating factor.
University of Tennessee (Knoxville), Department of Zoology, N. Greenburg.

Metabolism of platelet activating factor in human blood cells.
University of California (San Diego), Division of Rheumatic Diseases, S. I. Wasserman.

L. G. Littlefield
Studies on radiation-induced chromosome damage in humans.
National Cancer Institute, Environment Epidemiology Branch (Bethesda), C. Land and R. Kleinerman.

Evaluations of radiation-induced lesions in human lymphocytes. exposed to low-energy fission spectrum neutrons.
Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straube, and A. Carrano; Los Alamos National Laboratory (Los Alamos), L. Devan; Radiation Effects Research Foundation (Hiroshima), A. Awa.

Coordinated research program in routine use of chromosomal aberration analysis in occupationally exposed radiation workers.
International Atomic Energy Agency (Vienna), A. Bianco.

Cytogentic follow-up studies of a survivor exposed to ^{241}Am.
Hanford Environmental Health Foundation (Washington), B. D. Breitenstein.

C. C. Lushbaugh
Epidemiology of human carcinogenesis.
University of Alabama (Birmingham), Department of Epidemiology, P. Cole.

Comprehensive epidemiology of DOE atomic workers.
University of North Carolina (Chapel Hill), Department of Epidemiology, C. M. Shy, M. A. Ibrahim, and H. A. Tyroler.

Study of mortality among workers at the Savannah River Plant.
Los Alamos National Laboratory Epidemiology Group, G. Wilkinson, G. Voelz; E. I. DuPont de Nemours & Company, G. Poda.

Study of mortality among former Manhattan Engineer District sites at Linde Corporation and the Electro Metallurgical Laboratory.
Union Carbide Corporation, Corporate Epidemiology Department, S. G. Austin and T. A. Lincoln.

C. C. Lushbaugh and L. C. Washburn
Diethylenetriaminepentaacetate (DTPA) project.
U.S. Department of Energy contractors, 40 coinvestigators.

F. Snyder
Biological effects of unique phospholipid analogs.
Boston College (Chestnut Hill, Massachusetts), Department of Chemistry, J. Hajdu.

Mass spectrometry of unique lipids.
Oak Ridge National Laboratory, Analytical Chemistry Division, G. L. Glish.

Nuclear magnetic resonance studies of unique lipids.
Oak Ridge National Laboratory, Chemistry Division, B. M. Benjamin.

Synthesis of lipid analogs and their precursors.
University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Piantadosi.

Effects of a new group of antihypertensive phospholipids on cardiovascular function.
University of Tennessee (Knoxville), Department of Psychology, J. E. Lawler and J. W. Hubbard.

The role of intracellular transfer proteins in the cellular movement of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet aggregating phospholipid).
Western Carolina University (Cullowhee), Department of Biology, R. H. Lumb.

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine.
NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein.

Selective cytotoxic activity of alkyllysophospholipids in tumor cells.
Technische Universität München (West Germany), Division of Oncology, W. E. Berdel.

Pulmonary properties of an antihypertensive phospholipid.
University of British Columbia (Vancouver), Pulmonary Research Laboratory, R. R. Schellenberg.

L. C. Washburn
Pharmacological studies using ^{11}C-labeled compounds.
University of Kentucky (Lexington), School of Pharmacy, G. A. Digenis.

^{11}C-Labeled amines for pulmonary function studies.
University of Alberta (Edmonton), Faculty of Pharmacy and Pharmaceutical Sciences, A. A. Noujaim, and Department of Medicine, S. F. P. Man.

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy.
The Wistar Institute (Philadelphia), Z. S. Steplewski.

1028588

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1028589

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University of Texas at Austin
Texas Christian University
Texas Woman's University
Tulane University
Tuskegee Institute
Vanderbilt University
University of Virginia
Virginia Commonwealth University
Virginia Polytechnic Institute and State University
West Virginia University
College of William and Mary

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Oak Ridge Associated Universities **Medical and Health Sciences Division**



Research Report *1983 1984*

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The Medical and Health Sciences Division conducts research on biomedical problems involving the respiratory, vascular, pancreatic, and gastrointestinal systems. Emphasis is on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Research programs in the division are focused on the following areas: (a) preclinical and clinical development of new positron radiopharmaceutical agents for in vivo diagnosis of diseases in humans, (b) biochemical aspects of cellular lipid mediators and membrane lipids in disease processes, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), (e) food chain transport studies of synfuels and actinides, and (f) interdisciplinary investigations of the marmoset as a primate model for spontaneous colon cancer and other human diseases, particularly those associated with energy-related toxicants. Predoctoral and postdoctoral training is provided in each research program area. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a treatment and decontamination facility and a demonstration and training site.

Oak Ridge Associated Universities is a private, not-for-profit association of 49 colleges and universities. It is a contractor of the U.S. Department of Energy, conducting research and educational programs in the areas of energy, health, and the environment for DOE, other private and governmental organizations, and ORAU's member institutions.

ORAU-242, April 1985

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

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Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes
Printed copy: A04
Microfiche copy: A01

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Oak Ridge Associated Universities

Medical and Health Sciences Division

Research Report *1983 1984*

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Preface

Late in 1948, Dr. Marshall H. Brucer was asked to form a medical division for the Oak Ridge Institute of Nuclear Studies, the predecessor of Oak Ridge Associated Universities. Dr. Brucer was named the division's first chairman. Over the ensuing years and through fiscal year 1984, the division has had three chairmen: Dr. Brucer, Dr. Gould Andrews, and Dr. C. C. Lushbaugh.

On October 1, 1984, the fourth chairman, Dr. William W. Burr, Jr., was appointed. This year's research report depicts this leadership succession. During the tenure of each chairman, the emphasis within the program has reflected scientific advancements and the specific needs of the time, but the continuity of the division's research efforts has been maintained.

Early on, the division evaluated newly available radionuclides for potential uses in medicine. Initially, the major emphasis was on therapeutic applications. By 1951 the division had designed, built, and tested a cobalt-60 teletherapy unit and sent it to M. D. Anderson Hospital in Houston, Texas, for clinical trials. The division evaluated radionuclides potentially useful for teletherapy and studied other theraputic uses of the substances. Division researchers investigated the distribution of many radionuclides in various species of animals.

The division did much to advance methodology and instrumentation in the field of nuclear medicine. Training and collaborative research programs were established for physicians and other scientists. In an effort to bring consistency to clinical measurements of iodine-131, staff members developed calibration kits. During this period the division also became a center for work on rare earth elements.

Dr. Gould Andrews became chairman in 1961, when Dr. Brucer retired. In 1958 the division had been called on for medical management of the health problems of eight men exposed to radiation in an accident at the Y-12 Plant, a responsibility that focused the interest of the division on the management of medical care for persons accidentally exposed to radiation. Partly as a result of the Y-12 accident, the division devoted a major effort to investigations of human total-body irradiation to improve the treatment of certain types of leukemia and lymphoma. Related research was undertaken on the hematologic and histopathologic effects of radiation, and an immunology program was initiated. The division established a colony of marmosets in support of this work.

The primary emphasis in the biochemical area shifted from rare earth elements to a broader interest in lipid biochemistry, an emphasis that has continued to play an important role in the division's research program. In 1964 a cytogenetics laboratory was established, followed in 1968 by a cellular biology program. A sensitive whole-body counter was built in 1964. The nuclear medicine program directed research attention to gallium-67 and the use of positron-emitting, short half-life radionuclides, particularly carbon-11.

Much of the clinical cancer research in the division ended in 1975, when the Atomic Energy Commission (AEC) was incorporated into the Energy Research and Development Administration (ERDA). In the same year, Dr. Lushbaugh succeeded Dr. Andrews as division chairman. Most of the research interests of the division continued under ERDA and its successor, the Department of Energy, but the scope of the division work was broadened to

1028595

include concern for health effects associated with various other energy technologies besides nuclear.

A major new program in epidemiology began in 1976 and is today the largest program in the division. Studying a portion of employees of DOE and its predecessors, the division's Center for Epidemiologic Research is a major component of DOE's Health and Mortality Study. The Radiation Emergency Assistance Center/Training Site (REAC/TS) was established the same year for training physicians, nurses, paramedical personnel, and health physicists in the health care management of victims of radiation accidents, for maintaining accident registries, and for conducting research on biological dose assessment through cytogenetic methods. At the request of DOE, the division continues to have a responsibility for managing the Food and Drug Administration's investigational new drug approval for the use of calcium and zinc diethylenetriaminepentaacetic acid (DPTA) as a chelating agent and in managing a registry of their use. Studies on the transport of radionuclides through the food chain were added to the programs of the division, and the nuclear medicine section began to use an Emission Computerized Axial Tomograph (ECAT) in clinical trials in 1977.

The division will continue to exercise the strengths and capabilities necessary to meet the needs of DOE and other agencies and to move in new directions as different problems and priorities evolve in biomedical research.

Accounts of the research progress of all the division's programs in FY 1984 make up the following report. Detailed descriptions of these efforts can be found in the published papers listed on pages 21–27. Requests for reprints can be sent to one of the authors or to the office of the chairman.

W. W. Burr, Jr., Chairman
C. C. Lushbaugh, Chairman (Outgoing)
Fred Snyder, Associate Chairman

Table of Contents

Scope and Highlights of Research Programs 5
- Biochemistry 5
- Preclinical and Clinical Nuclear Medicine 8
- Cytogenetics 11
- Food Chain Transport 13
- Marmoset Research Program 14
- Radiation Emergency Assistance Center/Training Site 15
- Epidemiology 17

Publications 21
- Full-length Journal Articles 21
- Chapters, Review Articles, Other Publications, and Videotapes 23
- Published Abstracts of Papers Presented at Meetings 26
- Miscellaneous Talks Presented at National and International Scientific Conferences 27

Summaries of Published Papers 29
Training Programs 39
Non-DOE-Funded Research 41
Collaborative Research 43
Administration, Committees, and Staff 46

1028596

1948
1961



Marshall H. Brucer, Chairman 1948–1961

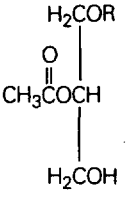
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Scope and Highlights of Research Programs

Biochemistry

Major research projects in the Medical and Health Sciences Division's biochemistry program at Oak Ridge Associated Universities focus on bioactive lipid molecules, such as platelet activating factor (PAF), that mediate diverse cellular functions and on the role of ether-linked lipids in biological membranes. In addition, recent studies have been conducted with a group of unnatural PAF analogs that exhibit highly selective antineoplastic activity toward tumor cells. The specific biochemical problems pursued in this program are closely aligned to normal physiological processes or to disorders in disease models used to study cancer, hypertension, and inflammation. Highlights of significant research accomplishments in biochemistry are summarized in the subsequent paragraphs of this section.

A neutral lipid with antihypertensive activity. Last year, the biochemistry staff discovered a neutral lipid (known to be a precursor of PAF) that possessed both antihypertensive and platelet aggregation activities. Characterization of the hypotensive response in SHR genetic hypertensive rats revealed that the essential structural requirements for the hypotensive activity of the neutral lipid was an *O*-alkyl moiety at the *sn*-1 position and either an acetyl or propionyl group at the *sn*-2 position of glycerol.



Other neutral lipid analogs, such as 1-acyl-2-acetyl-, 1-alkyl-2-acyl-, 1-alkyl-3-acetyl-, 1-alkyl-2,3-diacetyl-, and 1-alkyl- glycerols, were inactive. The active species were about a thousandfold less potent than an equivalent amount of PAF, but the lowering of blood pressure lasted for a significantly longer period. The hypotensive response obtained with the alkylacetylglycerols was similar to an uncharacterized antihypertensive neutral renal lipid (ANRL) described by other investigators.

The metabolism of the hypotensive neutral lipid was investigated by incubating [^{3}H]alkylacetylglycerols with rabbit platelets for 20 min. Six metabolites, identified on the basis of chemical and enzymatic reactions combined with thin-layer or high-performance liquid chromatographic analyses, were (% ^{3}H) alkylacetylglycerophosphocholine (PAF) (7.5%), alkylacyl(long chain)glycerophosphocholine (12.6%), alkylacetylglycerophosphoethanolamine (8.3%), alkylacetylglycerophosphate (6.4%), alkylacyl(long chain)glycerophosphoethanolamine (5.4%), and alkylglycerols (12.6%). These results indicate that the metabolic pathway for alkylacetylglycerols involves reactions catalyzed by choline- and ethanolamine-phosphotransferases, an acetylhydrolase, an acyltransferase, and a phosphotransferase. The step responsible for the biosynthesis of PAF could explain the hypotensive activities of the alkylacetylglycerols.

Enzymatic evidence has been obtained to support the following

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sequence of reactions for the de novo biosynthesis of alkylacetylglycerols in rat spleen microsomes.

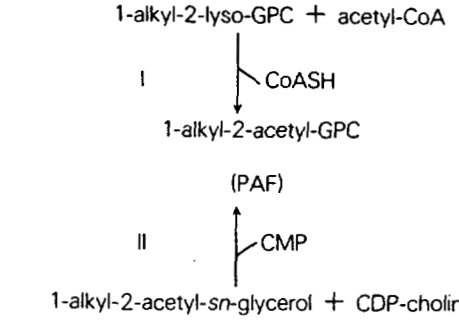
(I) 1-alkyl-2-lyso-*sn*-glycero-3-P → 1-alkyl-2-acetyl-*sn*-glycero-3-P

+ acetyl-CoA + CoASH

(II) 1-alkyl-2-acetyl-*sn*-glycero-3-P → 1-alkyl-2-acetyl-*sn*-glycerol + P_i

Reaction I is catalyzed by an acetyltransferase and Reaction II by a phosphohydrolase. The capacity of cells to produce alkylacetylglycerols via a branch of the main alkyl-glycerolipid pathway suggests the antihypertensive neutral lipid could be of physiological importance as another factor to consider in the regulation of blood pressure. Also, since alkylacetylglycerols are substrates for the dithiothreitol-insensitive cholinephosphotransferase in PAF biosynthesis, the finding that the alkyl-lyso analog of phosphatidic acid can be converted to alkylacetylglycerols by an acetylation and dephosphorylation step lends credence to the importance of the alternate cholinephosphotransferase pathway in PAF biosynthesis in certain cells.

Biosynthesis of PAF. The two enzymatic routes known for the biosynthesis of PAF were originally described by this laboratory. The reaction most extensively studied is catalyzed by an acetyltransferase (Reaction I). An alternate pathway that uses the neutral antihypertensive lipid (alkylacetylglycerols) is catalyzed by a dithiothreitol-insensitive cholinephosphotransferase (Reaction II).



Recent results from this laboratory have indicated the acetyltransferase activity is regulated by a protein kinase that converts the acetyltransferase to an active phosphorylated form and by a phosphohydrolase that inactivates the enzyme by removing the phosphate.

Collaborative experiments with S. Wasserman (University of California, San Diego) demonstrated that chemotactic agents are very effective stimuli of acetyltransferase activities in eosinophils isolated from patients with eosinophilia. Exposure of eosinophils to ECF-A, C5a, formyl-methionylleucylphenylalanine, or ionophore 23187 stimulated acetyltransferase activity up to fourfold in a dose-, time-, and extracellular Ca^{2+}/Mg^{2+}-dependent manner. The increased release of PAF by eosinophils when activated by chemotactic agents indicates these cells participate in

inflammatory reactions through the stimulation of acetyltransferase activity by such agents to produce PAF.

During inflammatory stimulation of rat alveolar macrophages, both PAF and arachidonic acid metabolites are produced from the common precursor, 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine. Although the release of arachidonic acid from diacyl phospholipids also occurs in the stimulated macrophages, the concept of alkylarachidonoyl-glycerophosphocholine serving as a shared precursor of both the PAF and arachidonate classes of mediators provides an important new basis for designing experiments to develop a better understanding of how such diverse lipid mediators are regulated during inflammatory reactions.

Catabolism (inactivation of PAF). PAF is inactivated when the acetate moiety is hydrolyzed by an acetylhydrolase. The serum-enzyme has been found among a wide variety of mammals and lower vertebrates; however, acetylhydrolase activity is conspicuously absent in animals of the Aves and Amphibia classes. The product of the acetylhydrolase reaction, "lyso"-PAF (1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine), is rapidly reacylated in most cells with a long chain fatty acid. Although the significance of "lyso"-PAF has recently been questioned by another laboratory, our data, based on kinetics and isotopic dilution of the lyso intermediate, have provided strong evidence to demonstrate "lyso"-PAF is an obligatory intermediate formed during the inactivation of PAF. Moreover, when PAF is inactivated by both rabbit platelets and rat alveolar macrophages, we found arachidonic acid (approximately 80%) to be the main fatty acid used in the reacylation of the "lyso"-PAF. These observations further emphasize the close association of PAF and arachidonic acid metabolism in platelet and alveolar macrophages.

PAF analogs as selective antineoplastic agents. Structural analogs of PAF with an acetamide or *O*-methoxy substituent at the *sn*-2 position have been shown to possess highly selective cytotoxic properties toward human leukemia cells in cell culture when compared to responses obtained with polymorphonuclear neutrophils and skin fibroblasts from humans. Substitution of a 20 carbon branched-chain phytanyl moiety in place of the straight chain alkyl moiety at the *sn*-1 position of various ether-linked phospholipid analogs caused a three- to ten-fold reduction in their cytotoxic potency toward the leukemic cells. Although natural L-PAF was relatively innocuous in terms of its cytotoxic activity, the close structural similarities between the unnatural cytotoxic alkyl analogs and native PAF suggest that the cytotoxic response in tumor cells could be due to the interference of some essential cellular function(s) mediated by PAF. Future studies in this project are directed toward elucidating the biochemical mechanisms that might explain the selective antineoplastic activities of the methoxy- and acetamide-substituted *sn*-2 analogs of PAF.

Technical developments for lipid analysis. A novel method was developed for the quantitative analysis of subclasses of phospholipids by thin-layer chromatography and molecular species of each subclass by high-performance liquid chromatography. A phospholipid class is first converted to diradylglycerols by phospholipase C treatment so that benzoate derivatives can be formed; the latter can then be quantitated spectrophotometrically at 230 nanometers. The diacyl-, alkylacyl-, and alk-1-enylacyl- benzoates of glycerol are easily resolved by thin-layer chromatography, whereas the molecular species among each subclass can be separated and quantitated on-line by

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high-performance liquid chromatography. This analytical approach is applicable to studies of a wide variety of biologically important lipids, such as phosphatidylcholine, phosphatidylinositol, PAF, plasmalogens, and diacylglycerols.

In collaboration with C. Piantadosi and J. Surles (University of North Carolina, Chapel Hill), this laboratory accomplished during the past year the complete chemical synthesis of PAF and related analogs containing unsaturation at the *sn*-1 alkyl chain. This method makes it possible to prepare PAF with defined unsaturated alkyl moieties that can be tritiated for use in metabolic studies. Biological studies of the synthetic PAF preparations revealed insertion of double bonds in the alkyl group of PAF did not alter its platelet aggregating properties.

Preclinical and Clinical Nuclear Medicine

Objectives of these related research programs are to develop new radiopharmaceutical agents and to carry out phase I and II testing of such materials in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of appropriate imaging agents, tests of these substances in animals for effectiveness and toxicity, and efficacy trials in humans according to U.S. Food and Drug Administration rules and guidelines. Emphasis is placed on the use of positron-emitting radionuclides to permit tomographic imaging.

Positron decay produces annihilation photons that can be detected outside the body by coincidence counting. By viewing these emissions through various angles with a positron-emission computerized tomograph, it is possible to produce three-dimensional, cross-sectional images of the tissue distribution of radiopharmaceuticals labeled with positron emitters. The images obtained are similar to autoradiograms but, unlike autoradiography, the positron tomographic technique is noninvasive. The images obtained by positron tomography reflect differences in tissue function rather than differences in tissue density as is the case with x-rays. Thus, they can be used specifically to detect metabolic alterations associated with disease processes. The clinical nuclear medicine program uses tomography to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications in neoplastic and pancreatic diseases with emphasis on more quantitative evaluation of the in vivo distribution of such radiopharmaceuticals.

The facilities of the clinical nuclear medicine program have been expanded and improved through the addition and implementation of new software interactive programs for ORAU's ECAT II™ positron tomographic scanner. These new programs will improve quantitative imaging with resultant better interpretation of regions of interest, especially in the thorax.

Amino acids with positron-emitting carbon-11 substituted for stable carbon-12 offer distinct advantages for studying protein synthesis and protein turnover in vivo, since ^{11}C-labeled agents are indistinguishable physiologically and chemically from their unlabeled counterparts. The laboratory's long-standing interest in ^{11}C-labeled amino acids continues. However, because of the shutdown of the Oak Ridge National Laboratory (ORNL) 86-inch cyclotron, this research is now carried out in collaboration with the Medical and Chemistry Departments of Brookhaven National Laboratory (BNL) (Drs. A. B. Brill, S. C. Srivastava, J. S. Fowler, and A. P. Wolf).

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Several years ago we developed a general method for the synthesis and purification of ^{11}C-carboxyl-labeled amino acid racemates (mixtures of the naturally occurring L-isomer and the unnatural D-isomer). For truly physiologic studies of the pancreas, the ^{11}C-labeled L-amino acid must be separated from the mixture by a process that is compatible with the 20.4-min half-life of carbon-11. Optical resolution of DL-[^{11}C]tryptophan and DL-[^{11}C]valine has been accomplished by high-performance liquid chromatography (HPLC) using a conventional reverse-phase HPLC column and a chiral mobile phase.

Preclinical animal studies have shown that the L-isomers of tryptophan and valine are most suitable for the evaluation of pancreatic function. In addition, the unnatural D-forms of these amino acids when used in conjunction with the L-forms appear to be of value in the differential diagnosis of pancreatic cancer. The Investigational New Drug (IND) applications for both the L- and the D-isomers of ^{11}C-labeled tryptophan and valine have been approved by the U.S. Food and Drug Administration for clinical phase I and phase II testing in patients with pancreatic cancer. Preliminary clinical investigations of the L-isomers using the ECAT II™ positron tomograph showed that L-[^{11}C]tryptophan is the most useful agent for imaging the pancreas. Studies have been initiated using BNL's PETT III positron tomograph to compare the L- and D-isomers of ^{11}C-labeled tryptophan and valine in pancreatic cancer patients. Our laboratory recently became the first to administer D-[^{11}C]valine to a patient with pancreatic carcinoma. This preliminary study with the D-enantiomer showed lower uptake in the liver than observed previously with the racemic mixture and an increased accumulation in the region of the tail of the pancreas that was consistent with tumor uptake at that site.

The unnatural ^{11}C-labeled alicyclic amino acid, [^{11}C]l-aminocyclobutanecarboxylic acid ([^{11}C]ACBC), is nonmetabolizable and has excellent tumor-localizing properties. [^{11}C]ACBC, therefore, has potential as a unique tool for measuring amino acid transport into tumors, since it may reflect the tumor's metabolic and proliferative state. In collaboration with BNL, we are evaluating the potential of [^{11}C]ACBC to monitor tumor therapy by positron tomographic quantitation of the tumor uptake before, during, and after chemotherapy or radiotherapy.

Another unnatural, nonmetabolizable amino acid, α-aminoisobutyric acid (AIB), has been shown by our research group and others to be taken up by tumor tissue with a specificity similar to that of ACBC. We have also shown with the ^{14}C-labeled compound that a monofluoromethyl derivative of AIB (DL-α-monofluoromethylalanine) gives the same tumor uptake as the parent AIB. Labeling DL-α-monofluoromethylalanine with fluorine-18 would provide a tumor-imaging agent labeled with a positron emitter that has a longer half-life than carbon-11 (110 min vs 20.4 min) and which can be produced using either an accelerator or a reactor. We expect that clinically useful quantities of fluorine-18 as aqueous fluoride ion will soon become available to our program via the Oak Ridge National Laboratory Tandem Van de Graaff accelerator. Development of methods for rapid synthesis and purification of 18F-labeled DL-α-monofluoromethylalanine is in progress.

Another research effort is directed toward the development of radiopharmaceuticals for positron tomographic studies of pulmonary function. One of the major metabolic activities of the lungs involves regulation of various biogenic amines. ^{11}C-Labeled straight-chain primary aliphatic amines

1028602

are sequestered very selectively by lung endothelial cells. However, the rapid metabolic loss of the label from the smaller amines in this series makes it difficult to quantitate their pulmonary uptakes accurately. We have found, however, that a long straight-chain amine, octadecylamine, has a high pulmonary uptake with no metabolic loss from the lung between 1 and 30 min after injection. This amine and others were investigated in a radiation-induced disease model via assessment of amine metabolism as measured by tissue distribution studies and $^{14}CO_2$ exhalation through a volume-controlled metabolic cage.

Because of the shutdown of the ORNL 86-inch cyclotron, we are in the process of identifying useful agents labeled with other positron-emitting radionuclides. An IND application for ^{64}Cu citrate as an agent for positron tomographic detection and staging of soft tissue tumors was recently approved by the U.S. Food and Drug Administration. We are also evaluating in animal models the potential of ^{64}Cu-labeled agents for the diagnosis and study of other disease processes.

We have recently initiated a study of the potential of radiolabeled monoclonal antibodies for treatment of cancer. Antibodies to tumor-associated antigens have been considered as leading candidates for the long-sought "magic bullet." However, only since the discovery and refinement of the monoclonal antibody technique (production of antibodies in tissue culture by lymphocytemyeloma hybridomas) have homogeneous, high-purity antibodies been available in sufficient quantity for this possibility to be tested. Monoclonal antibodies labeled with radioiodine have been investigated with some success for cancer diagnosis and therapy; however, in vivo deiodination is a considerable drawback to use of this type of radiolabeled agent. In collaboration with Dr. Zenon Steplewski of the Wistar Institute (Philadelphia), we are studying the potential of chelate-conjugated monoclonal antibodies to permit radiolabeling with metal ion radionuclides. Techniques for covalent attachment of the chelating agent diethylenetriaminepentaacetic acid (DTPA) to monoclonal antibodies have been developed, as well as purification techniques for the radiolabeled antibodies. We have demonstrated good tumor uptake of a monoclonal anticolorectal antibody labeled with gamma-emitting indium-111 in nude mice bearing human colon tumors. We plan to extend this work to studies of the potential of radioimmunotherapy using various tumor-specific monoclonal antibodies labeled with alpha-emitting fermium-255 and beta-emitting yttrium-90.

The research interests of this program have been broadened to include the cardiovascular system and hypertension. Investigation of differences in the cotransport of sodium and potassium by erythrocytes from normotensive and hypertensive subjects is being actively pursued. In addition, we are in the process of establishing a small colony of New England Deaconess Hospital rats (courtesy of the National Hypertension Association). These rats develop spontaneous pheochromocytomas and will also accept pheochromocytoma tumor implants. Therefore, they are potentially useful as animal models for testing new tumor-localizing agents. In the areas of clinical pharmacology/clinical hypertension, we have completed preliminary plans and are ready to initiate a collaborative trial with the University of Tennessee Memorial Research Center and Hospital to assess the efficacy of a new calcium entry blocker for treatment of hypertension.

Chelation therapy with DTPA drugs is the method of choice for

1028603

decorporation of internally deposited actinide contaminants. As part of the division's project to coordinate national use of DTPA (see REAC/TS section), the Radiopharmaceutical Development and Biological Chemistry programs collaborate in research on new, highly lipophilic chelating agents. We have shown that Ca-DTPA encapsulated in liposomes is more effective than the free form of the drug in removing colloidal ytterbium-169, a model contaminant, from rats. However, liposomes of the commonly used multilamellar vesicle (MLV) form cause spleen weight increases in rats, which has hindered further application of these agents. We have recently shown that Ca-DTPA encapsulated in a new type of liposome, called large unilamellar vesicles (LUV), gives the same removal of ytterbium-169 as the MLV but without the splenomegaly problem observed with the MLV.

Cytogenetics

Research activities in the cytogenetics program are focused on two main areas: (1) development and application of cytogenetic methods for biological dose estimation in persons having recent or long-term exposures to ionizing radiation; (2) continuation of basic research efforts aimed at defining the mechanisms involved in the induction, expression, and persistence of chromosome damage in somatic and germ cells following exposures to ionizing radiation or to mutagenic or carcinogenic chemicals. These objectives are accomplished through systematic collection and evaluation of cytogenetic data from human populations, in vitro experiments using human cells, and evaluations of radiation and chemical toxicity in experimental animals used as surrogates for humans.

The analysis of radiation-induced chromosome lesions in cultured lymphocytes is the most sensitive biological method currently available for estimating the absorbed dose in persons having recent exposures to penetrating radiations. As an adjunct to consultations by the Radiation Emergency Assistance Center/Training Site during last year the cytogenetics group provided biological dose estimates for fourteen persons, including nine adults and one child having suspected exposures to gamma radiation in the recent cobalt-60 accident that occurred in Juarez, Mexico. The results of extensive analyses of chromosome aberrations and micronuclei in lymphocytes demonstrated that eight of these individuals received substantial radiation exposures. These heavily exposed persons included not only men who worked at the contaminated Yonke Fenix junkyard but also community residents who lived in a crowded residential area where a contaminated pickup truck was parked for several days. Additional examinations are being considered, and plans for a long-term follow-up are being made.

In addition to evaluations in exposed persons, we have continued basic research studies to define more precisely the dose response relationships for radiations having differing linear energy transfer (LET). As an invited participant in the International Atomic Energy Agency's Coordinated Research Program on "the use of chromosome aberrations in biological dosimetry," our laboratory derived an in vitro dose response curve for chromosome aberrations in first division human lymphocytes exposed to cobalt-60 gamma radiation. Similar curves are being generated by laboratories in 10 other countries for inter-laboratory comparisons and standardization of in vitro dose response data. We are also collaborating with scientists at the Radiation Effects Research

1028604

Foundation and at Lawrence Livermore and Los Alamos National Laboratories in determining the dose response relationship for aberration induction in lymphocytes exposed to fission spectrum neutrons generated by a replica of the "Little Boy" assembly. It is anticipated that this curve will be useful in interpreting cytogenetic follow-up data from Hiroshima A-bomb survivors who were exposed to attenuated spectra of mixed gamma radiation and fission spectrum neutrons in 1945.

We have also continued long-term follow-up studies on specific groups of persons exposed many years previously. Our study populations include selected patients having partial body radiotherapy and persons having accidental exposures to high-LET or low-LET radiation, including survivors of the Y-12, UT-AEC, and Hanford accidents that occurred in 1958, 1970, and 1976, respectively. We have recently completed evaluations of persistent radiation-induced chromosome aberrations in cultured lymphocytes from 300 persons who received radiation therapy for enlarged tonsils or for carcinoma of the cervix 20 to 50 years ago. These studies are being conducted in collaboration with investigators at the Radiation Epidemiology Branch of the National Cancer Institute under support of an NCI-DOE Interagency Agreement. Data on the types and frequencies of chromosome aberrations observed in over 55,000 lymphocyte metaphases from these irradiated and control subjects are being analyzed to determine whether the incidence of chromosome lesions observed many years after exposure can be correlated with the total dose patients received, the region of the body exposed, the age at time of exposure, or the number of years since exposure. We are currently assessing persistent chromosome aberrations in 300 selected patients who received multiple fluoroscopies for diseases of the lung or radiotherapy for enlarged thymus in the 1940s. Such cytogenetic evaluations in cohorts of persons exposed to radiation many years previously will eventually provide information on the usefulness of somatic cell data for predictions of the risk of late effects in exposed populations.

In conjunction with these efforts to define more precisely the cytogenetic sequelae of human exposure to radiation, we have continued basic research in mammalian cytogenetics and reproductive toxicology. From ongoing studies in various experimental animal models, we hope to obtain data that will provide criteria for the extrapolation of such results to humans.

Under support of the National Institute for Child Health and Human Development we have evaluated rabbit embryos for the transmission of chromosome aberrations induced in germ cells of female rabbits. These studies have demonstrated that the frequency of chromosome aberrations induced by certain clastogenic chemicals in somatic cells is predictive of the level of effect observed in the conceptus of the female. Our current studies are aimed at evaluating the sensitivity of the female germ cells to chemicals at various stages in their development.

Under an agreement with the National Toxicology Program we have made additional refinements in the previously developed protocol for the in vivo cytogenetic assay of chemicals. Procedures for sister chromatid exchanges and chromosome aberrations in mouse bone marrow cells have been standardized in cooperation with a similar program at Brookhaven National Laboratory. The minimal interlaboratory variation obtained by this procedure permits the simultaneous testing of different chemicals in the two laboratories and the direct comparison of results. Seven chemicals submitted

1028605

by the National Toxicology Program were completely evaluated in this system during the current year.

Food Chain Transport

The food chain transport program traces the transport of toxic organic compounds and radionuclides and their metabolites through the food chain. In 1984 the research focused specifically on the products of synfuel generation and actinide metabolism. The studies related to synfuel generation ended in midyear when the Environmental Protection Agency ended its risk analysis studies at Oak Ridge and Brookhaven National Laboratories.

Synfuel studies. The synfuel research supported the risk analysis groups at the two national laboratories by developing data for their food chain analysis of synfuels-related chemicals.

The ORAU research determined the uptake and biological retention of RAU (risk analysis unit) compounds in food-producing animals (dairy cattle, swine, and poultry) and yielded the transfer coefficients required for food chain exposure. Specific goals of this program were to determine the biological retention of representative chemicals following both acute and chronic oral administration to food-producing animals, to determine the accumulation and loss of these chemicals in consumable products following ı acute dose, to determine the accumulation in tissues when they are Jministered chronically, and to develop and employ practical methodology for the isolation and quantitation of selected RAU parent chemicals that are representative of the major chemical classes found in coal liquefaction/shale oil products and production wastes.

Radiotracer methodology was used to determine the accumulation of these compounds in such consumable products as milk, eggs, and meat. Tissues were also disrupted and fractionated to determine soluble versus tissue-bound radiolabeled compounds, to determine the portion of parent compounds present in tissue, and to estimate the metabolites of parent compounds.

^{14}C-Labeled aniline (aromatic amines excluding the N-heterocyclics RAU), and indole (the neutral N-, O-, S-heterocyclics RAU) were administered orally to dairy cattle, swine, and laying pullets as a single dose (acute) or daily for 31 days (chronic). The data from these experiments demonstrate that aniline and indole are retained in consumable meats, eggs, and milk in variable amounts. When used as ingredients in prepared foods, significant quantities of these chemicals can accumulate in these products.

Actinide studies. The long-term study of the metabolism of radionuclides continues to be supported by the Nevada Applied Ecology Group of the DOE Nevada Operations Office. This research is designed to evaluate the role of the food chain in exposure of humans to radionuclides from weapons testing and radioactive fallout.

The principal objective of this program is to develop a predictive capability for estimating the possible impact, expecially on humans, of the release of radionuclides upon the biosphere from any credible type of nuclear :tivity. These studies deal with internal emitters since it is highly probable ınat most human exposures will involve slow delivery of low total doses of radionuclides to a segment of the population.

To accomplish the primary objective of this program our studies have focused on the gastrointestinal absorption, tissue distribution and

1028606

redistribution, tissue retention, and excretion of specific radionuclides. Also important is the determination of any biological effects of the radiation in humans from the internally deposited radionuclides. Therefore, these studies include the evaluation of cytogenetic, spermatogenic, and oogenic effects, as well as the gonadal localization of representative radionuclides of interest.

^{241}Am-Citrate (5 mCi) was given orally to swine (miniature and domestic) that were killed 1, 2, 4, 8, 16, 24, 48, 72, 96 hr, and 30 days later to determine the tissue distribution of the radioactivity. Differences observed between miniature and domestic swine suggest a relationship exists between surface area to the extent ^{241}Am is absorbed in the gastrointestinal tract. Tissue distribution data showed the bulk of the radioactivity was in bone (50%), liver (20%), and soft tissues (30%). Cytogenetic investigations of the exposed animals revealed no significant increases in chromosomal anomalies over background at any time. In gametogenetic studies, alpha tracks were seen most frequently in the medullary portion of the ovary, but they occurred only randomly in the testes. Enhanced necrosis was observed in both the oocytes and gonocytes, but the total number of germ cells was unaffected.

Experiments are currently under way to determine the fate of ingested europium in swine. These data will be used to predict better the potential body burden and the radiation hazard that humans can receive from europium that enters the food chain.

Marmoset Research Program

In March, 1983, the Medical and Health Sciences Division assumed responsibility for operation of an ongoing National Cancer Institute (NCI) contract which was awarded to ORAU in July, 1982. The contract's objectives are (1) to maintain a breeding colony of *Saguinus oedipus oedipus* and to produce a maximum number of healthy animals at minimum cost; (2) to maintain experimental tamarins injected with oncogenic or potentially oncogenic substances in a biohazard containment area sufficient to protect breeding stock animals, personnel, and technical support from contamination; and (3) to provide professional and technical support for safely and accurately performing experimental protocols approved and requested by the National Cancer Institute. During the period of this contract, offspring have been successfully produced by using principally colony-born breeding pairs. Thus, despite the fact that wild-caught *S. o. oedipus* are no longer imported, investigators can anticipate that adequate numbers of tamarins can be available from captive sources such as the ORAU colony.

Research into the behavioral, endocrine, and nutritional determinants of reproductive competence in the cotton-top tamarin is proceeding with funding from NIH's Division of Research Resources (DRR). An objective is to define the importance of the social experience of rearing infant siblings to a female's subsequent maternal behavior. Reproductive aging in female tamarins is being examined to determine whether these species display endocrine, ovarian, or fertility changes similar to menopausal women. Preliminary results indicate a significant decline in ovarian follicular development with age and reduced fertility in aging cotton-top tamarin females.

During the spring of 1984, ORAU cohosted (along with NCI, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, and DRR from NIH) a workshop to evaluate the marmoset as a potential model for the investigation of gastrointestinal diseases. This meeting brought together

1026607

approximately 100 scientists with broad medical expertise to discuss intestinal diseases in the marmoset and colon cancer in the cotton-top tamarin and to compare these processes with those that occur in humans. Some differences were identified; for example, cancers develop as carcinomas in situ and not from polypoid adenomas. Numerous similarities—distribution of carcinomas in the large bowel, early metastases to regional lymph nodes—were recognized that make this animal appropriate for the study of disease relationships.

A number of collaborative research projects have been initiated and include efforts to define aspects of the marmoset as an experimental animal and to relate these findings to disease processes. Extramural investigations involve the following studies: T and B cell immunology, cell culture of tamarin colon cancers, sterol metabolism and fecal bile acid profiles, in vitro tetraploidy in skin fibroblasts as a marker of cancer susceptibility, genetic segregation and familial relationships in tamarin colon cancer, and idiopathic ulcerative colitis in colon cancer susceptibility and expression. Other studies are investigating the effect of coronaviruses, lectin binding, fecal toxins, carcinoembryonic antigens, serum enzymes, and fecal enzymes in colonic diseases of marmosets. We are characterizing the development of preneoplastic lesions and associated changes preceding the appearance of colon cancer and, in particular, assessing the causative role of colitis in tamarin colon cancer.

Considerable progress has been made in the β-glucuronidase project. sing the modified fecal β-glucuronidase assay, we have identified and separated the bacteria-derived enzyme activity from that formed by the colonic cells. By use of a sonication technique, we have further identified intracellular, extracellular, and total amounts of the enzyme activity. Activity levels increase with age and vary among the three tamarin species; however, the highest activities were found in *S. o. oedipus*, the tamarin with the greatest incidences of colon cancer. In adult *S. o. oedipus* with histologically confirmed colon cancer, the activity of the intracellular form of β-glucuronidase in colonic cells was 12 times higher than that of age-matched noncancer tamarins. This finding suggests that fecal β-glucuronidase may be a useful marker to indicate the presence of early colon cancer in tamarins and possibly in humans. We are now investigating this hypothesis in families of animals that have a high risk of cancer, both before they develop cancer and during its development.

Radiation Emergency Assistance Center/Training Site

The staff of the Radiation Emergency Assistance Center/Training Site (REAC/TS), a treatment center and training unit, continue to provide assistance as needed in the management of health problems associated with radiation accidents and to teach medical and paramedical personnel similar management skills. A radiological emergency response team consisting of approximately 30 physicians, nurses, health physicists, laboratory technicians, emergency coordinators, record clerks, and communications coordinators is on 24-hour call to render medical and health physics support by direct participation or consultation. With its capabilities for accident response EAC/TS is a definitive care center for DOE/ORO facilities and all other DOE ites nationwide and is medical backup to the continent-based nuclear Navy. REAC/TS provides a medical consultation team as part of the DOE/DOD Accident Response Group (ARG), is a medical backup to the commercial nuclear power generating industry, is a World Health Organization (WHO)

1028608

collaboration center for the Western Hemisphere, and conducts research in cytogenetic dosimetry, the radiobiology of radiation-induced injury, and the use of diethylenetriaminepentaacetic acid (DTPA) as an experimental drug for removing internally or topically deposited toxic or radioactive metals from tissue.

Program accomplishments within this mission are reflected by REAC/TS response to over 260 radiation accidents or incidents, including WHO assistance to the governments of Jamaica, Venezuela, and, most recently, Mexico. As a WHO collaboration center, REAC/TS provided direct assistance to the Mexican government and medical community of Juarez, Mexico, following the accidental exposure or contamination of over 200 private citizens following mishandling of a cobalt-60 source contained in a teletherapy unit. Cytogenetic dosimetric evaluations were completed on 10 persons suspected of having the highest exposures in the Juarez accident. Additionally, REAC/TS conducted cytogenetic evaluations in other persons having suspected accidental radiation exposures and coordinated clinical usage of DTPA administration to victims of actinide contamination.

During the past year, REAC/TS continued to offer courses and training programs directed to the medical management of radiation accidents. Participants included 68 physicians, 41 health physicists, and 77 paramedical professionals. Since 1976, a total of 66 courses have been completed in the REAC/TS facility and 1,302 individuals trained. Graduates of REAC/TS courses include personnel from DOE and DOE contractor facilities, other federal agencies (Environmental Protection Agency , Health and Human Services, Nuclear Regulatory Commision, Department of Defense, etc.), the military, private hospitals, Veterans Administration hospitals, commercial nuclear facilities, nuclear support facilities (e.g., commercial fuel fabrication), private consultants, and private practice physicians. Citizens from 11 foreign countries are represented among REAC/TS course graduates. Less formalized training and information were provided through talks, a special exhibit shown at various professional meetings, and publication of a quarterly newsletter.

Long-term follow-up of victims involved in earlier radiation accidents continued through the REAC/TS radiation accident registry. The registry system is divided into four component parts: (1) the U.S. Radiation Registry, (2) the DOE $\geq$ 5 Rem Study Registry, (3) the DTPA Registry, and (4) the Foreign Registry. The REAC/TS Registry has identified 236 radiation accidents worldwide involving 1,187 individuals, of whom 549 received significant exposures and/or contamination. Twenty-nine fatalities are included in the REAC/TS Registry. Within the U.S. Radiation Registry component, a follow-up of accident survivors of earlier serious radiation accidents continues. The DOE $\geq$ 5 Rem Study Registry contains data on 3,153 persons, among whom 2,972 are included in a study cohort of workers who received $\geq$ 5 rem per year between 1947 and 1978. The end point of such follow-up is to establish trends in morbidity and mortality. Current status of the DTPA Registry is that a physician in each of 47 facilities (44 in the U.S. and one each in Canada, England, and Spain) was authorized as a co-investigator on the Food and Drug Administration's investigational new drug application to administer calcium and zinc forms of DTPA. Within the Foreign Registry system, REAC/TS continues to maintain contact with physicians treating radiation accident victims outside the United States.

An additional component of the REAC/TS mission has evolved and has

1028609

recently become more visible as litigation about alleged radiation accidents has come to trial. REAC/TS staff are providing forensic medical consultation in compensation cases where it is alleged that radiation exposures are causally related to the subsequent manifestation of disease. The REAC/TS team provides consultation to legal counsel on the medical causation of health problems allegedly related to radiation exposure, the pathological accuracy of diagnosis of alleged radiation injuries, the adequacy of care received by patients with radiation-related injury or illness, and the validity and credibility of death certificate information. These consultations are provided on the basis of REAC/TS medical expertise and are not biased toward plaintiffs or defendants. The interaction of the staffs of REAC/TS and Epidemiology programs makes these consultations unique.

Epidemiology

The division's Center for Epidemiologic Research continues to evaluate health and mortality among workers employed at some time since 1942 in selected facilities of the U.S. Department of Energy (DOE) and its predecessors (Manhattan Engineer District [MED], Atomic Energy Commission [AEC], Energy Research and Development Administration [ERDA]) and their contractors in Oak Ridge and other locations nationwide. CER's studies and parallel studies of Hanford workers by the Hanford Environmental Research Foundation with the Pacific Northwest Laboratories and of plutonium workers by epidemiologists at Los Alamos National Laboratory evaluate health and mortality data on approximately 600,000 employees and former employees of some 76 facilities nationwide. Collectively these studies make up the DOE Health and Mortality Study (HMS).

The main objective of the part of the HMS assigned to ORAU is to examine relationships between occupational exposure to ionizing radiations from external and/or internal sources and subsequent health and mortality. The health effects of chemical toxicants, especially uranium and other toxic metals, also are being investigated as both primary and potentially confounding (with respect to radiation) occupational exposures. ORAU's specific responsibilities are for studies of workers within the Oak Ridge Operations area. This area includes workers at DOE facilities now operated by Martin Marietta Energy Systems, Inc., in Oak Ridge, Tennessee, and Paducah, Kentucky; workers at the Gaseous Diffusion Plant at Portsmouth, Ohio; and workers at the Feed Materials Plant operated by NLO, Inc., at Fernald, Ohio. Workers at the Savannah River Plant, operated by E. I. du Pont de Nemours & Company in Aiken, South Carolina, are included in a collaborative study being conducted by ORAU and Los Alamos National Laboratory.

A final analysis of mortality was completed for workers employed between 1943 and 1972 at the Oak Ridge National Laboratory with respect to their radiation exposure and the types of work done. Mortality studies of workers at the Oak Ridge Y-12 and Gaseous Diffusion Plants are in progress. Final analysis was completed of mortality among a subcohort of men exposed to elemental mercury while employed at Y-12 between 1953 and 1963. A previously reported case control study of lung cancer deaths among workers at the Tennessee Eastman Corporation's Oak Ridge facility is being updated. The updated study will include the 530 lung cancer deaths that have occurred in this population through 1979. Workers employed at all the DOE facilities in Oak Ridge are included in an ongoing study of present and former

1028610

workers employed during the World War II period. This study will consider the age-specific death rates, taking into account socioeconomic status and birth year in addition to occupational exposure to radiation. Case control studies of brain cancer and hematologic malignancies among all workers at DOE's Oak Ridge facilities also are in progress. A study designed to evaluate the appropriateness of using the residents in the surrounding counties as a comparison population for the worker populations at DOE's Oak Ridge facilities has been completed. A study of mortality among NLO, Inc., workers employed between 1952 and 1977 has also been completed.

The ORAU epidemiology group also is responsible for studies of the health effects of occupational exposure to uranium and uranium compounds at several sites outside Oak Ridge, including selected defunct sites of the Manhattan Engineer District. A recently completed study of mortality among former workers at a MED site at Linde Corporation was conducted in collaboration with Union Carbide Corporation's corporate epidemiology group. Also in progress is a similar study of former MED site workers at Mallinckrodt Chemical Works, St. Louis, Missouri.

A preliminary mortality analysis has been completed for the cohort of 3,100 workers reported to have equaled or exceeded the current annual occupational limit of 5 rems since 1947 while employed at DOE (and predecessor organizations) and contractor facilities nationwide. A follow-up study of morbidity in this population is in progress on a by-site basis.

Another objective of the program is to develop and apply various statistical methods and probabilistic models for the analysis of epidemiologic data and to evaluate critically the methods and results of studies on health effects in populations with occupational or other exposures to ionizing radiations and/or other industrial toxicants. Improved biostatistical methods for the use of multiple variables in cohort analyses have been adapted and applied in certain of the mortality studies. A methodology has been developed that uses radiation monitoring data to estimate an annual radiation dose to the lungs of individual workers exposed to uranium. Expert opinion was provided to DOE concerning the radioepidemiological tables being developed by the Ad Hoc Working Group of the National Institutes of Health as a basis for the compensation of cancer victims in the Utah civilian and uranium miner populations and among military personnel present at atomic tests at the Nevada Test Site.

To facilitate these studies and those segments of the HMS being conducted at Los Alamos and Hanford, we operate the DOE Death Certificate Retrieval Office which obtains information on the vital status and the causes of any deaths among former DOE employees. These data are being accumulated through the cooperation of the Social Security Administration (SSA) and the vital statistics departments of all 50 states. We also have developed several alternate data sources for following up persons whose vital status cannot be determined by the SSA. These sources include state death indexes, the National Death Index, state motor vehicle license departments, the Civil Service Commission, and the Veterans Administration.

The long-term goal of the overall study is to merge all employee rosters and associated exposure and other relevant data where it is epidemiologically feasible. This data base could provide (1) a considerable total of person-rems and years of follow-up necessary for assessment of risks associated with exposure to low levels of ionizing radiation, (2) a basis for continued

1028611

surveillance of DOE workers, and (3) a resource contributing to the resolution of medico-legal arguments and the establishment of regulatory standards for health protection. The data base also provides a retrospective basis for a registry of radiation workers in the United States.

In these studies we have the collaboration of the Epidemiology Departments of the Schools of Public Health of the University of North Carolina, Chapel Hill, and of the University of Alabama at Birmingham.

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Publications

Full-length Journal Articles

Albert, D. H., and Snyder, F.
Release of arachidonic acid from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, a precursor of platelet activating factor, in rat alveolar macrophages.
Biochim. Biophys. Acta. In press.

Blank, M. L.; Byrd, B. L.; Cress, E. A.; Washburn, L. C.; and Snyder, F.
Liposomal preparations of calcium- or zinc-DTPA have a high efficacy for removing colloidal ytterbium-169 from rat tissues.
Toxicology 30:275–281; 1984.

Blank, M. L.; Cress, E. A.; Lee, T-c.; Stephens, N.; Piantadosi, C.; and Snyder, F.
Quantitative analysis of ether-linked lipids as alkyl- and alk-1-enyl-glycerol benzoates by high-performance liquid chromatography.
Anal. Biochem. 133:430–436; 1983.

Blank, M. L.; Cress, E. A.; and Snyder, F.
Methanol toxicity and elevated formate levels in the marmoset.
Res. Commun. Subst. Abuse. 4:259–262; 1983.

Blank, M. L.; Cress, E. A.; and Snyder, F.
A new class of antihypertensive neutral lipid: 1-alkyl-2-acetyl-*sn*-glycerols, a precursor of platelet activating factor.
Biochem. Biophys. Res. Commun. 118:344–350; 1984.

Blank, M. L.; Lee, T-c.; Cress, E. A.; Malone, B.; Fitzgerald, V.; and Snyder,F.
Conversion of 1-alkyl-2-acetyl-*sn*-glycerols to platelet activating factor and related phospholipids by rabbit platelets.
Biochem. Biophys. Res. Commun. In press.

Blank, M. L.; Robinson, M.; Fitzgerald, V.; and Snyder, F.
A novel quantitative method for determination of molecular species of phospholipids and diglycerides.
J. Chromatogr. 298:473–482; 1984.

Brodsky, J. B.; Groer, P. G.; Liddell, R.; Ishimaru, T.; and Ichimaru, M.
Temporal analysis of a dose-response relationship: leukemia mortality in atomic bomb survivors.
Radiat. Res. 99:547–561; 1984.

Cabot, M. C.
Hydrolysis of novel diacylglycerol analogs and phorbol diesters by serum lipase.
Biochim. Biophys. Acta. In press.

Cabot, M. C.
Tumor promoting phorbol diesters: substrates for diacylglycerol lipase.

1028614

Biochem. Biophys. Res. Commun. 123:170–177; 1984.

Cabot, M. C.; Faulkner, L. A.; Lackey, R. J.; and Snyder, F.
Vertebrate class distribution of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase in serum.
Comp. Biochem. Physiol. 78B:37–40; 1984.

Chertok, R. J.; Kullgren, B.; and Burbank, D.
The effects of $CdCl_2$ on the maternal-to-fetal clearance of ^{67}Cu and placental blood flow.
Proc. Soc. Exp. Biol. Med. 176:138–142; 1984.

Clapp, N. K.; Henke, M. A.; Holloway, E. C.; and Tankersley, W. G.
Carcinoma of the colon in the cotton-top tamarin: A radiographic study.
J. Am. Vet. Med. Assoc. 183:1328–1330; 1983.

Clapp, N. K.; Henke, M. A.; London, J. F.; and Shock, T. L.
Enhancement of 1,2-dimethylhydrazine-induced large bowel tumorigenesis in BALB/c mice by corn, soybean and wheat brans. *Nutr. Cancer.* 6:77–85; 1984.

Clapp, N. K.; Lushbaugh, C. C.; Humason, G. L.; Gangaware, B. L.; and Henke, M. A.
Natural history and pathology of colon cancer in *Saguinus oedipus oedipus.*
Dig. Dis. Sci. In press.

Clapp, N. K., and Tardif, S. D.
Marmoset husbandry and nutrition.
Dig. Dis. Sci. In press.

Coffey, J. L.; Hayes, R. L.; Rafter, J. J.; Watson, E. E.; and Carlton, J. E.
Radiation dosimetry and chemical toxicity considerations for ^{99}Tc.
Health Phys. 46:418–422; 1984.

Cragle, D. L.; Hollis, D. R.; Qualters, J. R.; Tankersley, W. G.; and Fry, S. A.
A mortality study of men exposed to elemental mercury.
J. Occup. Med. In press.

Crawford-Brown, D., and Wilson, J.
Observations on very long-term removal of uranium compounds.
Health Phys. In press.

DuFrain, R. J.
Sister chromatid exchange distributions in rabbit lymphocytes treated with streptonigrin.
Environ. Mutagen. 5:813–824; 1983.

DuFrain, R. J.
Is colon cancer familial in cotton-top tamarins?
Cancer Genet. Cytogenet. In press.

DuFrain, R. J.; Littlefield, L. G.; Morrison, W. D.; Huff, V. D.; and Hutton, D.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. III. A postimplantation analysis of streptonigrin effects.
Mutat. Res. 127:73–79; 1984.

DuFrain, R. J.; McFee, A. F.; Linkous, S.; Jennings, C. J.; and Lowe, K. W.
In vivo SCE analysis using bromodeoxyuridine, iododeoxyuridine, and chlorodeoxyuridine
Mutat. Res. 139:57–60, 1984.

Eisele, G. R.
Naphthalene distribution in tissues of laying pullets, swine, and dairy cattle.
Bull. Environ. Contam. Toxicol. In press.

Eisele, G. R., and Erickson, B. H.
Gonadal retention and localization of ^{241}Am in orally exposed swine.
Health Phys. In press.

Erickson, B. H., and Martin, P. G.
Stem-spermatogonial survival and incidence of reciprocal translocations in the γ-irradiated boar.
Environ. Mutagen. 6:219–227; 1984.

Erickson, B. H., and Martin, P. G.
Reproductive and genetic effects of continuous prenatal irradiation in the pig.
Teratology 30:99–106; 1984.

Feuerstein, G.; Lux, W. E., Jr.; Snyder, F.; Ezra, D.; and Faden, A. I.
Hypotension produced by platelet-activating factor is reversed by thyrotropin-releasing hormone.
Circ. Shock 13:255–260; 1984.

Frome, E. L.
The analysis of rates using Poisson regression models.
Biometrics 39:665–674; 1983.

Frome, E. L.
Response to Nelder's reaction on Poisson rate analysis.
Biometrics In press.

Frome, E. L., and Checkoway, H.
Use of Poisson regression models in estimating incidence rates and ratios.
Am. J. Epidemiol. In press.

Groer, P. G.
Bayesian estimates for the rate of Three-Mile Island type releases.
Risk Analysis. In press.

Hayes, R. L.
The interaction of gallium with biological systems.
Int. J. Nucl. Med. Biol. 10:257–261; 1983.

Hoffman, D. R.; Hajdu, J.; and Snyder, F.
Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts.
Blood 63:545–552; 1984.

Hoffman, D. R.; Stanley, J. D.; Berchtold, R.; and Snyder, F.
Cytotoxicity of ether-linked phytanyl phospholipid analogs and related derivatives in human HL-60 leukemia cells and polymorphonuclear neutrophils.
Res. Commun. Chem. Pathol. Pharmacol. 44:293–306; 1984.

1028615

Keane, A. T., and Polednak, A. P.
Retention of uranium in the chest: implications of findings in vivo and postmortem
Health Phys. 44, Suppl. 1:391–402; 1983.

Lackey, R. J., and Cabot, M. C.
Acetate utilization and fatty acid metabolism in phorbol ester and dimethyl sulfoxide-differentiated human leukemia cells.
Cancer Lett. 20:291–297; 1983.

Lee, T-c.; Lenihan, D. J.; Malone, B.; Roddy, L. L.; and Wasserman, S. I.
Increased biosynthesis of platelet activating factor on activated human eosinophils.
J. Biol. Chem. 259:5526–5530; 1984.

Lenihan, D. J., and Lee, T-c.
Regulation of platelet activating factor synthesis: Modulation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase by phosphorylation and dephosphorylation in rat spleen microsomes. *Biochem. Biophys. Res. Commun.* 120:834–839; 1984.

Littlefield, L. G., Joiner, E. E., Colyer, S. P., DuFrain, R. J., and Washburn, L. C.
Evaluation of cellular proliferation and chromosome breakages after in vitro exposure of human lymphocytes to calcium or zinc DTPA.
Radiat. Res. 99:129–139; 1984.

Littlefield, L. G.; Joiner, E. E.; and Sayer, A. M.
Premature separation of centromeres in marrow chromosomes from an untreated patient with acute myelogenous leukemia.
Cancer Genet. Cytogenet. In press.

Lushbaugh, C.; Humason, G.; and Clapp, N.
History of colon cancer in *Saguinus oedipus oedipus*. *Dig. Dis. Sci.* In press.

Lushbaugh, C.; Humason, G.; and Clapp, N.
Histology of colitis: *Saguinus oedipus oedipus* and other marmosets.
Dig. Dis. Sci. In press.

Polednak, A. P., and Hollis, D. R.
Mortality and causes of death among workers exposed to phosgene in 1943–45. *Clin. Toxicol.* In press.

Russell, R. G.; Brian, D. A.; Lenhard, A.; Potgieter, L. N. D.; Gillespie, D.; and Clapp, N. K.
Coronavirus-like particles and campylobacter in marmosets with diarrhea and colitis.
Dig. Dis. Sci. In press.

Surles, J. R.; Wykle, R. L.; O'Flaherty, J. T.; Salzer, W. L.; Thomas, M. J.; Snyder, F.; and Piantadosi, C.
Total synthesis and 250 MHz ^{1}H-NMR spectra on platelet-activating factor and analogues containing unsaturation in the *sn*-1 alkyl chain.
J. Med. Chem. In press.

Tardif, S. D.
The relationship between social interactions and sexual maturation in female *Saguinus oedipus oedipus*.
Folia Primatol. 40:268–275; 1983.

Tardif, S. D.
Social influences on sexual maturation of female *Saguinus oedipus oedipus*.
Am. J. Primatol. 6:199–209; 1984.

Tardif, S. D.; Richter, C. B.; and Carson, R. L.
Effects of sibling-rearing experience on future reproductive success in two species of Callitrichidae.
Am. J. Primatol. 6:377–380; 1984.

Tardif, S. D.; Richter, C. B.; and Carson, R. L.
Reproductive performance of three species of Callitrichidae.
Lab. Anim. Sci. 34:272–275; 1984.

Urso, P., and Gengozian, N.
Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after *in utero* exposure to benzo(a)pyrene.
J. Toxicol. Environ. Health. In press.

Washburn, L. C.; Sun, T. T.; Byrd, B. L.; and Callahan, A. P.
Resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan by high performance liquid chromatography.
J. Labelled Compd. Radiopharm. In press.

Wirth, N.; Swanson, D.; Shapiro, B.; Nakajo, M.; Coffey, J. L.; Eckhauser, F.; and Owyang, C.
A conveniently prepared Tc-99m resin for semisolid gastric emptying studies.
J. Nucl. Med. 24:511–514; 1983.

Chapters, Review Articles, Other Publications, and Videotapes

Abd Elghany, N.
An epidemiological study of cancer mortality among workers exposed to occupational low levels of ionizing radiation.
Ph.D. Dissertation, University of North Carolina, Chapel Hill, 1983.

Burr, W. W., Jr.
Human experience and epidemiology.
In *Biokinetics and Analysis of Uranium in Man*, Proceedings of U. S. Uranium Registry Conference, 1984, Richland, Washington Hanford Environmental Health Foundation, Richland, Washington. In press.

Clapp, N. K.; Lushbaugh, C. C.; Humason, G. L.; Gangaware, B. L.; and Henke, M. A.
The cotton-top tamarin as an animal model of colorectal cancer metastasis.
In *The Biology and Treatment of Colorectal Cancer Metastasis*, edited by A. Mastromarino, Martinus Nijhoff Publishing, Hingham, Massachusetts. In press.

Coffey, J. L.; Watson, E. E.; Hübner, K. F.; and Stabin, M. G.
Radiopharmaceutical absorbed dose considerations.
In *Clinical Aspects of Nuclear Medicine Science*, edited by W. B. Hladik, III, G. B. Saha, and K. T. Stady. Baltimore; Williams and Wilkins Corp. In press.

Cragle, D. L.
The effects of genetic and environmental interaction in an epidemiologic investigation of colon cancer and water

1028616

quality.
Ph.D. Dissertation, University of North Carolina, Chapel Hill, 1983.

Cragle, D. L.; Hollis, D. R.; Shy C. M.; and Newport, T. H.
A retrospective cohort mortality study among workers occupationally exposed to metallic nickel powder at the Oak Ridge Gaseous Diffusion Plant.
In *Symposium on Nickel in the Human Environment,* International Agency for Research on Cancer, Lyon, France, 1983. In press.

Crook, J. E., and Mroczkowski, P. J.
Red cell Na-K co-transport: dose-response effects of furosemide (F) inhibition in black (BH) and white hypertensives (WH).
In *Proceedings of the First International Conference on Diuretics,* edited by J. B. Puschett. Amsterdam, The Netherlands: Elsevier Biomedical Press. In press.

DuFrain, R. J.
Probing sister chromatid exchange mechanisms with halogenated pyrimidines.
In *Sister Chromatid Exchange: 25 Years of Experimental Research,* edited by R. R. Tice, and A. Hollaender. Proceedings of the International Symposium on Sister Chromatid Exchange, Brookhaven, New York, 1983. New York: Plenum Press. In press.

Erickson, B. H.
Effects of ionizing radiation on mammalian spermatogenesis: A model for chemical effects.
In *Target Organ Toxicity Gonads,* edited by R. L. Dixon. New York: Raven Press. In press.

ne, E. L.
gression methods for binomial and Poisson distributed data.
In *Proceedings of the AAPM First Midyear Topical Symposium, Multiple Regression Analysis: Applications in the Health Sciences,* Mobile, Alabama, 1984. In press.

Frome, E. L., and McLain, R.
A macro for fitting nonlinear models to Poisson distributed data.
In Proceedings of the Eighth Annual SAS Users Group International Conference, Hollywood, Florida. In press.

Fry, R. J. M., and Fry, S. A.
Effects on humans of exposure to radioactive materials: late somatic effects.
In *Encyclopedia of Materials Science and Engineering.* New York: Pergamon Press. In press.

Gissel, K. C.; Hansard, M. S.; and Wray, M. L.
Health and mortality study: Error detection, reporting, and resolution system.
In *Statistical Methods and the Improvement of Data Quality,* edited by T. Wright, pp. 321–332. New York: Academic Press, 1983.

Groer, P. G. [Commentary]
Direct estimates of low-level radiation risks of lung cancer.
Yale J. Biol. Med. 56:47; 1983.

Hammond, B. F., and Martin, P. G.
C m-cadmium effects on bone density and strength of the femur from multiparous rats.
Nutr. Res. Suppl. In press.

Hayes, R. L.
Detection of malignancies with radionuclidies.
In *Recent Advances in Nuclear Medicine,* Vol. 6, edited by J. H. Lawrence, and S. Winchell, pp. 177–197, New York: Grune & Stratton, 1983.

Hodges, H. D. [Book Review]
Medical Radiation Biology, Second Edition edited by D. J. Pizzarello, and R. L. Witcoski, 164 pp. Philadelphia: Lea & Febiger, 1982.
J. Nucl. Med. Technol. 11:197; 1983.

Hodges, H. D. [Letter to the Editor]
New specialty for nuclear medicine technologists.
J. Nucl. Med. Technol. 12:897; 1984.

Hübner, K. F.
Decontamination procedures and risks to health care personnel.
Bull. N.Y. Acad. Med. 59:1119–1128; 1983.

Hübner, K. F.; Littlefield, L. G.; and DuFrain, R. J.
Biologische Indikatoren fur ionisierende Strahlen.
Dtsch. Med. Wochenschr. In press.

Hübner, K. F., and Lushbaugh, C. C.
Radiation injuries.
In *Critical Decisions in Trauma,* edited by E. E. Moore, B. Eiseman, and C. W. Van Way, III, pp. 452–457, St. Louis, The C. V. Mosby Co., Missouri: 1984.

Lee, T-c., and Snyder, F.
Function, metabolism, and regulation of platelet activating factor and related ether lipids.
In *Phospholipids and Cellular Regulation,* edited by J. F. Kuo. Boca Raton, Florida: CRC Press, Inc. In press.

Littlefield, L. G.
The analysis of radiation-induced chromosome lesions in lymphocytes as a biological method for dose estimation.
In *Proceedings of the NATO Working Group Meeting on the Assessment of Injury from Ionizing Radiation Warfare—October 18–22, 1982,* compiled by R. T. Devine, pp. 351–391. Bethesda, Maryland: Armed Forces Radiobiology Research Institute, 1983.

Littlefield, L. G., and DuFrain, R. J.
Cytogenetic aspects of human exposure to transuranics.
Trans. Am. Nucl. Soc. 46:320–321; 1984.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. M.; and DuFrain, R. J.
Persistence of SCE-inducing lesions after G_0 exposure of human lymphocytes to differing classes of DNA-damaging chemicals.
In *Sister Chromatid Exchange: 25 Years of Experimental Research,* edited by R. R. Tice, and A. Hollaender, Proceedings of the International Symposium on Sister Chromatid Exchange, Brookhaven, New York, 1983. New York: Plenum Press. In press.

Littlefield, G.; Joiner, E.; DuFrain, R. J.; Colyer, S.; and Breitenstein, B.
Six year cytogenetic follow-up study of an individual heavily contaminated with americium 241.

1028617

Dosimetry, Radionuclides and Technology, edited by J. J. Broerse, G. W. Barendsen, H. B. Kal, and A. J. van der Kogel, Proceedings of the Seventh International Congress of Radiation Research, Amsterdam 1983. Rijswijk, The Netherlands: Martinus Nijhoff Publishers, Section E3–05, 1983.

Lushbaugh, C. C. [Moderator]
Hübner, K. F.; Ricks, R. C.; Saenger, E. L.; and Wald, N.
Panel discussion: Section IV care of the radiation-exposed and injured.
Bull. N.Y. Acad. Med. 59:1141–1149; 1983.

Lushbaugh, C. C.
Chairman's concluding comments.
Bull. N.Y. Acad. Med. 59:1139–1140; 1983.

Lushbaugh, C. C.; Brown, D. G.; and Frome, E. L.
Longevity of irradiated burros.
In *Proceedings of the Twenty-Second Hanford Life Sciences Symposium,* 1983. Richland, Washington. In press.

Lushbaugh, C. C.; Hübner, K. F.; and Fry, S. A.
Effects of high doses of radiation.
In Proceedings of the Eighth Life Sciences Symposium, Radiation and Its Effects, Los Alamos, New Mexico.
In press.

Lushbaugh, C. C.; Hübner, K. F.; Fry, S. A.; and Beck, W. L.
Effects on humans of exposure to radioactive materials: acute effects.
Encyclopedia of Materials Science and Engineering. New York: Pergamon Press. In press.

Lushbaugh, C. C.; Humason, G. L.; and Clapp, N. K.
Colonic adenocarcinoma.
Comp. Pathol. Bull. 15:2,4; 1983.

McFee, A. F., and Sherrill, M. N.
Accumulation of SCEs in lymphocytes during chronic ingestion of a mutagen.
In *Sister Chromatid Exchange: 25 Years of Experimental Research,* edited by R. R. Tice, and A. Hollaender, Proceedings of the International Symposium on Sister Chromatid Exchange, Brookhaven, New York, 1983. New York: Plenum Press. In press.

Mani, K. V.
Assessment of Oak Ridge National Laboratory internal dosimetry data for DOE health and mortality study.
M.S. Thesis, University of North Carolina, Chapel Hill, 1983.

Ricks, R. C.
Response to a spill of radioactive material.
In *Encyclopedia of Materials Science and Engineering.* New York: Pergamon Press. In press.

Ricks, R. C.
Transport of irradiated or radioactively contaminated patients to the hospital. *Bull. N. Y. Acad. Med.* 59:1108–1118; 1983.

Ricks, R. C. [Project Coordinator]
Prehospital response to radiation accidents (25-minute color videotape).
Oak Ridge, Tennessee: Oak Ridge Associated Universities, 1984.

Ricks, R. C.
Prehospital Management of Radiation Accidents.
Oak Ridge, Tennessee: Oak Ridge Associated Universities, ORAU-223, 1984.

Ricks, R. C. [Project Coordinator]
Hospital emergency department response to radiation accidents (25-minute color videotape).
Oak Ridge, Tennessee: Oak Ridge Associated Universities, 1984.

Ricks, R. C.
Hospital Emergency Department Management of Radiation Accidents.
Oak Ridge, Tennessee: Oak Ridge Associated Universities, ORAU-224, 1984.

Schwarz, O. J., and Eisele, G. R.
Food chain transport of synfuels: experimental approaches for acquisition of baseline data.
In *Synthetic Fossil Fuel Technologies: Results of Health and Environmental Studies,* edited by K. Cowser, pp. 441–462. Boston: Butterworth, Inc., 1984.

Snyder, F.
Chemical and biochemical aspects of "platelet activating factor," a novel class of acetylated ether-linked choline-phospholipids.
Med. Res. Rev. In press.

Snyder, F.
Metabolism, regulation, and function of ether-linked lipids.
In Biochemistry of Lipids and Membranes, edited by D. Vance and J. Vance, Menlo Park, California: The Benjamin/Cummings Publishing Company. In press.

Snyder, F.; Blank, M. L.; Lee, T-c.; Woodard, D. S.; and Malone, B.
Metabolism of platelet activating factor: the role of alkylacetylglycerols in PAF biosynthesis.
Adv. Inflammation Res. In press.

Snyder, F.; Lee, T-c.; Blank, M. L; Malone, B.; Woodard, D.; and Robinson, M.
Platelet activating factor (PAF): alternate pathways of biosynthesis and the mechanism of activation.
In Proceedings of Kyoto Conference on Prostaglandins, New York: Raven Press. In press.

Snyder, F.; Lee, T-c.; and Wykle, R. L.
Ether-linked glycerolipids and their bioactive species: enzymes and metabolic regulation.
In *The Enzymes of Biological Membranes,* Second Edition, edited by A. Martonosi, New York: Plenum Publishing Co. In press.

Snyder, F.; Lee, T-c.; Blank, M. L.; Cabot, M. C.; Malone, B.; and Albert, D. H.
Enzymatic pathways for platelet activating factor.
In *Platelet-Activating Factor and Structurally Related Ether-Lipids,* edited by J. Benveniste, and B. Arnoux, Proceedings of the 1st International Symposium on Platelet-Activating Factor, 1983, Paris, pp. 253–261, Amsterdam, The Netherlands: Elsevier Biomedical Press, (INSERM Symposium No. 23), 1983.

1028618

Strom, D. J.
A strategy for assessing occupational radiation monitoring data from many facilities for use in epidemiologic studies.
Ph.D. Dissertation, University of North Carolina, Chapel Hill, 1983.

Published Abstracts of Papers Presented at Meetings

Berdel, W. E.; Greiner, E.; Fink, U.; Stavrou, D.; Reichert, A.; Rastetter, J.; Hoffman, D. R.; and Snyder, F.
Investigations into the correlation of the cytotoxic effect of alkyl-lysophospholipids on brain tumor cells of rats and their low content of alkyl-cleavage enzyme.
Onkologie 6:262; 1983.

Berger, C. D.; Eisele, G. R.; and Lane, B. H.
Validation of calibration factors for long-term ^{241}Am deposition measurements in humans.
Health Phys. 47:145; 1984.

Blank, M. L.; Cress, E. A.; and Snyder, F.
A new class of hypotensive neutral lipid: 1-alkyl-2-acetyl-*sn*-glycerols (a precursor of platelet activating factor). *Fed. Proc.* 43:555; 1984.

Boyd, L. M.; Ezra, D.; Snyder, F.; Feuerstein, G.; and Goldstein, R. E.
Coronary effects of platelet-activating factor.
Circulation 68, Suppl. III, 34; 1983.

Cabot, M. C.
A serum lipase that hydrolyzes phorbol esters.
J. Cell. Biochem. Suppl. 8A:286; 1984.

Clapp, N. K.; Henke, M. A.; Tankersley, W. G.; and Gangaware, B. L.
Increased fecal β-glucuronidase activity as a potential indicator for colon cancer in tamarins.
Proc. Am. Assoc. Cancer Res. 25:1; 1984.

Clapp, N. K., and Tardif, S. D.
The successful rearing of cotton-top tamarins using colony-born breeders.
Lab. Anim. Sci. In press.

Coffey, J. L.; Carlton, J. E.; Washburn, L. C.; and Hübner, K. F.
Effect of different Prussian blue preparations on the whole body retention of Cs-137 by rats.
Health Phys. 47:151; 1984.

Cragle, D. L.; Hollis, D. R.; Qualters, J. R.; Tankersley, W. G.; and Fry, S. A.
A mortality study of men exposed to elemental mercury.
J. Occup. Med. 26:687; 1984.

Crook, J. E., and Mroczkowski, P. J.
Dose response effects of furosemide inhibition on erythrocyte sodium-potassium co-transport in white and black normotensives and hypertensives.
Clin. Res. 32:204A; 1984.

DuFrain, R. J., and Batson, J. S.
SCE evaluation in lymphocytes from chimeric tamarins.
Mamm. Chromosome Newslett. In press.

DuFrain, R. J.; Joiner, E. E.; and Littlefield, L. G.
Cytogenetic evaluations in long-term heavy smokers. II. SCE analysis in lymphocytes.
Environ. Mutagen. 6:478; 1984.

DuFrain, R. J.; Littlefield, L. G.; Batson, J. S.; Jennings, C. J.; Linkous, S.; and Morrison, W. D.
Cytogenetic studies on somatic and female germ cells of adult and neonatal rabbits (*Oryctolagus cuniculus* L.).
Mamm. Chromosome Newslett. 24:146; 1983.

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action.
Biometrics. In press.

Hoffman, D. R., and Snyder, F.
Selective cytotoxicity and metabolism of analogs of platelet activating factor in normal and leukemic cells.
Fed. Proc. 43:1459; 1984.

Lee, T-c.; Malone, B.; and Snyder, F.
Inactivation of platelet activating factor (PAF): evidence for lyso-PAF as a key intermediate.
Fed. Proc. 43:461; 1984.

Lenihan, D. J., and Lee, T-c.
Modulation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine: acetyl-CoA acetyltransferase by phosphorylation/dephosphorylation in vitro.
Fed. Proc. 43:461; 1984.

Littlefield, L. G.; Colyer, S. P.; Sayer, A. S.; and DuFrain, R. J.
Comparisons of baseline and mutagen-induced SCEs in "early dividing" and "late dividing" lymphocyte populations.
Mamm. Chromosome Newslett. 24:152; 1983.

Littlefield, L. G.; Joiner, E. E.; and DuFrain, R. J.
Cytogenetic evaluations in long-term heavy smokers. I. Chromosome aberrations in first division lymphocyte metaphases.
Environ. Mutagen. 6:477; 1984.

Littlefield, L. G.; Sayer, A.; Colyer, S.; Joiner, E. E.; Outlaw, J.; and Dry, L.
Persistent radiation-induced chromosome lesions in lymphocytes of the Y-12 accident survivors: Evaluations of 25 years post-exposure.
Mamm. Chromosome Newslett. In press.

Martin, P. G., and Erickson, B. H.
Reproductive and teratological effects of chronic ingestion of 7,12-dimethylbenzanthracene in the mini-pig.
Teratology 29:45A; 1984.

McFee, A. F.
Cytogenetic sensitivity of domestic animals to ingested mutagenic chemicals.
J. Dairy Sci. 66, Suppl. 1:247; 1983.

McFee, A. F., and Sherrill, M. N.
Sister chromatid exchanges induced in lymphocytes of sheep and swine by chronic feeding of a mutagen.
Mamm. Chromosome Newslett. 24:153; 1983.

Robinson, M., and Snyder, F.
Metabolism of platelet activating factor in alveolar macro-

1026619

ıages by a deacylation-reacylation pathway with evidence ıor a 2-lyso intermediate.
Fed. Proc. 43:1458; 1984.

Strom, D. J.; Crawford-Brown, D. J.; and Tankersley, W. G.
Preperation of 30 years' personnel monitoring data from a uranium fabrication plant for use in epidemiologic studies.
Health Phys. 47:123; 1984.

Sun, T. T.; Washburn, L. C.; Byrd, B. L.; Hayes, R. L.; and Callahan, A. P. HPLC resolution of ^{11}C-labeled amino acid racemates.
Int. J. Appl. Radiat. Isot. In press.

Tardif, S. D., and Clapp., N. K.
Reproductive longevity in tamarin females (*Saguinus sp.*).
Lab. Anim. Sci. In press.

Tardif, S. D.; Clapp, N. K.; and Lushbaugh, C. C.
Histological comparison of the ovaries of young and aging tamarins (*Saguinus sp.*).
Am. J. Primatol. 6:433; 1984.

Tsai, S. C.; Eisele, G. R.; Kirk, P. G.; Payne, J. L.; and Urso, I.
Biotransformation and retention of synfuel components in hens, pigs, and cows.
DOE (U.S. Dept. Energy) *Symp. Ser.* In press.

Tsai, S. C.; Eisele, G. R.; Kirk, P. G.; Payne, J. L.; and so, I.
.etention of synfuel components in hens, pigs, and cows.
Environ. Toxicol. Chem. In press.

Washburn, L. C.; Lingen, J. K.; Sun, T. T.; and Byrd, B. L.
Synthesis and tumor uptake studies of ^{14}C-labeled -amino-ısobutyric acid analogs.
J. Labelled Compd. Radiopharm. In press.

Washburn, L. C.; Wallace, R. T.; Byrd, B. L.; and Sun, T. T.
Factors affecting the lung uptake and retention of primary aliphatic amines.
Clin. Nucl. Med. 8:P60; 1983.

Washburn, L. C.; Wallace, R. T.; Byrd, B. L.; Sun, T. T.; Coffey, J. L.; and Hübner, K. F.
C-11-Labeled octadecylamine, a potential agent for positron tomographic pulmonary metabolism studies.
J. Nucl. Med. 25:P126–P127; 1984.

Woodard, D. S.; Lee, T-c.; and Snyder, F.
Biosynthesis of platelet activating factor via two enzymatic pathways in rat kidney tissue.
Fed Proc. 43:1655; 1984.

Miscellaneous Talks Presented at National and International Scientific Conferences

ɔok, J. E., and Mroczkowski, P. J.
reased red cell Na-K ATPase activity following exposure ıo a new calcium entry blocker -nitrendipine.
International Symposium on Calcium Entry Blockers and Tissue Protection, 15–16 March 1984 Rome, Italy.

Groer, P. G., and Blum, S.
Bayesian estimation and comparison of mortality rates.
6th International Congress of the International Radiation Protection Association, 7–12 May 1984, Berlin, West Germany.

Groer, P. G., and Blum, S.
Dose-response curves and cell killing.
32nd Annual Scientific Meeting of the Radiation Research Society, 25–29 March 1984, Orlando, Florida.

Littlefield, L. G., and Hübner, K. F.
Mexican cobalt-60 accident.
Health Physics Society Annual Meeting, 7 June 1984, New Orleans, Louisiana.

Ricks, R. C.
Victim management in technological hazard situations.
The 1983 National EMS Conference, 11 October 1983, Charleston, West Virginia.

Ricks, R. C.
The management of radiological emergencies.
1984 National Disaster Conference, 2 February 1984, Orlando, Florida.

Ricks, R. C.
Effects of a radioactive material accident.
Emergency department management of a radiation disaster.
Containment and decontamination of a radioactive materials accident.
Medical treatment procedures for radiation injuries.
First Annual Central Valley Disaster Planning Symposium on Hazardous Materials, 26 June 1984, Fresno, California.

Ricks, R. C.
Scope of the problem.
Basic radiation physics.
Radiological monitors.
Contamination control.
Prehospital response.
Radiological emergency team organization and leadership.
Preparation of the radiation emergency response area.
Second Annual Emergency Services Symposium/Radiation Accidents Management, 1–2 August 1984, Birmingham, Alabama.

Snyder, F.
Metabolism of platelet activating factor: the role of alkylacetylglycerols in PAF biosynthesis.
III International Congress of Inflammation, 3–7 September 1984, Paris, France.

Snyder, F.
Enzymatic pathways for platelet activating factor.
Federation of American Societies for Experimental Biology Immunopharmacology Conference, July 1–6, 1984, Saxtons River, Vermont.

Tankersley, W. G., and Checkoway, H.
Procedure for assignment of chemical hazards indices for use in epidemiology studies.
American Industrial Hygiene Conference, 22–27 May 1983, Philadelphia, Pennsylvania.

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C. C. Lushbaugh, Chairman 1975–1984

1028621

Summaries of Published Papers

Blank, M. L.; Byrd, B. L.; Cress, E. A., Washburn, L. C.; and Snyder, F.
Liposomal preparations of calcium- or zinc-DTPA have a high efficacy for removing colloidal ytterbium-169 from rat tissues.
Toxicology 30:275–281; 1984.

Results from this study demonstrate that liposomes increase the effectiveness of chelating agents in removing heavy metals from contaminated tissues in vivo. We compared the ability of free and liposomal preparations of Ca- and Zn-diethylenetriaminepentaacetic acid (DTPA) to remove ^{169}Yb from tissues of rats previously injected intravenously with either soluble or colloidal forms of ^{169}Yb. Although single injections of liposomal Zn-DTPA were better than the free chelator in reducing the body burden of ^{169}Yb administered in a soluble form (citrate), the liposomal preparations of Zn-PA or Ca-DTPA were even more effective in removing the Yb that had been injected as the colloidal form. However, a second injection of liposomal Zn-DTPA given 8 days after the initial treatment was not as effective in removing ^{169}Yb as a second injection of the free chelator. Whether injected in the free or liposomal forms, Ca-DTPA was more effective than Zn-DTPA in removing the colloidal ^{169}Yb. Significantly lower amounts of colloidal ^{169}Yb remained in the liver, kidney, muscle, bone, and blood of rats after injection of the liposomal preparations of Ca- or Zn-DTPA than in the corresponding organs of the controls ($P < 0.01$). The liposomal preparations were also more efficient in reducing the retention of colloidal ^{169}Yb in bone and blood than the free chelators ($P < 0.01$).

Blank, M. L.; Cress, E. A.; Lee, T-c.; Stephens, N.; Piantadosi, C.; and Snyder, F.
Quantitative analysis of ether-linked lipids as alkyl- and alk-1-enyl-glycerol benzoates by high-performance liquid chromatography.
Anal. Biochem. 133, 430–436, 1983

Methodology for the quantitative and qualitative analyses of alkyl- and alk-1-enyl-glycerols derived by Vitride reduction of ether-linked glycerolipids in the presence of an internal standard was developed. The procedure involved preparation of nzoate derivatives that were subsequently analyzed by gh-performance liquid chromatography with detection at 228 nm. Separation of the glycerol ether dibenzoates on a C_{18} reverse-phase column allowed for the simultaneous quantitation and the determination of chain length of both

1028622

alkyl- and alk-1-enyl-glycerols in a single chromatographic run of less than 15 min. The method was accurate (<10% error), reproducible, and sensitive (<1 μg per component). Application of the method to the analysis of phospholipids from L-M cells grown in the presence and absence of elaidic acid demonstrated that the cells incorporated a portion of the *trans* acid supplement (presumably via the fatty alcohol) into the side chain of both alkyl- and alk-1-enyl-glycerol-containing phosphatides.

Blank, M. L.; Cress, E. A.; and Snyder F.
Methanol toxicity and elevated formate levels in the marmoset. *Res. Commun. Subst. Abuse* 4:259–262; 1983.

Two white-lipped marmosets (small, New World monkeys) were examined as a possible model for studies of methanol toxicity in humans. Their blood was analyzed for pH, pCO_2, and formate after they received oral doses of 2 to 4 g methanol/kg body weight. We did not find evidence of increased metabolic acidosis in either animal even though there were high levels of formate in the blood after methanol ingestion. Therefore, although the marmoset appears to be an unlikely model for investigating the complete methanol toxicity syndrome that occurs in humans, it does offer a unique animal system to examine the mechanisms responsible for the accumulation of formate as an underlying causative factor in some of the specific biological responses elicited by methanol.

Blank, M. L.; Cress, E. A.; and Snyder, F.
A new class of antihypertensive neutral lipid: 1-alkyl-2-acetyl-*sn*-glycerols, a precursor of platelet activating factor.
Biochem. Biophys. Res. Commun. 118:344–350; 1984.

A new type of neutral lipid is described that possesses hypotensive activity in genetic hypertensive (SHR) and normotensive (WKY) rats. 1-Alkyl-2-acetyl-*sn*-glycerols and 1-alkyl-2-propionyl-*sn*-glycerols are both equally effective in eliciting the hypotensive response. Requirement for the 1-alkyl and 2-acetyl or 2-propionyl structure of the active isomer was documented by the negative responses obtained with closely related neutral lipid analogs (1-alkyl-2-acyl-, 1-alkyl-3-acetyl-, 1-acyl-2-acetyl-, 1-alkyl-2,3-diacetyl-, and 1-alkyl-glycerols). Although less potent than PAF (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine), the 1-alkyl-2-acetyl-*sn*-glycerols produce a response of significantly longer duration and may have fewer immediate side effects than PAF. The mechanism for the biological activity is unknown; however, we have demonstrated previously that the enzymatic synthesis of 1-alkyl-2-acetyl-*sn*-glycerols to PAF occurs via a specific cholinephosphotransferase and therefore the observed blood pressure response might be due to the conversion of the neutral lipid precursor to PAF in vivo.

Blank, M. L.; Robinson, M.; Fitzgerald, V.; and Snyder, F.
A novel quantitative method for determination of molecular species of phospholipids and diglycerides.
J. Chromatogr. 298:473–482; 1984.

Novel methodology is described for the quantitative analysis of subclasses (alk-1-enylacyl, alkylacyl, and diacyl types) and molecular species within each subclass of glycerophosphatides. Diradylglycerols from phospholipase C hydrolysis of the phospholipids are converted to benzoate derivatives, the benzoates are separated into their respective subclasses by thin-layer chromatography, and quantitated by measuring absorbance at 230 nm. Molecular species within individual subclasses are separated using a combination of argentation thin-layer chromatography and reverse-phase high-performance liquid chromatography with direct, on-line quantitation at 230 nm. We applied the methodology to the analysis of ethanolamine phosphatides from beef brain and were able to quantitate the three diradylglycerol subclasses (alk-1-enyl, alkylacyl, and diacyl types) as well as approximately 29 molecular species within each of these subclasses. This new quantitative approach for the analysis of specific molecular species of glycerolipids should be applicable to studies involving a variety of biologically important lipids, such as phosphatidylcholine, phosphatidylinositol, platelet activating factor, plasmalogens, and neutral type glycerolipids including diacylglycerols.

Brodsky, J. B.; Groer, P. G.; Liddell, R.; Ishimaru, T.; and Ichimaru, M.
Temporal analysis of a dose-response relationship: leukemia mortality in atomic bomb survivors.
Radiat. Res. 99:547–561; 1984.

A data analysis that incorporates time dependencies is demonstrated for the dose response of leukemia mortality in the atomic bomb survivors. The time dependencies are initially left unspecified and the data on leukemia mortality—up to the end of 1978—are used to infer them. Several findings based on T65 revised doses (T65DR) are obtained. First, it is shown that the fits to the data of time-independent L(linear in γ dose)-Q (quadratic in γ dose)-L (linear in neutron dose), L-L, and Q-L dose-response models are significantly improved ($P<0.001$) by using the corresponding time-dependent dose-response models. Second, it is shown that the increased risk of leukemia mortality due to γ irradiation decreases in time while the increased risk due to neutron exposure decreases more slowly, if at all, in time. Consequently, relative biological effectiveness (RBE) of neutrons is shown to increase in time ($P=0.002$) and the current definition of RBE as a time-independent quantity is therefore challenged. It is demonstrated with time-dependent models that the L-L model has a poor fit ($P=0.01$) to the data for the first 7 years of study, but has an adequate fit for the remaining 21 years. In contrast the Q-L model has an adequate fit for the entire follow-up period ($P>0.30$).

1028623

oot, M. C.
Tumor promoting phorbol diesters: substrates for diacylglycerol lipase.
Biochem. Biophys. Res. Commun. 123:170–177; 1984.

Enzyme activity in rat serum was examined utilizing the potent tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and various glycerolipids as substrates. The serum activity was specific for hydrolysis of the long chain tetradecanoate moiety of TPA, hydrolyzed mono- and diacylglycerols, but was not effective against triacylglycerols, cholesterylesters, or phospholipids. Heating the enzyme preparation at 56°C for 1 min was dually effective in reducing the hydrolysis of both TPA and dioleylglycerol by 83–86% of control levels. The potent diacylglycerol lipase inhibitor, RHC 80267, inhibited the hydrolysis of TPA in the 0.2–1.0 μM range and was also a potent blocker of monoacyl- and diacylglycerol hydrolysis. In substrate competition studies, exogenous unlabeled TPA was added to the [^{14}C]dioleylglycerol-containing reaction mixture; however, this produced an approximate 3-fold stimulation of [^{14}C]dioleylglycerol hydrolysis. Although we have not established whether the hydrolysis of TPA and diacylglycerol is the work of one enzyme, the effectiveness of the specific lipase inhibitor, RHC 80267, demonstrates that diacylglycerol ·se can utilize TPA as substrate. This point is of interest in (of the theory that phorbol esters act by mimicry of the natural lipid mediator, diacylglycerols.

Cabot, M. C.; Faulkner, L. A.; Lackey, R. J.; and Snyder, F.
Vertebrate class distribution of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase in serum.
Comp. Biochem. Physiol. 78B:37–40; 1984.

Serum from numerous mammals and lower vertebrates contains an enzyme activity that is specific for the hydrolysis of the acetate moiety of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF, platelet activating factor). Acetylhydrolase (EC 3.1.1.47, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetohydrolase) was found in all mammalian sera with activity ranging from 11 (fetal calf) to 178 (rabbit) pmol acetate liberated/1 serum/min. The enzyme is not present in avian serum but is a constituent of reptiles and bony fishes.

Chertok, R. J.; Kullgren, B.; and Burbank, D.
The effects of $CdCl_2$ on the maternal-to-fetal clearance of ^{67}Cu and placental blood flow.
Proc. Soc. Exp. Biol. Med. 176:138–142; 1984.

Copper is an essential element while Cd is an extremely ·: heavy metal of questionable biological usefulness. Cadn has been reported to interfere with the metabolism of Cu, be teratogenic, and decrease blood flow in the fetal placenta. Because of these reported biological interactions of Cd and Cu, this investigation was conducted to determine the effects of Cd on placental transport of ^{67}Cu and placental blood flow in the guinea pig. All guinea pigs used were 60 ± 1 days pregnant. A placental perfusion technique was used to measure the maternal-to-fetal clearance of ^{67}Cu and $^{3}H_2O$ across the placenta. The clearance of $^{3}H_2O$ served as an indicator of placental blood flow on the maternal side of the circulation. The results indicated that an intravenous injection of 1 mg Cd/kg body weight resulted in an immediate increase in the clearance of ^{67}Cu which declined over the next 8 min to an elevated level compared to the extrapolated best-fit curve of control values. This IV injection of $CdCl_2$ concomitantly reduced the maternal-to-fetal clearance of $^{3}H_2O$ across the placenta. In conclusion, an acute exposure of the pregnant female to $CdCl_2$ results in an increased maternal-to-fetal clearance of ^{67}Cu and a reduced placental blood flow that can alter the supply of nutrients to the developing embryo or fetus, and therefore modifies normal development.

Clapp, N. K.; Henke, M. A.; Holloway, E. C.; and Tankersley, W. G.
Carcinoma of the colon in the cotton-top tamarin: a radiographic study.
J. Am. Vet. Med. Assoc. 11:1328–1330; 1983.

The cotton-top tamarin (*Saguinus oedipus oedipus*) has large numbers (28/41 deaths in 1981–1982 in our colony) of spontaneous carcinomas of the colon. Only one tumor of the small intestine and no large bowel tumors have been observed in several hundred necropsies of other tamarin species and marmosets in our colony, whereas 74 have been found in about 300 *S. oedipus oedipus* necropsies. Tamarins were prepared for contrast radiography by fasting them for about 18 hours; however, 16% corn syrup in water was provided ad libitum during this period. A 10- to 15-ml warm water enema was then given 2 to 3 hours before radiography. Each animal was tranquilized with undiluted ketamine (15 to 30 mg/kg, IM) and restrained by taping it to the film cassette. The colon was filled with barium sulfate through a Foley catheter with an inflated bulb that prevented leakage of the barium sulfate and/or air. After evacuation of the barium sulfate, pneumoperitoneum was utilized to permit visualization of the serosal surface. This expanded radiographic technique may be used productively in other laboratory animals for diagnostic purposes as well as other aspects of clinical small animal veterinary medicine.

Clapp, N. K.; Henke, M. A.; London, J. F.; and Shock, T. L.
Enhancement of 1,2-dimethylhydrazine-induced large bowel tumorigenesis in BALB/c mice by corn, soybean, and wheat brans.
Nutr. Cancer 6:77–85; 1984.

This study was designed to determine the effects of four well-characterized dietary brans on large bowel tumori-

1028624

genesis induced in mice with 1,2-dimethylhydrazine (DMH). Eight-week-old barrier-derived male BALB/c mice were fed a semisynthetic diet with 20% bran added (either corn, soybean, soft winter wheat, or hard spring wheat) or a no-fiber-added control diet. Half of each group was given DMH (20 mg/kg body weight/week, subcutaneously for 10 weeks) beginning at 11 weeks of age. Surviving mice were killed 40 weeks after the first DMH injection. Tumors were not found in mice not subjected to DMH. In DMH-treated mice, tumors were found almost exclusively in the distal colon. Tumor incidences were as follows: controls, 11%; soybean group, 44%; soft winter wheat group, 48%; hard spring wheat group, 58%; and corn group, 72%. Tumors per tumor-bearing mouse ranged from 1.4 to 1.6, except in the corn group, which had 2.1. A positive correlation was found between percentage of neutral detergent fiber in the brans and tumor incidences but not between the individual components of cellulose, hemicellulose, or lignin. The enhancement of DMH-induced large bowel tumorigenesis by all four bran types may reflect a species and/or mouse strain effect that is bran-source related. These data emphasize the importance of using well-defined bran in all "fiber" studies.

DuFrain, R. J.
Sister chromatid exchange distributions in rabbit lymphocytes treated with streptonigrin.
Environ. Mutagen. 5:813–824; 1983.

Streptonigrin (NSC-45383), a direct-acting clastogen which induces SCEs in vivo and chromosome aberrations both in vivo and in vitro, was evaluated for SCE induction in both G_0 and stimulated rabbit lymphocytes. Determinations were made for 16 cultures from seven female rabbits. These included controls, as well as cells exposed to 90 μg/kg in vivo, cells pulse-treated with 50 ng/ml in vitro, and a culture continuously exposed to 5 ng/ml in vitro. For all cultures the SCE/cell frequency was determined from 20 complete (44 chromosome) metaphases and, in selected cultures, SCEs on individual chromosomes (880 per culture from 20 cells) were enumerated to determine SCE/chromosome frequency and the chromosomal distribution of SCEs. Analysis of variance and least significant difference tests of the $\sqrt{x}$ transformed SCE/cell data show that cells exposed to streptonigrin while dividing have significantly higher ($P < 0.01$) frequencies (over double the control 5.3 SCE/cell value) whereas treated G_0 cells were not significantly different from the controls. Dispersion analysis of both SCE/cell and SCE/chromosome data confirms the adequacy of the Poisson distribution for spontaneous or baseline but not streptonigrin-induced SCEs.

DuFrain, R. J.; Littlefield, L. G.; Morrison, W. D.; Huff, V. D.; and Hutton, D.
Evaluation of chemically induced cytogenetic lesions in rabbit oocytes. III. A postimplantation analysis of streptonigrin effects.
Mutat. Res. 127:73–79; 1984.

The frequency of consistent structural chromosome aberrations was determined for 108 fetuses (day 14 of gestation) from 20 female rabbits treated before conception with 90 μg/kg of streptonigrin (NSC-45383). The findings by karyotype analysis of 1.85% of the fetuses with consistent aberrations was compared with 6.32% frequency previously observed in 6-day (preimplantation) blastocysts from identically treated females and found to be significantly reduced. The interpretation of the findings is that the developmental events of implantation and placentation are effective in eliminating from further gestation the majority of the rabbit conceptuses with consistent structural chromosome abnormalities. The data are also discussed relative to the predictive nature of somatic cell chromosome damage in women of childbearing age being an estimator of risk of producing offspring with structural chromosome aberrations.

The data show that preconceptional treatment of female rabbits with streptonigrin caused a shift in the cytogenetically determined sex ratio of the 14-day fetuses, but not of the 6-day blastocysts. The significant decrease in relative numbers of males observed is consistent with the induction of lethal mutations on the X chromosome. This finding is discussed in conjunction with the timing and parental specificity of X chromosome inactivation during mammalian embryonic development.

DuFrain, R. J.; McFee, A. F.; Linkous, S.; Jennings, C. J.; and Lowe, K. W.
In vivo SCE analysis using bromodeoxyuridine, iododeoxyuridine, and chlorodeoxyuridine.
Mutat. Res. 139:57–60; 1984.

Sister-chromatid exchange (SCE) frequencies were determined for mouse and rabbit bone-marrow cells following incorporation of 3 different halogenated analogues of thymidine. For both species the SCE frequency was highest for chlorodeoxyuridine, lowest for iododeoxyuridine and intermediate for bromodeoxyuridine. The study demonstrates that halogenated pyrimidine analogues other than brominated compounds can be used for in vivo SCE analysis and that their effects on baseline SCE frequencies are qualitatively similar to results of previous in vitro studies.

Erickson, B. H., and Martin, P. G.
Stem-spermatogonial survival and incidence of reciprocal translocations in the γ-irradiated boar.
Environ. Mutagen. 6:219–227; 1984.

To assess the effects of γ-radiation on stem-cell survival and incidence of reciprocal translocations, boar testes were irradiated with 100, 200, or 400 rad. Stem-cell survival was markedly affected by 100 rad (51% of control) and reduced to 34% of control by 400 rad. Production of differentiating spermatogonia was all but completely interrupted by 200 rad and spermatogonial renewal was incomplete at 12 weeks. From the state of the seminiferous epithelium at 12

1028625

weeks, estimates of the percentage of permanent impairment of sperm-producing capacity ranged from 20 ± 6 (100 rad) to 67 ± 10 (400 rad). Incidence of translocations peaked at 200 rad and the number occurring at 100 and 400 rad was similar. Kinetics of porcine spermatogonial renewal differs considerably from those of the rodent and, relative to the rodent, this may account for the boar's higher sensitivity to stem-cell killing and lower sensitivity to translocations.

Erickson, B. H., and Martin, P. G.
Reproductive and genetic effects of continuous prenatal irradiation in the pig.
Teratology 30:99–106; 1984.

The stem germ-cells of the prenatal pig are highly vulnerable to the cytotoxic effects of ionizing irradiation. This study was conducted to determine whether sensitivity to killing was also marked by a sensitivity to mutation and how prenatal depletion of the germ-cell population affects reproductive performance. Germ cell populations were reduced by continuously irradiating sows at dose-rates of either 0.25 or 1.0 rad/day for the first 108 days of gestation. The prenatally irradiated boars were tested for sperm-producing ability, sperm abnormalities, dominant lethality, reciprocal translocations and fertility. Prenatally irradiated females were allowed to bear and nurture one litter, then tested for dominant lethality in a second litter. Germ cell survival and follicular development were assessed in their serially sectioned ovaries. Sperm production was not significantly affected in the 0.25 rad boars; boars irradiated with 1.0 rad per day produced sperm at only 17% of the total level. Incidence of defective sperm was 4.9% and 11.1% in the 0.25 and 1.0 groups, respectively. Four of the 1.0 rad boars were infertile, but prenatal irradiation apparently caused neither dominant lethality nor reciprocal translocations in fertile males. Number of oocytes was reduced to 66 ± 7% of control in the 0.25 rad gilts, but reproductive performance was unaffected and no dominant lethality was observed. Only 7 ± 1% of the oocytes survived in the 1.0 rad group. Reproductive performance was normal for the first litter, but 4 of the 23 tested were infertile at the second litter and a significant incidence of dominant lethality was observed. Whether this was a direct irradiation effect or the consequence of severe oocyte depletion is discussed. Diminished reproductive capacity rather than mutation appears to be the major consequence of continuous prenatal irradiation in the pig.

Feuerstein, G.; Lux, W. E., Jr.; Snyder, F.; Ezra, D.; and Faden, A. I.
Hypotension produced by platelet-activating factor is reversed by thyrotropin-releasing hormone.
Circ. Shock 13:255–260, 1984.

Platelet-activating factor (PAF), a vasoactive phospholipid implicated in anaphylactic reactions, causes severe hypotension in experimental animals that is highly resistant to pharmacological therapy. In the present studies, we showed that PAF (1 nmol/600 g body weight, IV) produced profound hypotension in unanesthetized guinea pigs that was promptly and completely reversed by thyrotropin-releasing hormone (TRH) (2 mg/kg, IV) or by the synthetic TRH analog MK771 (2 mg/kg, IV). TRH also reversed this hypotension when administered intracerebroventricularly (ICV) at a dose (0.02 mg/kg) that was systemically ineffective. The opiate receptor antagonist naloxone (5 mg/kg) was less effective than TRH in reversing the cardiovascular consequences of PAF administration. These data suggest that TRH reverses PAF-induced shock through central receptor-mediated mechanisms. This therapeutic action of TRH may partially account for the beneficial cardiovascular effects of this peptide in anaphylactic shock.

Frome, E. L.
The analysis of rates using Poisson regression models.
Biometrics 39:665–674; 1983.

Models are considered in which the underlying rate at which events occur can be represented by a regression function that describes the relation between the predictor variables and the unknown parameters. Estimates of the parameters can be obtained by means of iteratively reweighted least square (IRLS). When the events of interest follow the Poisson distribution, the IRLS algorithm is equivalent to using the method of scoring to obtain maximum likelihood (ML) estimates. The general Poisson regression models include log-linear, quasilinear and intrinsically nonlinear models. The approach considered enables one to concentrate on describing the relation between the dependent variable and the predictor variables through the regression model. Standard statistical packages that support IRLS can then be used to obtain ML estimates, their asymptotic covariance matrix, and diagnostic measures that can be used to aid the analyst in detecting outlying responses and extreme points in the model space. Applications of these methods to epidemiologic follow-up studies with the data organized into a life-table type of format are discussed. The method is illustrated by using a nonlinear model, derived from the multistage theory of carcinogenesis, to analyze lung cancer death rates among British physicians who were regular cigarette smokers.

Hayes, R. L.
The interaction of gallium with biological systems. *Int. J. Nucl. Med. Biol.* 10:257–261; 1983.

The element gallium, although it has no known trace element function, interacts with biological systems in many unique ways. When administered intravenously at low levels in the trivalent form, it initially binds to plasma proteins, principally transferrin. These plasma protein binding sites are saturable, and, when milligram quantities of gallium are administered, deposition of gallium in bone is enhanced and

1028626

its urinary excretion is greatly increased. Gallium uptake in soft tissue at the same time decreased. In the absence of saturation or blocking by other agents, the eventual tissue istribution of gallium is mainly controlled by the binding of gallium to plasma proteins. When carrier-free ^{67}Ga is administered, it is concentrated to a remarkable degree in malignancies and in inflammatory processes. Other factors that affect the gross tissue distribution of ^{67}Ga include viability, age, sex, pregnancy, lactation, exposure to ionizing radiation, and food intake. At the subcellular level ^{67}Ga is predominantly associated with lysosomes and small lysosomal-like particles. In addition to being bound to transferrin, it has been suggested that the association of ^{67}Ga with ferritin, siderophores, lactoferrin, and a 4-5 x 104 dalton glycoprotein may be of biological importance. There is considerable controversy about the mechanism(s) that may be involved in the preferential uptake of ^{67}Ga by malignancies and inflammatory lesions. Hyperpermeability of the plasma membrane, transferrin receptor sites, and endocytotic processes are some of the factors that may be involved.

Hoffman, D. R.; Hajdu, J.; and Snyder, F.
Cytotoxicity of platelet activating factor and related alkyl-phospholipid analogs in human leukemia cells, polymorphonuclear neutrophils, and skin fibroblasts.
Blood 63:545–552; 1984.

A series of 11 alkyl-phospholipid analogs, structurally related to platelet activating factor (L-PAF), were analyzed for cytoxic activity in human leukemic (HL-60) cells, human polymorphonuclear neutrophils, and Detroit 551 human skin fibroblasts. The order of selectiveness of the analogs in their cytotoxic response toward HL-60 cells in comparison to neutrophils is 1-alkyl-2-acetamide-GPC* > 1-alkyl-2-methoxy-GPC > D-PAF > 1-acyl-2-lyso-GPC > 1-alkyl-2-lyso-GPC > L-PAF. A time-sequenced progression of events caused by the most potent cytotoxic alkyl-phospholipid analogs was characterized by (a) a rapid decrease in the cellular uptake and incorporation of ^{3}H-thymidine into DNA that was detectable 4 hours after exposure to the analog, (b) a release of lactate dehydrogenase activity into the media at 8 hours after exposure, and (c) a decrease in cell number due to cell death that begins at 12 hours after exposure. Treatment of HL-60 cells with 1-alkyl-2-methoxy-GPC for 1 hour destroyed 40% of the cells after a subsequent 24-hour incubation period. The varied biologic activities of L-PAF, including how it affects serotonin release from platelets, blood pressure in rats, and cytotoxic responses in normal and leukemic cells, are discussed in relation to its D-enantiomer, 3-alkyl-2-acetyl-GPC, and the 2-acetamide analog. This report characterizes the kinetic events of the cellular responses in both normal and HL-60 cells in relation to the antineoplastic activities of unnatural ether-linked phospholipid analogs that are structurally related to L-PAF.

Hoffman, D. R.; Stanley, J. D.; Berchtold, R.; and Snyder, F.
Cytotoxicity of ether-linked phytanyl phospholipid analogs and related derivatives in human HL-60 leukemia cells and polymorphonuclear neutrophils.
Res. Commun. Chem. Pathol. Pharmacol. 44:293–306; 1984.

A cytotoxic activity, highly selective for neoplastic cells, is expressed by 1-alkyl-2-methoxy-*sn*-glycero-3-phosphocholine and by other derivatives closely related to the chemical structure of platelet activating factor. The antineoplastic potencies of a new series of analogs tested in HL-60 human leukemia cells and human polymorphonuclear neutrophils are reported. The degree of cytotoxicity was documented according to the ability of each analog to 1) destroy leukemic or normal cells or 2) to release lactic acid dehydrogenase from these cells. An index of selectivity of the analogs for their cytotoxicity toward leukemia cells is presented. Substitution by the twenty carbon branched-chain phytanyl moiety in place of the straight chain alkyl ether-linked group at the *sn*-1 position of various phospholipid analogs resulted in a three- to tenfold reduction in their cytotoxic potency of HL-60 leukemia cells. The enantiomeric isomers (D-forms) of several of the analogs possessed slightly greater cytotoxic activity in leukemia cells than the L-isomers. Alkyl-phospholipid analogs possessing the *sn*-2-acetyl (platelet activating factor) or *sn*-2-propionoyl substituents, both biologically active in their ability to aggregate platelets and to induce hypotension, were relatively innocuous in terms of the measured cytotoxic responses in both HL-60 cells and neutrophils.

Keane, A. T., and Polednak, A. P.
Retention of uranium in the chest: implications of findings in vivo and postmortem.
Health Phys. 44, Suppl. 1:391–402; 1983.

An unusually protracted retention of uranium in the chests of certain workers at a uranium processing plant has been reported in the literature. This finding has implications for the protection of current uranium workers and for health-effects studies of early workers. It is shown that the limited data obtained postmortem that have been reported do not reveal a significant unusually protracted retention of uranium in the pulmonary region or in the tracheobronchial lymph nodes. Probable explanations of the disparate findings are discussed. Data are presented on mortality from cancers of lymphatic tissues among males who worked in the period from 1943 to 1947 at the Y-12 Plant, Tennessee-Eastman Corp., Oak Ridge. No significantly increased mortality from these cancers was evident.

Lackey, R. J., and Cabot, M. C.
Acetate utilization and fatty acid metabolism in phorbol ester

1028627

nd dimethyl sulfoxide-differentiated human leukemia cells.
Cancer Lett. 20:291–297; 1983.

Cultured human promyelocytic leukemia cells were incubated with [1-^{14}C]acetate to assess the predominant mode of fatty acid snythesis (de novo vs. chain elongation) in these cells and in chemically differentiated populations. 12-O-Tetradecanoylphorbol-13-acetate (TPA) and dimethyl sulfoxide (DMSO) will induce differentiation of this leukemic cell line to macrophage- and granulocyte-like cells, respectively. Human peripheral and rat peritoneal macrophages were employed as controls for the TPA-induced counterpart. Labeling of the carbonyl carbon (C-1) was determined by Schmidt degradation and used to distinguish between chain elongation of pre-existing cellular fatty acids and de novo synthesis. Cultured leukemia cells and the TPA-derived macrophage only incorporated from 16% to 23% of the total radioactivity into the C-1 position, indicating an operable de novo pathway. Cells differentiated by exposure to DMSO displayed a preference for the chain elongation mechanism (89% ^{14}C in C-1 position) of fatty acid synthesis. Both rat peritoneal and human peripheral macrophages likewise incorporated >80% of the radioactivity in the C-1 position of the fatty acyl chains. Thus, DMSO-treated leukemia cells resemble normal differentiated cells, whereas phorbol ester-induced cells, in contrast, retain ochemical features of the undifferentiated cancer cell.

Lee, T-c.; Lenihan, D. J.; Malone, B.; Roddy, L. L.; and Wasserman, S. I.
Increased biosynthesis of platelet activating factor in activated human eosinophils.
J. Biol. Chem. 259:5526–5530; 1984.

1-Alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase catalyzes the conversion of biologically active lysophospholipid to bioactive platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) by an acetylation reaction. The activity of this enzyme in eosinophils isolated from patients with eosinophilia is stimulated (up to fourfold) in a dose-, time-, and Ca^{2+}/Mg^{2+}-dependent manner after exposure to the eosinophil chemotactic factor of anaphylaxis (ECF-A), C5a, formyl-methionyl leucylphenylalanine (fMLP), or ionophore A23187. The three naturally occurring chemotactic factors (ECF-A, C5a, and fMLP) cause a rapid and transient increase of enzyme activity, with a maximum at 1 or 3 minutes, whereas ionophore A23187 maintains an elevated level for up to 15 minutes. The activity of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase, an enzyme that catalyzes the breakdown of PAF to lyso-PAF, is not affected by C5a, fMLP, or ionophore A23187. The presence of PAF in eosinophils was established by demonstrating the lipid nature of the compound, the R_f value being identical with that of synthetic 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine on thin layer chromatograms, and by its ability to induce serotonin release from rabbit platelets. Furthermore, ECF-A, C5a, fMLP, and ionophore A23187 all induce the secretion of PAF from eosinophils. These findings suggest that the generation and release of PAF could be a consequence of eosinophil chemotactic activation and may thus function in inflammatory and allergic reactions in which eosinophils participate.

Lenihan, D. J., and Lee, T-c.
Regulation of platelet activating factor synthesis: Modulation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase by phosphorylation and dephosphorylation in rat spleen microsomes.
Biochem. Biophys. Res. Commun. 120:834–839; 1984.

1-Alkyl-2-lyso-*sn*-glycero-3-phosphocholine:acetyl-CoA acetyltransferase plays an important regulatory role in the biosynthesis of platelet activating factor, a potent bioactive mediator. We tested the hypothesis that the activity of acetyltransferase may be modulated by enzymatic phosphorylation and dephosphorylation. The results showed that acetyltransferase activity in rat spleens was two- to threefold higher in microsomes isolated in the presence of F^- than in those isolated in the presence of Cl^-. The microsomal acetyltransferase could be activated by preincubation of microsomes, isolated in the presence of Cl^-, with ATP, Mg^{2+}, and the soluble fraction from rat spleen. Addition of phosphatidylserine, diacylglycerols, plus Ca^{2+} further enhanced the activity. The increase in the activity of acetyltransferase was abolished by treatment of the activated microsomes with alkaline phosphatase. Conversely, the activity of acetyltransferase can be reactivated in the alkaline phosphatase-treated microsomes with incubation conditions that favor phosphorylation. Therefore, our findings suggest that acetyltransferase activity is regulated by reversible activation/inactivation through phosphorylation/dephosphorylation.

Littlefield, L. G.; Joiner, E. E.; Colyer, S. P.; DuFrain, R. J.; and Washburn, L. C.
Evaluation of cellular proliferation and chromosome breakages after in vitro exposure of human lymphocytes to calcium or zinc DTPA.
Radiat. Res. 99:129–139; 1984.

The analysis of mitotic indices (MI) and chromosome breakages in metaphases of 50-hour lymphocyte cultures exposed to the calcium or zinc chelates of diethylenetriamine pentaacetic acid (DTPA) demonstrated (1) an 80% reduction in MI in cultures from three women but no reduction in those from two men after in vitro exposure to CaDTPA in concentrations as low as ~10 μg/ml culture medium, and complete suppression of mitoses in cultures from men and women after exposure to 40 μg/ml CaDTPA; (2) minor suppression in MI in cultures from women and none in those

1028628

from men after exposure to 40 or 80 μg/ml ZnDTPA; (3) no ring or dicentric chromosomes in 1,700 metaphases from DTPA-treated cultures. Likewise, in other experiments we observed no differences in the frequency or distributions of rings and dicentrics in lymphocyte cultures from two persons after in vitro exposure to 250-R ^{60}Co gamma radiation in the presence or absence of 10 μg/ml CaDTPA or 10 or 80 μg/ml ZnDTPA. These data indicate that while accurate estimates of the frequencies of radiation-induced rings and dicentrics in lymphocytes can be made in actinide-contaminated persons undergoing DTPA chelation therapy, blood samples for cytogenetic cultures should not be obtained from chelated patients until the compound has been cleared from the blood plasma.

Schwarz, O. J., and Eisele, G. R.
Food chain transport of synfuels: experimental approaches for acquisition of baseline data.
In *Synthetic Fossil Fuel Technologies: Results of Health and Environmental Studies*, (Cowser, K., ed.), pp. 441–462, Boston: Butterworth, Inc. 1984.

Experiments designed to define the extent of uptake, retention, and distribution of radiolabeled naphthalene, α-naphthol, and 7-methylbenz(c)acridine in consumable meats, eggs, milk, and plants are described. The data from these experiments demonstrate the retention of variable but significant amounts of these compounds and/or their metabolites, indicating that they have the potential to enter the food chain. The animal experiments with swine, chickens, and dairy cattle include both acute and chronic studies. Plant experiments measured the extent of uptake and transport via a root port-of-entry mechanism in selected vegetable crops (i.e., lettuce, *Lactuca sativa*; onion, *Allium sepa*; pea, *Pisum sativum*). A hydroponic-based system was used to provide "worst case" exposure conditions.

Snyder, F.; Lee, T-c.; Blank, M. L.; Cabot, M. C.; Malone, B.; and Albert, D. H.
Enzymatic pathways for platelet activating factor In *Platelet-Activating Factor and Structurally Related Ether-Lipids* edited by J. Benveniste and B. Arnoux, Proceedings of the 1st International Symposium on Platelet-Activating Factor, 1983, Paris, pp. 253–261. Amsterdam, The Netherlands: Elsevier Biomedical Press, (INSERM Symposium No. 23), 1983.

Biosynthesis of the ether bond in lipids is catalyzed by alkyl-DHAP synthase from acyl-DHAP and long chain fatty alcohols via a unique substitution reaction. Subsequent steps lead to the formation of 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, the substrate for the phospholipase A2 catalyzed reaction that forms 1-alkyl-2-lyso-GPC; this lysophospholipid serves as the substrate for the acetyltransferase that forms PAF. An alternate pathway involving a highly specific cholinephosphotransferase with 1-alkyl-2-acetyl-*sn*-glycerol as a substrate also exists for PAF biosynthesis. The inactivation of PAF involves acetylhydrolase and acyltransferase activities that produce the inactive form of PAF (1-alkyl-2-acyl-*sn*-GPC). This report also demonstrates that serum acetylhydrolase levels vary widely among different animal species.

Tardif, S. D.
The relationship between social interactions and sexual maturation in female *Saguinus oedipus oedipus*.
Folia Primatol. 40:268–275; 1983.

Four female *Saguinus oedipus oedipus* were paired with strange, unrelated males while five remained in their natal family group. A comparison of the frequency of selected social interactions involving these females when sexually immature versus mature was made. The frequency of sexual and affiliative interactions in male-female pairs was basically unchanged by the female's maturation. Mother-daughter interactions were also not changed in a consistent fashion by the daughters' maturation. Females with strange, unrelated males, either alone or as part of their natal group, displayed increases in marking behavior in association with sexual maturation.

Tardif, S. D.
Social influences on sexual maturation of female *Saguinus oedipus oedipus*.
Am. J. Primatol. 6:199–209; 1984.

The hypothesis tested was that *Saguinus oedipus oedipus* females housed with adult males would mature sexually at an earlier age than females remaining in their natal family groups. Six females were housed with strange, unrelated males. Five females remained in their natal groups. Blood samples were taken twice weekly, and the plasma was assayed for progesterone. Sexual maturation was operationally defined as that age at which plasma progesterone levels became consistently detectable. Females housing with males did mature at an earlier age than females remaining in their natal groups. In addition, it was noted that the presence or absence of a healthy, reproductive mother in the natal group was not related to the daughters maturation age. However, whether the natal group as a whole inhibited maturation of young females or whether an unrelated male accelerated maturation, or both, remains unknown.

Tardif, S. D.; Richter, C. B.; and Carson, R. L.
Effects of sibling-rearing experience on future reproductive success in two species of Callitrichidae.
Am. J. Primatol. 6:377–380; 1984.

The survival rate for offspring of mothers who either had or did not have previous experience rearing younger siblings was compared in two callitrichid species, *Callithrix jacchus* and *Saguinus oedipus*. Offspring of mothers with

1028629

bling-rearing experience had a higher survival percentage ian offspring of inexperienced mothers in both species. While 50–60% of offspring of inexperienced *C. jacchus* mothers survived, no offspring of inexperienced *S. oedipus* mothers survived. The results suggest that sibling-rearing experience is necessary to the development of maternal behavior in *S. oedipus*, but not necessary to the development of maternal behavior in *C. jacchus*. Effects of previous sibling-rearing experience of *S. oedipus* fathers on offspring survival were also examined. Whether the father had rearing experience was not related to the survival of their offspring.

Tardif, S. D.; Richter, C. B.; and Carson, R. L.
Reproductive performance of three species of Callitrichidae.
Lab. Anim. Sci. 34:272–275; 1984.

The reproductive performance of three species of Callitrichidae housed under identical conditions, including relatively spacious caging and visual screening, was compared. The three species were *Callithrix jacchus*, *Saguinus fuscicollis*, and *Saguinus oedipus*. *Callithrix jacchus* was the most reliable breeder, with the lowest percentage of stillborn and the highest post-weaning survival. While the reproductive performance of *S. oedipus* was better than that generally reported for other colonies, the reproductive performance of the *Saguinus* species was still poorer than that of *C. jacchus*. e two *Saguinus* species did not differ significantly in either rcentage of stillborn or post-weaning survival. None of the species showed infertility due to lactation, but the mean interbirth interval was significantly longer for *S. oedipus* than for either *C. jacchus* or *S. fuscicollis*.

Wirth, N.; Swanson, D.; Shapiro, B.; Nakajo, M.; Coffey, J. L.; Eckhauser, F.; and Owyang, C.
A conveniently prepared Tc-99m resin for semi-solid gastric emptying studies.
J. Nucl. Med. 24:511–514; 1983.

A polystyrene resin suitable for semi-solid gastric emptying studies was rapidly (<20 min) and conveniently prepared using commercially available reagents. Using the outlined procedure, Chelex-100 resin bound Tc-99m with greater than 98% labeling efficiency. The resulting Tc-99m Chelex-100 resin demonstrated excellent in vitro and in vivo stability. The clinical application of Tc-99m Chelex-100 resin, mixed with oatmeal, was tested in normal subjects and in various patient groups, including diabetic autonomic neuropathy, pyloric obstruction, postoperative dumping syndrome, and morbidly obese patients before and after gastroplasty.

1028630

1984



William W. Burr, Jr., Chairman

1028631

Training Programs

Training programs in the Medical and Health Sciences Division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. Special short-term courses dealing with radiation health problems are available for biomedical personnel.

Graduate students participating in Ph.D. training programs come from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama (Birmingham), and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals. Advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by Oak Ridge National Laboratory, the University Programs Division at ORAU (sponsored by DOE), and individual research grants.

Postdoctoral training continues to be an important part of the Division's research efforts. In the past, funds for support of postdoctoral fellows have been available from a variety of sources: the Damon Runyon-Walter Winchell Cancer Fund, the Andrew W. Mellon Foundation, a National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health and American Cancer Society grants, and divisional funds.

Summer research opportunities make it possible for academic staff and advanced undergraduate students from colleges and universities throughout the United States to participate in any of the division's research programs. The DOE-sponsored summer program is administered through the University Programs Division at ORAU.

Short-term courses in the management of medical problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of ten REAC/TS courses were given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

The following individuals participated in the training programs at the Division during the past year:

1028632

1. **Predoctoral students**
   Arvind Carpenter* (Epidemiology)
   University of Alabama
   Donna Cragle† (Epidemiology)
   University of North Carolina
   Elizabeth Dupree Ellis‡ (Epidemiology)
   University of North Carolina
   Daniel Strom* (Environmental Sciences and Engineering)
   University of North Carolina
   Jefferson Surles* (Medicinal Chemistry)
   University of North Carolina
   Donna Woodard‡ (Biochemistry)
   University of Tennessee
2. **Students pursuing MPH degree**
   Shirley Fry‡ (Epidemiology)
   University of North Carolina
3. **Postdoctoral fellows**
   Dennis R. Hoffman (Biochemistry)
   Mitchell E. Robinson (Biochemistry)
   Eng-Lay Tan (Biochemistry)
   Jerome Wilson (Epidemiology)
4. **Visiting scientists**
   Joyce E. Harris (Radiopharmaceuticals)
   Knoxville College
5. **College student research participants**
   Sheryl L. Byrkit (Administration)
   University of Tennessee, Knoxville
   Kathleen M. Darcy (Cytogenetics)
   St. Joseph's College, Brooklyn, N.Y.
   Daniel W. Lawson (Nuclear Medicine)
   University of Tennessee, Knoxville
   Richard J. Lin (Radiopharmaceuticals)
   Princeton University, Princeton, N.J.
   Andrew M. Liu (Epidemiology)
   The Johns Hopkins University, Baltimore, Maryland
   Julie L. Reihsen
   Southwestern Missouri State University, Springfield
6. **High school vocational education students**
   Deddrick Brown (Field Site)
   Oak Ridge High School
   Lisa Freeman (Biochemistry)
   Clinton Senior High School
   Robin Gregory (Epidemiology)
   Anderson County High School
   Gary Jackson (Epidemiology)
   Oak Ridge High School
   Linda Manning (Biochemistry)
   Anderson County High School
   Rhonda Scott (Epidemiology)
   Oak Ridge High School

* Portion of thesis research being done at the Division.
†Non-thesis work.
‡Thesis research being completed at the Division.

1028633

Non-DOE-Funded Research

N. Clapp
Operation of a marmoset colony for cancer research. National Institutes of Health, National Cancer Institute $216,594* (23 June 1984 to 22 July 1985; last year of three-year award)

D. Cragle
Minority high school student research apprentice program. National Institutes of Health, Division of Research Resources $3,000* (1 March 1984 to 28 February 1985; current year of continuing award)

J. Crook
Red cell transmembrane transport. Medical College of Wisconsin $11,925‡ (2 February 1984 to 31 July 1984)

G. Eisele
Actinide metabolism studies. Nevada Operations Office/U.S. Department of Energy‡ $225,000† (1 October 1984 to 30 September 1985; seventh year of eight-year award)

Food chain transport of synfuels. U.S. Environmental Protection Agency-U.S. Department of Energy Interagency Agreement $314,400† (1 October 1984 31 July 1985; last year of four-year award)

S. Fry
Feasibility of a mortality study of workers in nuclear power plants. Electric Power Research Institute, Inc. $19,104† (1 March 1984 to 30 April 1984)

K. F. Hübner
^{11}C-Amino acids/positron ECT for pancreatic studies. National Pancreatic Cancer Project, National Cancer Institute $80,734* (1 July 1983 to 30 June 1984; last year of three-year award)

L. G. Littlefield
Evaluation of induced cytogenetic lesions in oocytes. National Institute of Child Health and Human Development $101,331* (1 August 1983 to 31 July 1984; last year of nine-year award)

Studies of radiation-induced chromosome damage in humans. National Cancer Institute–U.S. Department of Energy Interagency Agreement $145,000† (1 September 1984 to 30 September 1985; last year of four-year award)

C. C. Lushbaugh
Biomedical research support grant. National Institutes of Health, Division of Research Resources $29,372* (1 April 1984 to 31 March 1985; current year of continuing award)

A. McFee
Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations. National Cancer Institute–U.S. Department of Energy Interagency Agreement $256,2185† (30 September 1984 to 29 September 1985; first year of two-year award)

1028634

F. Snyder

Ether lipids in cancer: enzyme mechanisms and inhibitors. National Cancer Institute $65,466* (1 May 1984 to 30 April 1985; last year of five-year award)

Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells. American Cancer Society $86,331† (1 January 1984 to 31 December 1984; first year of two-year award)

A new class of biologically active phospholipids. National Heart, Lung, and Blood Institute $67,730* (1 April 1984 to 31 March 1985; fourth year of five-year award)

S. Tardif

Determinants of reproductive competence in tamarins. National Institutes of Health, Division of Research Resources $39,571* (5 September 1984 to 4 September 1985; first year of three-year award)

*Direct costs (current year)
†Total costs, i.e., direct plus overhead (current year)
‡Not under DOE prime contract

1028635

Collaborative Research

M. C. Cabot
Lipid requirements for protein kinase C activation. National Cancer Institute (Bethesda), S. Jaken.

N. K. Clapp
Fecal bile acid analysis in marmosets. Beth Israel Medical Center (New York), B. Cohen.

In vitro tetraploidy in skin fibroblasts of colon cancer tamarins. Cornell Medical College (New York), B. S. Danes.

Genetic segregation and familial cancer relationships in colon cancer tamarins. Creighton University (Omaha), W. Kimberling and H. Lynch.

Coronaviruses in colitis and colon cancer. University of Tennessee (Knoxville), College of Veterinary Medicine, D. Brian.

Carcinoembryonic antigens in colon tumors. Harvard Medical School (Boston), P. Thomas.

Lectin binding in colitis and colon cancer in cotton-top tamarins. VA Medical Center (Ann Arbor), C. R. Boland.

Culture of tamarin colon cancer cells. Baylor College of Medicine (Houston), M. Stain.

Fecal toxins in tamarin colon disease. University of Tennessee Memorial Research Center (Knoxville), J. Fuhr.

Idiopathic ulcerative colitis in marmosets. University of Nevada School of Medicine (Reno), O. Peck and J. Wood.

In vitro culture of tamarin colonic tumors. Oak Ridge National Laboratory (Oak Ridge), E. Perkins.

A study of CEA and LDH_k in colon-cancer-bearing tamarins. Roswell Park Memorial Institute (Buffalo), N. Petrelli.

J. E. Crook
Production of positron-emitting radionuclides using the Tandem van de Graaff accelerator. Oak Ridge National Laboratory, Physics Division, P. D. Miller, Jr.

Development of software for quantitative positron tomography.

The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, G. Smith.

Vanderbilt University (Nashville), Department of Biomedical Engineering, P. H. King.

^{11}C-Amino acids/positron ECT for pancreatic studies. Brookhaven National Laboratory, Medical Department, A. B. Brill.

Clinical investigation of newer antihypertensive agents. The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, Peasley, and Department of Family Practice, G. Shocklett.

Synthesis of labeled compounds using organoborane techniques. University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka.

1028636

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp.

Use of radionuclides in animal models of human disease. The University of Tennessee Center for the Health Sciences (Knoxville) Department of Radiology, K. F. Hübner.

R. J. DuFrain

Species comparisons in cytogenetic assays. Oak Ridge National Laboratory, Biology Division, R. J. Preston.

Statistical analysis and Poisson regression studies in biological dosimetry. Oak Ridge National Laboratory, Computer Science Division, E. L. Frome.

T-c. Lee

Effect of stress on the metabolism of platelet-activating factor. University of Tennessee (Knoxville), Department of Zoology, N. Greenburg.

Metabolism of platelet-activating factor in human blood cells. University of California (San Diego), Division of Rheumatic Diseases, S. I. Wasserman.

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Environment Epidemiology Branch (Bethesda), C. Land and R. Kleinerman.

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons.

Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straube, and A. Carrano.

Los Alamos National Laboratory (Los Alamos), L. Devan.

Radiation Effects Research Foundation (Hiroshima), A. Awa.

Coordinated research program in routine use of chromosomal aberration analysis in occupationally exposed radiation workers. International Atomic Energy Agency (Vienna), A. Bianco.

Cytogenetic follow-up studies of a survivor exposed to ^{241}Am. Hanford Environmental Health Foundation (Washington), B. D. Breitenstein.

C. C. Lushbaugh

United States Transuranium Registry. Hanford Environmental Health Foundation, B. D. Breitenstein.

C. C. Lushbaugh and N. K. Clapp

Marmoset T and B cellular immunology. Meharry Medical College (Nashville), P. Patel.

C. C. Lushbaugh and S. A. Fry

Epidemiology of human carcinogenesis. University of Alabama (Birmingham), Department of Epidemiology, W. D. Flanders.

Comprehensive epidemiology of DOE atomic workers. University of North Carolina (Chapel Hill), Department of Epidemiology, C. M. Shy.

1028637

Study of mortality among former Manhattan Engineer District sites at Linde Corporation and the Electro Metallurgical Laboratory. Union Carbide Corporation, Corporate Epidemiology Department, S. G. Austin and T. A. Lincoln.

C. C. Lushbaugh and L. C. Washburn
Diethylenetriaminepentaacetate (DTPA) project. Department of Energy contractors, 40 coinvestigators.

F. Snyder
Biological effects of unique phospholipid analogs. California State University (Northridge), Department of Chemistry, J. Hajdu.

Mass spectrometry of unique lipids. Oak Ridge National Laboratory, Analytical Chemistry Division, G. L. Glish.

Nuclear magnetic resonance studies of unique lipids. Oak Ridge National Laboratory, Chemistry Division, B. M. Benjamin.

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Piantadosi.

Effects of a new group of antihypertensive phospholipids on cardiovascular function. University of Tennessee (Knoxville), Department of Psychology, J. E. wler and J. W. Hubbard.

ıhe role of intracellular transfer proteins in the cellular movement of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet aggregating phospholipid). Western Carolina University (Cullowhee), Department of Biology, R. H. Lumb.

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein.

Selective cytotoxic activity of alkyllysophospholipids in tumor cells. Technische Universitat Munchen (West Germany), Division of Oncology, W. E. Berdel

Pulmonary properties of an antihypertensive phospholipid. University of British Columbia (Vancouver), Pulmonary Research Laboratory, R. R. Schellenberg

S. Tardif
Reproductive aging of female tamarins. University of Winsconsin (Madison), Department of Psychology, C. T. Snowdon and T. E. Ziegler.

Skeletal age changes and neonatal growth in tamarins. Southwest Foundation for Biomedical Research, San Antonio, Texas, D. Glassman.

L. C. Washburn
Pharmacological studies using 11C-labeled compounds. University of Kentucky (Lexington), College of Pharmacy, G. A. Digenis.

^{11}C-labeled amines for pulmonary function studies. University of Alberta (Edmonton), Faculty of Pharmacy and Pharmaceutical Sciences, A. A. ujaim, and Department of Medicine, S. F. P. Man.

ıadiolabeled monoclonal antibodies for cancer diagnosis and therapy. The Wistar Institute (Philadelphia), Z. S. Steplewski.

Production of ^{11}C-labeled amino acids. Brookhaven National Laboratory, Medical Department, S. C. Srivastava and Chemistry Department, J. S. Fowler.

1028638

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1028639

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[9]Part-time Continuing
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1028640

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1984/1985

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The Medical and Health Sciences Division conducts research on biomedical problems involving the respiratory, vascular, pancreatic, and gastrointestinal systems. Emphasis is on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Research programs in the division are focused on the following areas: (a) preclinical and clinical development of new positron radiopharmaceutical agents for in vivo diagnosis of diseases in humans, (b) biochemical aspects of cellular lipid mediators and membrane lipids in disease processes, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), (e) food chain transport studies of synfuels and actinides, and (f) interdisciplinary investigations of the marmoset as a primate model for spontaneous colon cancer and other human diseases, particularly those associated with energy-related toxicants. Predoctoral and postdoctoral training is provided in each research program area. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a treatment and decontamination facility and a demonstration and training site.

Oak Ridge Associated Universities is a private, not-for-profit association of 49 colleges and universities. It is a contractor of the U.S. Department of Energy, conducting research and educational programs in the areas of energy, health, and the environment for DOE, ORAU's member institutions, other colleges and universities, and other private and governmental organizations.

ORAU-260, March 1986

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

NOTICES

The opinions expressed herein do not necessarily reflect the opinions of the sponsoring institutions of Oak Ridge Associated Universities.

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Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes
Printed copy: A04
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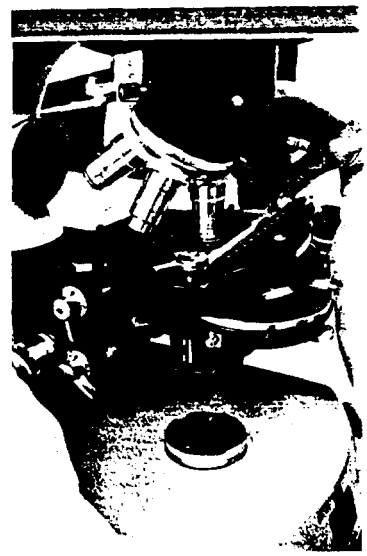
Medical and Health Sciences Division

Research Report 1984/1985



Cover photograph—
Electron microscope photograph of normal intestinal mucosa showing principal cell types.

1028645



- An interim report by the epidemiology group on cancer mortality among former workers at the Manhattan Engineer District site of the Linde Corporation showed no association between increases in mortality and exposure to radiation or uranium dust.
- Researchers in the Center for Epidemiologic Research developed computer software, called "PCAUS," that uses radioepidemiologic tables from the National Institutes of Health to calculate the probability that an existing cancer of an individual previously exposed to radiation might be radiation-induced.
- Biochemistry staff demonstrated that dual pathways with different functions exist for the biosynthesis of platelet activating factor (PAF), a potent cellular mediator involved in inflammatory and hypotensive responses. One of the pathways is involved in inflammatory responses, whereas the other route appears to be necessary for maintaining physiological levels of PAF for blood pressure control and other cellular functions.
- In the biochemistry program a novel group of antitumor PAF analogs, a new generation of membrane targeted anticancer agents, was found to exert selective cytotoxicity toward tumors by inhibiting the uptake of small essential nutrient molecules across the plasma membrane of cancer cells.
- The division has two patents pending with the U.S. Patent Office on two bioactive phospholipids, "platelet activating factor" and a related neutral ether-linked lipid; the chemical structures of both compounds were identified by the biochemistry group and shown to be potent, naturally occurring compounds for lowering blood pressure.
- Improved techniques were developed in the radiopharmaceutical development program for labeling DTPA-conjugated tumor-associated monoclonal antibodies with the potential therapeutic radionuclides, fermium-255, einsteinium-253, and yttrium-90.

1028646

- In the nuclear medicine program, copper-64 citrate was identified as a potential new agent for positron emission tomographic myocardial imaging.
- Experiments by the cytogenetics group to determine cytogenetic effects of cryopreservation on irradiated lymphocytes revealed no significant differences between frozen and freshly prepared lymphocytes in the recovery of metaphases with radiation-induced chromosomal aberrations. In other experiments with human lymphocytes, protein cross-linking agents with high specificity for lysine residues were shown to induce pronounced depressions in mitotic activity, but minimal increases in sister chromatid exchanges.
- In studies to optimize the breeding of marmosets and tamarins for research, the marmoset research group found that the maternal care of infants is directly related to the age of their mothers; experiments on the reproductive aging of female tamarins are expected to determine whether marmosets display endocrine, ovarian, and fertility changes similar to menopausal women. In the colon cancer project, mucins were identified in colitis and colon cancer in tamarins; such mucins are also found in humans with these diseases.
- The Radiation Emergency Assistance Center/Training Site staff continued follow-up studies of the Ciudad Juárez radiation accident in Mexico, including cytogenetic analysis of cells from irradiated persons to determine their exposure dose. REAC/TS continued to serve the Federal Emergency Management Agency in providing training courses on radiation hazards.
- The food chain transport group completed a study that demonstrated six toxic compounds related to the production of synthetic fuels would appear in consumable meats, eggs, and milk if the food-producing animals were exposed to such agents. Other studies on radionuclides administered orally to farm animals indicate that europium (-152, -153, and -154) is almost entirely excreted from exposed animals, whereas cesium-137, which is located in soft tissue, would render meat from such animals unfit for human consumption.
- During its first year of operation under division management, the ORAU technical assistance group to the Committee on Interagency Radiation Research and Policy Coordination hired staff, assisted in the establishment of advisory panels on policy and science with representatives from various federal agencies, and identified priorities on radiation issues.
- In addition to DOE support, the division has continued to successfully compete for research funds from a variety of government and private agencies (see page 55 for listing). Especially noteworthy is that the NCI contract to breed and raise marmosets for research was renewed; this colony is maintained by the division as a national primate resource for biomedical researchers throughout the United States.

W. W. Burr, Jr., Chairman
Fred Snyder, Associate Chairman
Medical and Health Sciences Division

1028647

contents

Scope of Research Programs 1

Biochemistry 2

Preclinical and Clinical Nuclear Medicine 7

Cytogenetics 14

Food Chain Transport 19

Marmoset Research 23

REAC/TS 27

Epidemiology 30

Committee on Interagency Radiation Research & Policy Coordination (CIRRPC) 36

Publications 39

Full-length Journal Articles 39

Chapters, Review Articles, Other Publications 41

Published Abstracts of Papers Presented at Meetings 43

Miscellaneous Papers or Talks Presented at National and International Scientific Conferences 44

Summaries of Published Papers 44

Training Programs 52

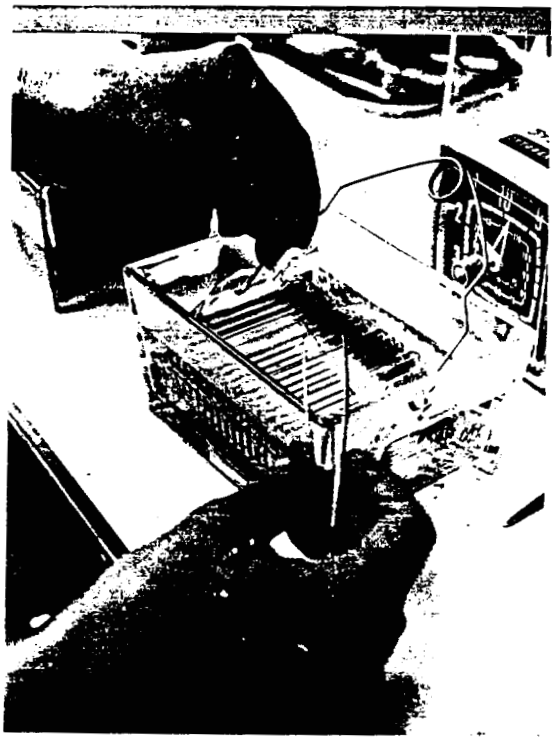
Non-DOE-Funded Research 55

Collaborative Research 56

Administration, Committees, and Staff 58

1028648

scope of research programs



1028649



Biochemistry

Research in the biochemistry program concerns three main areas: (1) phospholipid cellular mediators (platelet activating factor; i.e., PAF and related lipids), (2) antitumor ether-linked phospholipid analogs, and (3) the role of enzymes in lipid metabolism and cell membrane function. Highlights of each project are summarized in the following paragraphs; specific details can be found in the publications listed elsewhere in this report. Emphasis is on the metabolism, regulation, and function of lipids in mammalian cells, particularly in relationship to the mode of action of the biologically active phospholipids and the reaction mechanisms catalyzed by enzymes involved in glycerolipid metabolism. Biological systems used in these investigations include enzyme preparations, subcellular organelles, membrane vesicles, cultured cells, and animal models. The overall aim of the program in biological chemistry is to establish the role of glycerolipids in health and in the pathogenesis of cancer, hypertension, inflammation, and respiratory disease.

1028650

Phospholipid cellular mediators. This past year a number of the properties of enzymes involved in an alternative pathway for PAF biosynthesis were characterized. Specific reaction steps that make up this alternative route (via a cholinephosphotransferase) were established for (1) the acetylation of 1-alkyl-2-lyso-*sn*-glycero-3-P by a microsomal acetyltransferase with acetyl-CoA as the acetate donor, (2) dephosphorylation of the acetylated product (1-alkyl-2-acetyl-*sn*-glycero-3-P) by a phosphohydrolase to form alkylacetylglycerols, a hypotensive lipid, and (3) the final step in the conversion of the alkylacetylglycerols to PAF by a dithiothreitol-insensitive cholinephosphotransferase.

In a separate investigation, results documented that the substrate specificity of the acetyltransferase that utilizes 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine correlates very well with the structural features required for expression of the biological activities of PAF and its analogs in intact cells and in vivo. For example the acetyltransferase utilizes acyl-CoA's with carbon chain lengths greater than three carbon atoms much less effectively than either acetyl- or propionyl-CoA's, and the preference of the acetyltransferase for acetylating the lyso alkyl-phospholipid substrate occurs according to the following order: P-choline> P-dimethylethanolamine> P-monomethylethanolamine> P-ethanolamine.

Enzymatic experiments on the catabolism of PAF in rabbit platelets, rat endothelial cells, and rat alveolar macrophages under nonstimulating conditions firmly documented that lyso-PAF (1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) is the initial intermediate formed when PAF is inactivated. The reaction steps for the inactivation of PAF observed in all three cell types includes its conversion to lyso-PAF, which is then acylated to form "acyl"-PAF, a stored precursor of PAF. It is interesting that platelets produce barely detectable amounts of lyso-PAF when radiolabeled exogenous PAF is added to the cells, whereas under

identical conditions, both alveolar macrophages and endothelial cells produce and release relatively large quantities of lyso-PAF. These results further substantiate that the inactivation steps for PAF are common to all cell types investigated so far, but that the relative quantities of metabolites produced and released to the extracellular compartment differ.

Investigations in this project have clearly demonstrated that dual pathways exist for the biosynthesis of PAF. The cholinephosphotransferase pathway appears to play a prominent role in maintaining physiological levels of PAF for normal cellular functions, whereas the route involving 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase as the final reaction step is stimulated by agents that produce the excessive amounts of PAF responsible for inflammatory, anaphylactic, and allergic reactions.

Another important result was the discovery of a unique CoA-independent acyltransferase that selectively transfers arachidonic acid from diacyl cholinephospholipids to 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine, the intermediate produced during the inactivation of PAF by acetylhydrolase. Properties of the microsomal arachidonoyl transacylase have been characterized in a membrane fraction isolated from rabbit alveolar macrophages. This highly selective transacylase activity appears to account for the sequestering of arachidonic acid into the ether-linked lipid pool that can also serve as a precursor of PAF.

A number of physiological and pharmacological experiments were completed in collaborative projects with other research groups. Studies done with Dr. J. E. Lawler and co-workers at the University of Tennessee, Knoxville, indicate PAF reduces the mean blood pressure via a dose-related decrease in pe-

1028652

ripheral vascular resistance and cardiac index in conscious genetic hypertensive (SHR) and normal (WKY controls) rats. Results of studies done with Dr. G. Feuerstein and collaborators (Uniformed Services University of the Health Sciences) revealed that the hypotension produced by PAF is not due to a secondary effect of cysteinyl leukotrienes. The tachycardia evolved by PAF appears to be moderated by sympathetic activation and β-adrenegic receptors and supports the major role played by the sympathetic system and β-adrenoceptors in the recuperation from PAF-induced shock. These experiments also demonstrated that serum acetylhydrolase is an important regulatory system for controlling PAF levels in the vascular compartment.

Antitumor ether-linked phospholipid analogs. Several unnatural structural analogs of platelet activating factor have been shown to possess highly selective cytotoxic activities toward a number of different types of tumor cells. The alkyl-acetamide- and alkylmethoxy-glycerophosphocholines are two of the most potent antineoplastic lipid analogs yet identified. Our investigation of their biochemical mode of action indicates that these agents are plasma-membrane targeted and differ from conventional antineoplastic chemotherapy drugs that exert their lethal effects on tumor cells via inhibition of nucleic acid metabolism. Experimental results with a tritiated methoxy analog of PAF have shown it is



poorly metabolized in both tumor and normal cells; approximately 90 percent of the analog remained structurally unchanged when the analog was incubated with cell cultures for a 24-hr period. It is interesting that an enrichment of the radioactive analog occurs in the plasma membrane fraction of HL-60 cells. However, the most striking preliminary findings related to their mechanism of action suggest these ether-linked phospholipid analogs exert their antitumor activity by inhibiting the uptake of small essential nutrient molecules (e.g., amino acids, fatty acids, choline, etc.) into the tumor cells.

Future studies in this area are aimed at identifying the nature of the interaction between the antitumor ether-linked phospholipids and specific membrane components. The goal of these experiments is to identify the mechanism(s) responsible for their interference with such a variety of transport systems associated with the plasma membranes of tumor cells.

The role of enzymes of lipid metabolism in cell membrane function. Researchers in this prcject have continued to investigate the mechanism of the reaction catalyzed by alkyldihydroxyacetone-P synthase, the enzyme responsible for the biosynthesis of the alkyl ether bond in glycerolipids. A 1000-fold purified preparation of alkyl-DHAP synthase was shown to also have the capability of catalyzing the hydrolysis of the acyl moiety of acyldihydroxyacetone-P, the direct precursor of the ether bond. These results further support the original reaction mechanism for the formation of the alkyl ether bond that was previously proposed in earlier published studies from our laboratory.

Another exciting recent development has been the detection of diarachidonoyl and other species of phospholipids that contain two polyunsaturated acyl chains. Moreover, the rapid labeling of these highly unsaturated diacyl species in rat testes and erythrocytes after pulsing with labeled arachidonic acid sug-

1028654

gests that these species serve as important intermediates in the transfer of arachidonic acid among phospholipid classes.

Earlier work from our laboratory had established that acyl-CoA reductase catalyzes the conversion of acyl-CoA's to the long-chain fatty alcohols that form the alkyl ether-linked moiety of glycerolipids. Current results indicate acyl-CoA reductase is an important regulatory enzyme in the biosynthetic pathway for ether lipids and that it is under hormonal control. Testosterone was shown to be able to increase acyl CoA reductase activity 16-fold and the activity of the cytosolic stimulatory protein for this enzyme was also selectively enhanced by hormonal treatment.



Preclinical and Clinical Nuclear Medicine

The major objectives of these related research programs are to develop new radiopharmaceuticals and to carry out phase I and II testing of such agents in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of potential agents for radiodiagnosis and radiotherapy, tests of these substances in animals for effectiveness and toxicity, and clinical efficacy trials in humans according to U.S. Food and Drug Administration rules

1028655

and guidelines.

Emphasis is placed on the use of positron emission tomography (PET) to permit high-resolution, three-dimensional images as well as to act as a noninvasive probe of biochemical functions. Thus, it can be used specifically to detect metabolic alterations associated with disease processes. The clinical nuclear medicine program uses PET to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications in cardiovascular and neoplastic diseases, especially of the pancreas, with emphasis on a more quantitative evaluation of the in vivo distribution of such radiopharmaceuticals.

The facilities of the clinical nuclear medicine program have continued to improve through the addition and implementation of new software interactive programs for ORAU's ECAT II® positron tomographic scanner. These new programs will enhance quantitative imaging resulting in improved interpretations of regions of interest, especially in the thorax.

Amino acids with positron-emitting carbon-11 substituted for stable carbon-12 offer distinct advantages for studying protein synthesis and protein turnover in vivo, since ^{11}C-labeled agents are physiologically and chemically indistinguishable from their unlabeled counterparts. The laboratory's long-standing interest in ^{11}C-labeled amino acids continues. However, because access to the Oak Ridge National Laboratory 86-inch cyclotron is no longer available, the 41-inch and 60-inch cyclotrons at Brookhaven National Laboratory (BNL) are utilized in collaboration with Drs. A. B. Brill, J. S. Fowler, and A. P. Wolf.

Several years ago we developed a general method for the synthesis and purification of ^{11}C-carboxyl-labeled amino acid racemates (mixtures of the naturally occurring L-isomer and the unnatural D-isomer). For truly physiological studies of the pancreas, the ^{11}C-labeled L-amino acid must be separated

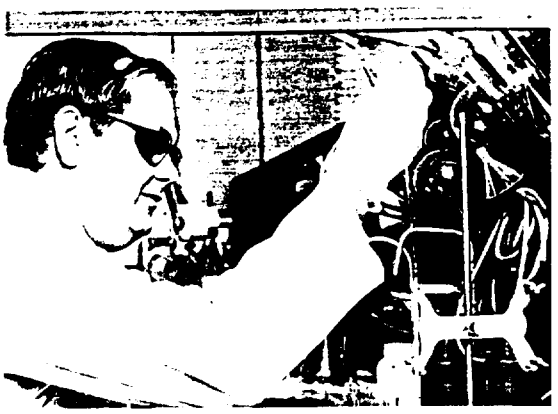
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from the racemic mixture by a process that is compatible with the 20.4 minute half-life of carbon-11. Optical resolution of DL-[^{11}C]tryptophan and DL-[^{11}C]valine have been accomplished by high-performance liquid chromatography (HPLC) using a conventional reverse-phase HPLC column and a chiral mobile phase.

Preclinical animal studies have shown that the L-isomers of tryptophan and valine are most suitable for the evaluation of pancreatic function. In addition, the unnatural D-forms of these amino acids used in conjunction with the L-forms appear to be of value in the differential diagnosis of pancreatic cancer. Investigational New Drug (IND) exemptions for both the L- and the D-isomers of ^{11}C-labeled tryptophan and valine have been granted by the U.S. Food and Drug Administration for clinical phase I and phase II testing in patients with pancreatic cancer. Preliminary clinical investigation of the L-isomers using the ECAT II® positron tomograph showed that L-[^{11}C]tryptophan is the most useful agent for imaging the pancreas. Studies were initiated using BNL's PETT III positron tomograph to compare the L- and D-isomers of ^{11}C-labeled tryptophan and valine in pancreatic cancer patients. Future plans call for the utilization of a multiwire proportional counter to increase the resolution of both tumor and pancreas. Our laboratory recently became the first to administer D-[^{11}C]valine and D-[^{11}C]tryptophan to patients with pancreatic carcinoma. These preliminary studies with the D-enantiomers showed lower uptake in the liver than observed previously with the racemic mixtures.

The unnatural ^{11}C-labeled alicyclic amino acid, [^{11}C]1-aminocyclobutane-carboxylic acid ([^{11}C]ACBC), is nonmetabolizable and has excellent tumor-localizing properties. [^{11}C]ACBC, therefore, has potential as a unique tool for measuring amino acid transport into tumors, since it may reflect the tumor's

1028657



metabolic and proliferative state. In collaboration with BNL, we are evaluating the potential of [^{11}C]ACBC to monitor tumor therapy by positron tomographic quantitation of the tumor uptake before, during, and after chemotherapy or radiotherapy.

We have shown with a ^{14}C-labeled compound that the unnatural, fluorine-containing amino acid DL-α-monofluoromethylalanine is taken up by tumor tissue with a specificity similar to that of ACBC. Labeling DL-α-monofluoromethylalanine with fluorine-18 would provide a tumor-imaging agent labeled with a positron emitter that has a longer half-life than carbon-11 (110 minutes vs 20.4 minutes) and which can be produced using either an accelerator or a reactor. We are currently utilizing the facilities of BNL in an effort to develop a synthetic route to this ^{18}F-labeled amino acid.

Another research effort is directed toward the development of radiopharmaceuticals for positron tomographic studies of pulmonary function. One of the major metabolic activities of the lungs involves regulation of various biogenic amines. ^{11}C-Labeled straight-chain primary aliphatic amines are sequestered very selectively by lung endothelial cells. However, the rapid metabolic loss of the label makes it difficult to accurately quantitate the pulmonary uptake. Our

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work in this area is currently focused on development of an ^{18}F-labeled large ω-substituted primary aliphatic amine. Based on our previous work, the metabolic breakdown of the larger amines in this series is much slower than that of the smaller ones. In addition, the presence of a radiofluorine substituent in the ω-position should result in a much-delayed loss of radiolabel compared to our previous work with amines labeled in the α-position with carbon-11. Therefore, we are attempting to develop a synthesis for ^{18}F-labeled 12-fluorododecylamine, again using the facilities of BNL.

We are in the process of identifying agents labeled with other positron-emitting radionuclides which will be useful in clinical research. An IND exemption for ^{64}Cu citrate as an agent for positron tomographic detection and staging of soft tissue tumors was recently granted by the U.S. Food and Drug Administration. We are also evaluating in animal models the potential of ^{64}Cu-labeled agents for the diagnosis and study of other disease processes. Our results with ^{64}Cu citrate as a myocardial imaging agent in normal and isoproterenol-infarcted canine hearts have been encouraging.

Support has recently been received from the National Cancer Institute for a study of radiolabeled monoclonal antibodies for cancer therapy. Antibodies to tumor-associated antigens have long been considered to have great potential for specifically directing radionuclides to tumors for both diagnostic and therapeutic applications. However, only since the discovery and refinement of the monoclonal antibody technique (production of antibodies in tissue culture by lymphocyte-myeloma hybridomas) have homogeneous, high-purity antibodies been available in sufficient quantity for this possibility to be tested. Monoclonal antibodies labeled with radioiodine have been investigated with some success for cancer diagnosis and therapy; however, in vivo deiodination is a consider-

1028659

able drawback to use of radiolabeled agents of this type. In collaboration with Dr. Zenon Steplewski of the Wistar Institute (Philadelphia), we are studying the potential of radiolabeled chelate-conjugated monoclonal antibodies for use in diagnosis and therapy of cancer. The chelating agent, diethylenetriaminepenta-acetic acid (DTPA), is covalently attached to monoclonal antibodies, permitting radiolabeling with a variety of metal ion radionuclides. We have demonstrated good tumor uptake of an anticolorectal carcinoma monoclonal antibody labeled with gamma-emitting indium-111 in nude mice bearing human colon tumors. This work has recently been extended to studies of the potential of radioimmunotherapy using various tumor-specific monoclonal antibodies labeled with the alpha-emitters, fermium-255 and einsteinium-253, and the beta-emitter, yttrium-90. We plan to further extend this work to studies using monoclonal antibodies labeled with the positron-emitting radionuclides, scandium-44 (recently made available via a titanium-44/scandium-44 generator developed at Los Alamos National Laboratory), copper-64, and gallium-68, for positron tomographic radioimmunodiagnosis of cancer.

The research interests of this program were recently broadened to include the cardiovascular system and hypertension. Investigation of differences in the cotransport of sodium and potassium by erythrocytes from normotensive and hypertensive subjects is being actively pursued. In addition, we have established a small colony of New England Deaconess Hospital rats (courtesy of the National Hypertension Association). These rats develop spontaneous pheochromocytomas and will also accept pheochromocytoma tumor implants. Therefore, they are potentially useful as animal models for testing new tumor-localizing agents. Because the clinical literature is equivocal regarding a gender dominance for the occurrence of pheochromocytomas, a series of studies addressing

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this question were conducted by a student research participant. Groups of castrated male and testosterone-treated female New England Deaconess Hospital rats had tumors implanted and arterial pressures monitored. A statistically significant earlier time of appearance of the tumors in the males was demonstrated. In the areas of clinical pharmacology/clinical hypertension, we have initiated a collaborative trial with the University of Tennessee Memorial Research Center and Hospital to assess the efficacy of a new calcium entry blocker for the treatment of clinical hypertension.

Chelation therapy with DTPA drugs is the method of choice for decorporation of internally deposited actinide contaminants. As part of the division's project to coordinate national use of DTPA (see REAC/TS section), the Nuclear Medicine and Biochemistry programs collaborate in research on new, highly lipophilic chelating agents. We have shown that Ca-DTPA encapsulated in liposomes is more effective than the free form of the drug in removing from rats colloidal ytterbium-169, a model contaminant. However, liposomes of the commonly used multilamellar vesicle (MLV) form cause spleen weight increases in

1028661

rats, which has hindered further application of these agents. We have recently shown that Ca-DTPA encapsulated in a new type of liposome, called large unilamellar vesicles (LUV), gives the same removal of ytterbium-169 as MLV but without the splenomegaly problem observed with MLV. Further work in this area will be an investigation of the optimal dosing schedule for contaminant removal.

Cytogenetics

During the last several years, one research objective of the cytogenetics program has been to develop and apply cytogenetic methods for dose assessment in persons having recent or previous radiation exposures. Since 1976, cytogenetic dose estimates have been provided for over 160 persons referred to REAC/TS because of suspected overexposures, and in many instances these evaluations proved to be the only means for knowing whether someone was irradiated. In FY 1985 14 dosimetry and follow-up evaluations were performed for REAC/TS.

The utility of the lymphocyte cytogenetic system as a biological indicator of exposure is well established in situations where persons have recently received acute whole body exposures to about 20 rad or more of x or gamma radiation. However, significant scientific questions remain regarding the applicability of cytogenetic systems for dose estimation in persons having chronic or fractionated exposures and in individuals exposed many years previously; basic research studies aimed at determining the utility of cytogenetic techniques for dosimetry many years after exposure are under way.

Considerable progress was made in our ongoing evaluations in persons who received partial body radiation exposures 20–40 years ago. In collaboration

1028662

with scientists at the Radiation Epidemiology Branch of NCI, and under the support of an NCI-DOE Interagency Agreement, we have now completed detailed cytogenetic studies in approximately 500 control and irradiated persons selected from various cohorts under study by NCI. The study populations include control subjects and persons who received radiation treatment for enlarged tonsils; women treated surgically or those who received fractionated and/or protracted radiotherapy for carcinoma of the cervix; and a population of women who underwent multiple fluoroscopic examinations during the course of treatment for diseases of the lung. Relative to findings in non-irradiated controls, a small but statistically significant increase in the frequency of lymphocytes bearing radiation-induced chromosome aberrations was observed in the population of persons who received 400–1200 rad localized radiation to the tonsil. In lymphocyte cultures from 98 cervical cancer patients who received average marrow doses of 750 rad, the numbers of metaphases with symmetrical and asymmetrical chromosome-type aberrations ranged from 0 to 12%. In spite of considerable variability in findings among women who received similar doses, preliminary statistical evaluations demonstrated that the mean frequencies of metaphases bearing radiation-induced aberrations varies as a linear function of total active bone marrow dose in women having radiotherapy for cervical cancer 20 to 40 years ago. During this year we completed cytogenetic evaluations of approximately 30,000 lymphocyte metaphases from 146 fluoroscopy patients, and we are currently assessing persistent aberrations in 200 persons who received radiotheraphy for enlarged thymus in the 1940s.

The cytogenetics group also continued basic research studies to more precisely define physical and biochemical factors affecting the induction, repair, and persistence of chromosomal aberrations and sister chromatid exchanges in

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human lymphocytes. A series of experiments was conducted to ascertain and quantify the effects of cryopreservation on baseline and radiation-induced chromosome aberrations. Human lymphocytes were exposed in vitro to 100, 200, or 400 rad of cobalt-60 gamma radiation and were cultured immediately after exposure or after one week's storage at -70°C. A slight depression in cellular proliferation and a significant increase in chromatid breakages were observed in cultures initiated from frozen lymphocytes. However, in preparations from both frozen and nonfrozen lymphocytes, the dose response relationships for radiation-induced dicentrics gave a best fit to the linear quadratic model ($Y = \alpha D + \beta D^2$, where Y is yield of dicentrics and D is dose) with no significant differences between either the alpha or beta terms for the two sets of cultures. This finding provides evidence that lymphocytes bearing radiation-induced chromosomal aberrations are not at selective risk for cell death as a result of cryopreservation.

Studies of SCE induction in human lymphocytes exposed to chemicals having different modes of action on DNA also continued. In collaboration with scientists at Tuskegee University, in vitro studies were initiated to determine the cytogenetic consequences of exposure of human lymphocytes to therapeutic imidates. These compounds are protein cross-linking agents that are highly specific for lysine residues. Studies with methyl acetimidate demonstrated that the



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reagent was highly cytotoxic at concentrations of 4 mM, while lower concentrations induced suppression of cell growth as measured by depressions in the mitotic index and lengthening of the cell cycle. However, the compound caused only minimal increases in SCEs at concentrations that caused pronounced alterations in cell cycle kinetics. In contrast to findings of highly significant increases in sister chromatid exchanges after exposure of human lymphocytes to DNA cross-linking agents such as mitomycin C, it appears that chemical agents that act specifically by cross-linking lysine residues in proteins are not efficient inducers of sister chromatid exchanges.

In addition to directly quantifying the cytogenetic consequences of radiation and chemical exposure in humans, we are attempting to better understand the basic sequelae of damage at the cellular and chromosomal level through the use of experimental animals. The utilization of various animal models permits data collection under specialized conditions to answer specific questions regarding cytogenetic and reproductive toxicity resulting from exposure to radiation and chemicals. The extrapolation of data from such in vivo and in vitro studies provides a basis for predicting the effects to be seen in cases of accidental exposure of humans to these agents.

Studies conducted under an Interagency Agreement with the National Toxicology Program are examining the effects of in vivo exposure to certain chemicals on cell proliferation rates and the induction of chromosomal changes in mouse bone marrow cells. In this cooperative study, conducted in conjunction with Brookhaven National Laboratory, numerous factors with a potential for influencing the outcome of cytogenetic evaluations under in vivo conditions have been identified and quantified in the animal system. This has allowed us to determine the importance of these variables and to develop a standardized sys-

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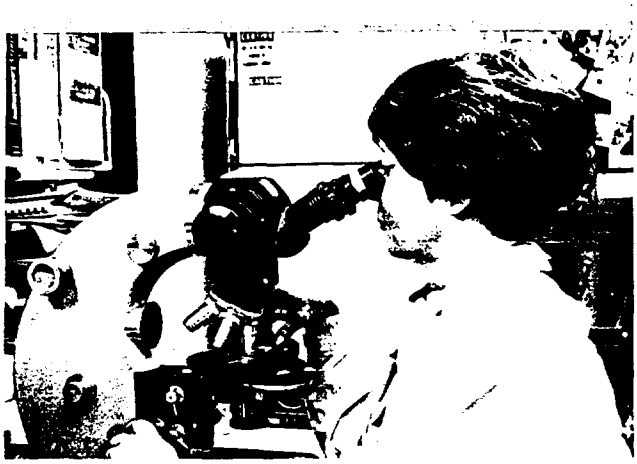
tem for the assay of chemical effects. The effectiveness of this approach has been evaluated in a study comparing the results obtained when our two laboratories independently tested the same four chemicals. The qualitative similarity of the results obtained demonstrated the ability to minimize interlaboratory variation, a factor which is a major contributor to the uncertainty surrounding many quantitative tests. Since our studies require a simultaneous evaluation of three different endpoints—chromosome aberrations, sister chromatid exchanges, and cell kinetics—they contribute to a better understanding of the mechanisms involved in the production of these effects. Portions of this work have also been part of a collaborative study by the International Program on Chemical Safety to evaluate the efficiency of various in vivo test systems.

Earlier studies had shown that extending the time between treatment and sacrifice resulted in higher aberration rates in bone marrow cells of mice injected with mitomycin C or dimethylbenzanthracene. A similar study was performed this year using 4-nitroquinoline. The similarity of the results obtained using these three chemicals, which have quite different actions, established the generality of the time effect on aberration production and validated the increased sensitivity of the assay obtained by sampling at later times. Since late sampling necessitates the use of bromodeoxyuridine to avoid scoring second division metaphases, we have also compared aberration levels in mice with and without bromodeoxyuridine implants and established that the chemical does not have an effect on aberration rates.

An unusual pair of related chemicals was examined in some detail for their aberration-inducing potential. Tests with 4-chloro-o-phenylenediamine had earlier identified it as a suspected clastogen. Further evaluations during the past year have shown it to be a rather unique chemical in that it causes no detectable damage in the majority of marrow cells but in up to 3 percent of the cells,

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depending on dose, causes extensive structural rearrangement of the chromosomes. The very closely related compound 4-nitro-o-phenylenediamine did not have this effect when simutaneously tested. It is uncertain at this time whether this all-or-none effect of the 4-chloro compound is due to a small population of cells with highly sensitive chromosomes or to differential penetration of the compound into selected cells.



Food Chain Transport

Synfuel studies. The Environmental Protection Agency (EPA) funded studies to evaluate the impact of effluents from coal liquefaction and shale oil production on the human food chain. Many of these effluents are known toxic substances, and many are closely related chemically to recognized carcinogens and mutagens. To determine the significance of the food chain as a vehicle for toxic agents, it is imperative to characterize the materials entering the system with appropriate food-producing animals. Accurate and precise data on the behavior and metabolism of these chemicals are needed for regulatory agencies

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to assess the possible health risks to humans. It is not reasonable or accurate to predict these risks by extrapolation from small laboratory animals and from experiments that are often unrealistic and peripheral to circumstances encountered in potential exposure of the public.

The environmental contamination level of a chemical may be only a partial indicator of potential levels in animals; the capability of biomagnification may allow animals to concentrate relatively small quantities of the chemical(s) and accumulate them in specific tissues of the body at levels greater than those encountered in the environment. The ingestion of contaminated feed and water is the primary route of animal exposure, and the consumption of contaminated milk, eggs, and meat is the primary route for humans. For example, American consumers eat well in excess of 200 pounds of meat and poultry per capita per year, making this a formidable potential route of human exposure.

The food chain transport program supported the risk analysis group by developing data for use in their food chain analysis of synfuels-related chemicals, a program that was terminated in FY 1985. A total of six chemicals were studied to determine the uptake and biological retention in dairy cattle, swine, and poultry and to yield the transfer coefficients required for estimates of exposure via the food chain.

Specific goals of the food chain transport program were to (1) determine the biological retention of representative chemicals following acute and chronic oral administration to food-producing animals, (2) determine the accumulation and loss of these chemicals in consumable products following an acute dose, (3) determine their retention in tissues when they are administered chronically, and (4) develop and employ practical methodology for the isolation and quantitation of selected organic chemicals that are representative of the major chemical

1028668

classes found in coal liquefaction and shale oil products and production wastes.

Radiotracer methodology was utilized to determine the accumulation of organic chemicals in consumable products such as milk, eggs, and meat. Tissues were also disrupted and fractionated to determine the amounts of radiolabeled compounds in soluble versus membrane-bound fractions, to determine the portion of parent compounds remaining in tissues, and to estimate the amount and nature of metabolites formed from the parent compound. The six chemicals studied were naphthalene, alpha naphthol, 7-methylbenz(c)acridine, indole, aniline, and benzo(a)pyrene.

Results of these experiments demonstrated that all of the six chemicals studied were retained in consumable meats, eggs, and milk. Retention levels and tissue distribution were influenced by species and route of exposure (acute versus chronic). Since almost the entire animal is utilized and many animal products are being recycled into alternative food products (dried eggs in baking products, for example) which have a long consumer shelf life, the possibility of prolonged exposure of humans to low levels of chemicals must be recognized.



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Actinide studies. The Nevada Applied Ecology Group of the Nevada Operations Office of the Department of Energy supports studies on the metabolism of radionuclides of specific interest to the Nevada Test Site. This research is designed to evaluate the role of the food chain in exposure of humans to radionuclides from the testing of nuclear weapons and radioactive fallout.

To determine potential health hazards to man from ingestion of contaminated foods requires comprehensive experiments on absorption, tissue localization, retention, and excretion times. Most human exposures involve slow chronic delivery and accumulation of the radionuclides with few outward manifestations. The principal objective of the ORAU actinide program is to develop predictive estimates on the impact of these and other radionuclides on the human food chain and the risks to humans.

To accomplish these objectives, ORAU studies have focused on gastrointestinal absorption, tissue distribution and redistribution, retention, and subsequent excretion. Swine are used in these studies because they are a major animal species used as food by humans, and they are similar to humans both physiologically and anatomically. Thus, this animal model not only allows evaluation of the food chain but also yields valuable data on the tissue distribution and retention of radionuclides that are applicable to human evaluations.

Data on the distribution of orally administered ^{241}Am citrate in swine (miniature and domestic) showed the majority of the radioactivity to be incorporated into bone (50 percent), liver (20 percent), and other soft tissues (30 percent). Cytogenetic evaluations showed no significant increases in chromosomal anomalies. Gametogenetic evaluations revealed alpha tracks in the medullary portion of the ovary and randomly in the testes.

A one-year chronic exposure of swine to orally administered europium-152,

1028670

-153, -154 and cesium-137 has just been completed. Although the data analyses are not yet completed, preliminary results indicate that the site of cesium deposition is the soft tissues, with the majority of radioactivity retained in muscle. Over 99 percent of the europium was excreted; little was detectable in any soft tissue, and only low levels were observed in bone. Therefore, it appears that europium absorption is insignificant under our experimental conditions. In contrast, the ingestion of radioactive cesium could render such animals unfit for human consumption.

Marmoset Research

In June 1985, the Medical and Health Sciences Division was awarded a National Cancer Institute contract for three years to continue breeding and raising marmosets for cancer research. The contract's objectives are (1) to maintain a breeding colony of *Saguinus oedipus oedipus* and to produce a maximum number of healthy animals at minimum cost, (2) to provide facilities for experimental studies of oncogenic substances in tamarins, and (3) to provide professional and technical support for others to safely and accurately perform experimental protocols approved and requested by the National Cancer Institute. During the previous contract period, the reproductive efficiency of the cotton-top tamarin colony continued to improve as primarily colony-born animals were used as breeding pairs. In addition, the National Foundation for Ileitis and Colitis awarded marmoset-colony support ($25,000/yr for three years) beginning in September 1985.

1028671



Research concerned with the behavioral, endocrine, and nutritional determinants of reproductive competence in the cotton-top tamarin is conducted through support from the Division of Research Resources of National Institutes of Health. The objective is to define the importance of the social aspects of rearing infant siblings and the subsequent maternal behavior of females. Results from this study have revealed that certain demographic factors (e.g., age of mothers) are related to the maternal care of infants. Reproductive aging in female tamarins is being examined to determine whether these species display endocrine, ovarian, or fertility changes similar to menopausal women. Preliminary results indicate that a significant decline in ovarian follicular development occurs with age and reduced fertility in aging cotton-top tamarin females. Collaborative studies with the University of Wisconsin assess through radioimmune assays the hormonal status of females ranging from prepubertal stages through those in reproductive decline. Two of the aged females examined had low ovarian steroid levels and elevated amounts of luteinizing hormone similar to what has been reported in menopausal women.

Proceedings of the Marmoset Workshop that was cohosted by ORAU, NCI, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Divi-

1028672

sion of Research Resources, and the National Foundation for Ileitis and Colitis in April 1984 was in the December-1985 issue of *Digestive Diseases and Sciences* (Supplement). The approximately 100 scientists and doctors who attended this conference discussed various intestinal diseases in the marmoset and colon cancer in the cotton-top tamarin and compared these processes with those that occur in humans. An obvious goal was to evaluate the marmoset as a potential model for the investigation of gastrointestinal diseases. Although some differences were identified and numerous similarities were recognized, the consensus opinion of participants was that the diseases of this primate were of such importance that further investigation was merited and could contribute to our understanding of mechanisms responsible for human diseases.

Collaborative research projects with extramural investigators are designed to characterize the biology, biochemistry, physiology, nutritional aspects, and behavioral characteristics of the marmoset as an experimental animal and to relate these findings to disease processes. These investigations involve the following studies: T and B cell immunology, cell culture of tamarin colon cancers, sterol metabolism and fecal bile acid profiles, in vitro tetraploidy in skin fibroblasts as a marker of cancer susceptibility, genetic epidemiology and immunological capabilities in familial relationships in tamarin colon cancer, and idiopathic ulcerative colitis in colon cancer susceptibility and expression. Other projects are investigating the effect of coronaviruses, lectin binding, fecal toxins, carcinoembryonic antigens, serum enzymes, and fecal enzymes in colonic diseases of marmosets.

In collaboration with gastroenterologists at the University of Nevada School of Medicine, ORAU personnel have begun systematic visualization and biopsy of tamarin and marmoset colonic tissues to determine the pathogenesis of colitis

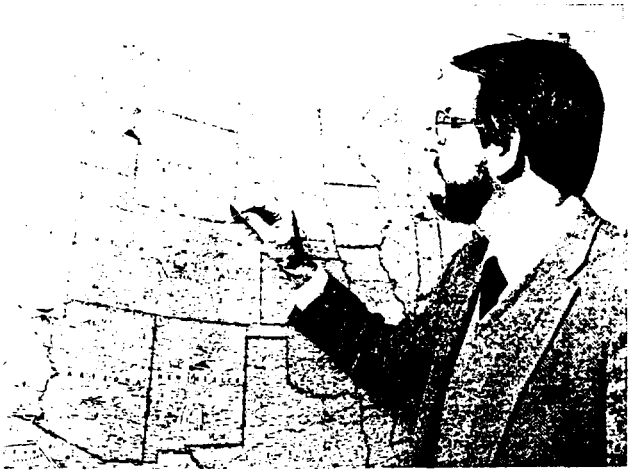
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and its relationship with the development of colon cancer in cotton-top tamarins. This information is being correlated with observations from tissues of necropsied animals to identify contributing factors in these related diseases. To date, the strongest correlation exists between the presence of acute colitis and colon carcinoma. Collaborative efforts with other scientists have identified oncodevelopmental mucins in both colitis and colon cancer in tamarins; these mucins are also found in humans with the same diseases.

An exciting relationship for collaborative research is in the developmental stages with the Harbor-UCLA School of Medicine. If NIH funds are received to establish a center for the study of inflammatory bowel disease at UCLA, the marmoset model for the investigation of the disease will be used to correlate genetic epidemiologic and immunologic parameters with disease processes that are observed clinically.



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REAC/TS

The Radiation Emergency Assistance Center/Training Site (REAC/TS) program continues to provide multiphasic support for medical management of radiation accidents. Support in the areas of 24-hr emergency medical response, teaching/training, radiation accident registries, medical follow-up of accident survivors, and forensic medical consultation are provided through this program. The REAC/TS radiation emergency response team, consisting of physicians, nurses, health physicists, laboratory technicians, an emergency coordinator, communications coordinators, and record clerks, provide in-house emergency medical assistance and consultative support. REAC/TS has recently been redesignated as the Western Hemisphere collaboration center for four additional years by the World Health Organization (WHO). Groups affiliated with REAC/TS also conduct research in cytogenetic dosimetry and the use of diethylenetriaminepentaacetic acid (DTPA) as a chelator for removal of internally-deposited toxic and/or radioactive metals from tissue.

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Program accomplishments within the REAC/TS mission are reflected by 45 calls for assistance in FY 1985, including 6 accidents, 38 incidents (nonaccidents), and 1 assistance request by a foreign country. In this regard, REAC/TS provided both in-house and onsite accident response. REAC/TS continued to provide advice and consultation as follow-up to the Ciudad Juárez, Mexico, cobalt-60 accident under our WHO collaboration center designation. Working with representatives of the Mexican Ministry of Health and the Pan American Health Organization (a component of WHO), REAC/TS staff physicians and a cytogeneticist have continued the follow-up of Mexicans receiving the highest radiation doses in the Ciudad Juárez accident; additional cytogenetic dosimetric studies are planned for the future. REAC/TS also conducted cytogenetic evaluations on other persons suspected of accidental exposure to ionizing radiation.

Clinical use of DTPA continued to be coordinated by REAC/TS with re-evaluation (by whole body counting) of individuals undergoing DTPA chelation therapy for known inhalation of americium-241. Results of such whole body counting evaluations are used to determine the efficacy of therapy and to determine a need for continued use of DTPA in persons internally contaminated.

During the past year, REAC/TS continued to offer courses of instruction in medical management of radiation accidents to medical, paramedical, and health physics personnel. Participants included 73 physicians, 52 nurses, 36 health physicists, and 14 others for a total of 175 individuals. Since 1976 a total of 76 courses have been offered at REAC/TS with 1,477 health professionals trained. REAC/TS course graduates comprise a broad base of occupational backgrounds and locales within the United States and 11 foreign countries. REAC/TS staff also participated in other formal training endeavors sponsored

1028676

by government, private industry, medical/health physics societies, hospitals, and other various professional meetings.

REAC/TS continues to develop its close association with the Federal Emergency Management Agency (FEMA). In FY 1986, FEMA will provide funding support for conducting REAC/TS training courses in radiation accident management. REAC/TS staff continue to assist FEMA programs conducted at FEMA's Emergency Management Institute (FEMA/EMI), Emmitsburg, Maryland, through teaching and development of course materials. REAC/TS also assists FEMA/EMI in evaluation and updating of their existing courses in radiological training.

Through the REAC/TS Registry system, long-term follow-up of persons involved in earlier radiation accidents continues. The REAC/TS Registry at present contains information on 719 events (accidents or incidents) involving over 6,200 persons. The registry has identified 258 serious radiation accidents worldwide involving 1,206 persons and 31 acute fatalities. Through the DOE ≥5 Rem Study Registry, a subcomponent of the central REAC/TS Registry, follow-up continues for the purpose of establishing trends in morbidity and mortality. The DTPA user registry has expanded to include 52 facilities with 45 physicians reporting annually on 497 persons who have received at least one dose of DTPA chelate (calcium or zinc) for actual or suspected internal contamination. Information on foreign radiation accidents continues to be added to the registry system through contact with physicians and health physicists in various foreign countries and through close liaison with our sister WHO collaboration center in Paris.

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Epidemiology

The primary objective of this multidisciplinary program is to examine the effects on morbidity and mortality of chronic exposure to low levels of ionizing radiations from external and/or internal sources alone or in the presence of chemical toxicants, particularly uranium and other toxic metals that potentially may confound or modify the effects of exposure to radiation among workers in the nuclear industry. A related objective is to evaluate the health and mortality experience of workers to identify untoward effects, if any, of their employment at some time since 1942 at selected facilities of the U.S. Department of Energy (DOE) and its predecessor agencies (Manhattan Engineer District [MED], Atomic Energy Commission [AEC], Energy Research and Development Administration [ERDA]) and their contractors.

Additional programmatic objectives are (1) to develop and apply improved statistical methods, including probabilistic models, for the analysis of epidemiologic data, (2) to critically evaluate the methods and results of studies of health effects among other populations exposed to ionizing radiations, occupationally or otherwise, and to other industrial toxicants, (3) to advance methodologies in the areas of data collection and records management and of occupa-



1028678

tional hazards assessment, and (4) to develop and apply improved computer systems to ensure the high quality and validity of the data bases and to facilitate the processing, manipulation, and analysis of large volumes of data in support of ongoing programmatic activities.

We are pursuing these objectives as part of the overall Health and Mortality Study of DOE Workers that has an estimated total population of 600,000 present and former workers. The part of the Health and Mortality Study assigned to ORAU includes all workers (ca 200,000)—males, females, whites, nonwhites—at DOE's research and development and production facilities operated by Martin Marietta Energy Systems, Inc., in Oak Ridge, Tennessee, and Paducah, Kentucky; at the Gaseous Diffusion Plant in Portsmouth, Ohio, operated by Goodyear Atomic Corporation; at the Fernald Feed Materials Plant (FFMP) in Fernald, Ohio, now contracted to Westinghouse Corporation; and at the Savannah River Plant (SRP) in Aiken, South Carolina, operated by E. I. du Pont de Nemours and Company. Our responsibilities also include epidemiologic studies of workers (ca 3000) at former MED uranium processing and refining facilities in upstate New York and St. Louis, Missouri, and of workers (ca 3000) reported to have received externally $\geq$5 rems in a year while employed at any DOE or contractor facility since 1947. For practical reasons, our studies currently focus primarily on the white male workers among these facilities' populations. However, follow-up of vital status is maintained for all race and sex groups in anticipation of studying these groups in the future.

In the past year, we have been working to complete (1) final analysis of mortality through 1979 among white males employed at the Oak Ridge Y-12 plant since 1947, (2) an updated analysis of lung cancer deaths among white males employed at the Y-12 plant between 1943 and 1947, (3) continuing analysis of

1028679

nonmalignant respiratory disease through 1977 among white male workers at FFMP, (4) analysis of primary brain cancer deaths through 1979 among workers employed at the Oak Ridge Y-12 plant and Oak Ridge National Laboratory since 1943, and (5) initial analysis of mortality through 1980 among SRP employees. Previously developed methods for the estimation of radiation doses from internally deposited uranium and other potential hazards, and for ranking individual workers according to their potential for exposure to uranium (based on job titles and department) are being applied in some of these analyses. In an ongoing study that encompasses all workers at DOE's facilities in Oak Ridge, age-specific mortality rates among workers employed only during World War II are being evaluated using the Mortality Analysis System (MAS) being developed by the biostatistics and computer staffs to expedite the evaluation using external or internal comparison populations of the effects on mortality rates of different levels of variables of interest (in the current analysis these include socioeconomic status, birth year, duration of employment, length of follow-up, and potential for occupational exposure to radiation). Reports of the results of these several analyses are anticipated in 1986. An interim report presented results of an analysis of cancer mortality among former workers at the MED site at Linde Corporation. In this study, which was conducted in collaboration with Union Carbide Corporation's corporate epidemiology group, we found no association between increases in overall mortality or mortality due to specific diseases of interest and the workers' exposure to radiation or uranium dust.

A computer based follow-up and telephone interview program was developed and has been initiated to obtain data on morbidity and other relevant medical, occupational, and social factors from workers reported to have

1028680



received ≥5 rems in a year while employed at DOE or contractor facilities and matched comparison subjects. The purpose of this survey, which is being conducted on a facility basis, beginning with those in DOE's Oak Ridge Operations area, is to determine if there are certain illness excesses among this population and, if so, if they are related to occupational exposure to radiation; also to be determined is whether morbidity provides a more sensitive measure of the human health effects of low level radiation than mortality.

In addition to continuous updating of mortality among all worker populations in the ORAU portion of the Health and Mortality Study, work in progress also includes data preparation for initial or updated analyses of mortality among (1) workers of the gaseous diffusion plants in Oak Ridge, Paducah, and Portsmouth, (2) former MED site workers at the Mallinckrodt Chemical Works, St. Louis, Missouri, (3) FFMP workers, and (4) welders at all Oak Ridge plants. A study of mortality among chemical and reactor operators at all Oak Ridge plants has been initiated in response to workers' concerns.

Previously analyzed and reported data on the mortality experience of Oak Ridge Gaseous Diffusion Plant workers monitored for exposure to metallic nickel were contributed to an epidemiologic study sponsored by the U.S.

1028681

Environmental Protection Agency and other national and international institutions in Canada and Europe. The objective of this study is to evaluate existing epidemiologic data to determine whether exposure to specific forms of nickel increases the risk for cancer development. This investigation is being directed by an international steering committee on which ORAU is represented.

Expert opinion on the probability of causation was given to DOE and the Scientific Sub-Panel on Radioepidemiologic Tables of the Committee on Interagency Radiation Research and Policy Coordination. A major accomplishment of this work has been the development by CER staff of computer software (PCAUS) that uses the Radioepidemiological Tables of the National Institutes of Health Ad Hoc Working Group as a basis for calculating the probability that an existing cancer of an individual previously exposed to radiation was radiation-induced. The software has been copyrighted by ORAU for use by individuals and institutions needing to evaluate claims of allegedly radiation-induced cancers.

CER staff were members of (1) a Centers for Disease Control Epidemiologic Studies Review Panel at Savannah River Plant, (2) the Committee on the Biological Effects of Internally Deposited Alpha-Emitting Radionuclides, and (3) Committees of the National Council on Radiation Protection and Measurements on Influence of Radiation Quality on Dose Response Relationships and Radiation Exposure and Potentially Related Injury.

Problems associated with determining and evaluating the uncertainties associated with radiation monitoring data and with the categorization by overall potential for exposure to occupational hazards, and the use of such data in epidemiologic analyses, are being addressed using data prepared for studies of workers at the Oak Ridge Y-12 plant and at FFMP for which air and personnel monitoring data are available.

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We continue to operate the Death Certificate Retrieval Office (DCRO) in support of the ORAU and other DOE epidemiology groups, primarily those at Los Alamos and Hanford who are conducting studies of other DOE worker populations as part of the Health and Mortality Study. Data on vital status and deaths among DOE employees are retrieved primarily through the Social Security Administration and the vital statistics departments of the states. Other sources of these data include the National Death Index, state death indexes, state motor vehicle records, the Civil Service Commission, the Veterans' Administration, and other large retirement systems. The DCRO staff has a lead responsibility in the development and implementation of the Tennessee State Cancer Registry, which will be a valuable source of cancer incidence data for prospective studies of workers at the Oak Ridge plants.

The long-term goal of the epidemiology program is to merge all employee rosters and associated exposure and other relevant data where it is epidemiologically feasible. This data base could provide (1) a sufficient cumulative population radiation dose and total years of follow-up necessary for a meaningful assessment of risks associated with exposure to low levels of ionizing radiation, (2) a basis for the continued surveillance of DOE workers' health experience, and (3) a resource contributing to the resolution of medico-legal arguments and the establishment of regulatory standards for health protection. The data base also provides a retrospective basis for a registry of radiation workers in the United States.

In these studies we have the collaboration of the Epidemiology Departments of the Schools of Public Health of the University of North Carolina, Chapel Hill, the University of Alabama at Birmingham, and the Department of Biostatistics and Epidemiology of the University of Cincinnati.

1028683



Committee on Interagency Radiation Research and Policy Coordination (CIRRPC)

The White House Office of Science and Technology Policy (OSTP) created the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) on May 25, 1984, to replace the Interagency Radiation Research Committee, the Radiation Policy Council, and the Committee on Interagency Radiation Policy of the Federal Coordinating Council for Science, Engineering, and Technology, all of whose charters had expired. CIRRPC coordinates radiation matters between agencies, evaluates radiation research, and provides advice to OSTP on the formulation of radiation policy. Eighteen federal agencies have designated representatives to CIRRPC's two panels, a policy panel and a science panel.

Since August 1, 1984, ORAU has provided the necessary personnel and facilities to serve as a secretariat to handle technical, administrative, and logistical functions for CIRRPC. ORAU/CIRRPC staff assist the science panel by providing research and analysis activities, scoping radiation-related issues, manag-

1028684

ing research contracted to other organizations, convening meetings and seminars, and publishing reports. ORAU/CIRRPC staff manage all the logistics for the meetings of the executive committee and both panels, including announcements, agendas, and minutes. It also keeps all the official records for CIRRPC and maintains a state of the art automated communication network among the members; CIRRPC has created a talent pool of over 400 experts in all fields pertinent to radiation to provide technical assistance to the science panel. ORAU contracts with the individual members of the talent pool and coordinates their involvement.

ORAU/CIRRPC staff consists of a program director, a senior technical manager, two technical specialists, a business officer, a meeting coordinator, a programmer, and three secretaries.

ORAU/CIRRPC staff isolated and identified priority issues after holding meetings of the executive committee with all member agencies and soliciting responses from Congress and private organizations involved in radiation issues.

To date, two important issues have been addressed (Veteran's Administration assessment of veterans exposed to ionizing radiation and the radioepidemiological tables) and efforts begun to address other complex issues. Currently, five science and two policy issues are being studied by subpanels created on health effects, the radioepidemiological tables, and the scientific basis for radiation protection standards. The policy subpanels include metrication of radiation units and policy impact of the radioepidemiological tables. Expert consultants to these subpanels are drawn from the CIRRPC talent pool. In addition, through a contract between ORAU and the National Committee on Radiation Protection and Measurements, CIRRPC is funding an assessment of the radiation exposure to the U.S. population.

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Four major publications were produced by CIRRPC in its first year of operation. The CIRRPC Science Panel completed three reports on issues studied at the request of member agencies: *CIRRPC Scientific Review of the Proposal: VA Assessment of Veterans with Military Service at Sites of Temporarily Augmented Ionizing Radiation, Science Panel Report No. 1*, September 7, 1984; *CIRRPC Scientific Report on the Draft Report of the Ad Hoc Working Group to Develop Radioepidemiological Tables, Science Panel Report No. 2*, November 6, 1984; and *CIRRPC Scientific Report on the Report of the Ad Hoc Working Group to Develop Radioepidemiological Tables, Science Panel Report No. 3*, June 1, 1985. In addition, the CIRRPC staff produced the *First Annual Report* on June 30, 1985, and has distributed 1300 copies to CIRRPC members, members of Congress, and interested parties in federal and state governments, industry-related organizations, and international organizations. CIRRPC has been able to draw upon the radiation expertise at ORAU, particularly in the planning of informational seminars, such as one on dosimetry, and in scoping issues such as non-ionizing radiation.



1028b88

publications

Full-length Journal Articles

Albert, D. H., and Snyder, F.
Release of arachidonic acid from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, a precursor of platelet activating factor, in rat alveolar macrophages
Biochim. Biophys. Acta 796, 92–101, 1984

Blank, M. L., Cress, E. A., Robinson, M., and Snyder, F.
Metabolism of unique diarachidonoyl and linoleoylarachidonoyl species of ethanolamine and choline phosphoglycerides in rat testes
Biochim. Biophys. Acta 833, 366–371, 1985

Blank, M. L., Lee, T-c., Cress, E. A., Malone, B., Fitzgerald, V., and Snyder, F.
Conversion of 1-alkyl-2-acetyl-*sn*-glycerols to platelet activating factor and related phospholipids by rabbit platelets
Biochem. Biophys. Res. Commun. 124, 156–163, 1984

Brown, A. J., Glish, G. L., McBay, E. H., and Snyder, F.
Alkyl-DHAP synthase mechanism: ^{18}O studies of the fatty acid release from acyl-DHAP
Biochemistry (in press)

Cabot, M. C.
Hydrolysis of novel diacylglycerol analogs and phorbol diesters by serum lipase
Biochim. Biophys. Acta 833, 330–335, 1985

Cabot, M. C.
Tumor promoting phorbol diesters: substrates for diacylglycerol lipase
Biochem. Biophys. Res. Commun. 123, 170–177, 1984

Cabot, M. C., and Jaken, S.
Structural and chemical specificity for diradylglycerols for protein kinase C activation
Biochem. Biophys. Res. Commun. 125, 163–169, 1984

Checkoway, H., Matthew, R. M., Shy, C. M., Watson, J. E., Jr., Tankersley, W. G., Wolf, S. H., Smith, J. C., and Fry, S. A.
Radiation, work experience, and cause specific mortality among workers at an energy research laboratory
Br. J. Ind. Med. 42, 525–533, 1985

Clapp, N. K., Lushbaugh, C. C., Humason, G. L., Gangaware, B. L., and Henke, M. A.
Natural history and pathology of colon cancer in *Saguinus oedipus oedipus*
Dig. Dis. Sci. (in press)

Clapp, N. K., and Tardif, S. D.
Marmoset husbandry and nutrition
Dig. Dis. Sci. (in press)

Cragle, D. L., Hollis, D. R., Qualters, J. R., Tankersley, W. G., and Fry, S. A.
A mortality study of men exposed to elemental mercury
J. Occup. Med. 26, 817–821, 1984

Crawford-Brown, D., and Wilson, J.
Observations on very long-term removal of uranium compounds
Health Phys. 47, 443–446, 1984

Daugherty, J. P., and Clapp, N. K.
Association of nitrosamine-derived radioactivity with nuclear and mitochondrial DNA in mice
GANN: Jpn. J. Cancer Res. 76, 197–201, 1985

DuFrain, R. J.
Is cancer of the colon familial in cotton-top tamarins?
Cancer Genet. Cytogenet. 14, 83–87, 1985

Eisele, G. R.
Naphthalene distribution in tissues of laying pullets, swine, and dairy cattle
Bull. Environ. Contam. Toxicol. 34, 549–556, 1985

Eisele, G. R.
A perspective on gossypol ingestion in swine
Vet. Human Toxicol. (in press)

Eisele, G. R.
Metabolism and effects of organic compounds in animals
Vet. Human Toxicol. (in press)

Eisele, G. R., and Ericksen, B. H.
Gonadal retention and localization of ^{241}Am in orally exposed swine
Health Phys. (in press)

Eisele, G. R., Tsai, S. C., Payne, J., Kyle, B., and Urso, I.
The distribution of orally administered ^{14}C-aniline in tissues of dairy cattle, swine and laying pullets
J. Anim. Sci. (in press)

Feuerstein, G., Lux, W. E., Jr., Ezra, D., Hayes, E. C., Snyder, F., and Faden, A. I.
Thyrotropin-releasing hormone blocks the hypotensive effects of platelet-activating factor in the unanesthetized guinea pig
J. Cardiovasc. Pharmacol. 7, 335–340, 1985

Feuerstein, G., Lux, W. E., Jr., Snyder, F., Ezra, D., and Faden, A. I.
Hypotension produced by platelet-activating factor is reversed by thyrotropin-releasing hormone
Circ. Shock 13, 255–260, 1984

Frome, E. L.
Response to Nelder's reaction on Poisson rate analysis
Biometrics 40, 1160–1162, 1984

Frome, E. L., and Checkoway, H.
Epidemiologic programs for computers and calculators
Use of Poisson regression models in estimating incidence rates and ratios
Am. J. Epidemiol. 121, 309–323, 1985

1028687

Hubbard, J. W., Cox, R. H., Lawler, J. E., Blank, M. L., and Snyder, F.
Cardiac output and peripheral vascular resistance during intravenous infusion of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious normotensive and spontaneously hypertensive rats
Res. Commun. Chem. Pathol. Pharmacol. (in press)

Lee, T-c.
Biosynthesis of platelet activating factor: substrate specificity of 1-acetyl-2-lyso-*sn*-glycero-3-phosphocholine:acyl-CoA acetyltransferase in rat spleen microsomes
J. Biol. Chem. 260, 10952–10955, 1985

Lenihan, D. J., Greenberg, N., and Lee, T-c.
Involvement of platelet activating factor in physiological stress in the lizard, *Anolis carolinensis*
Comp. Biochem. Physiol. 81C, 81–86, 1985

Littlefield, L. G., and Joiner, E. E.
Analysis of chromosome aberrations in lymphocytes of long-term heavy smokers
Mutat. Res. (in press)

Littlefield, L. G., Joiner, E. E., and Sayer, A. M.
Premature separation of centromeres in marrow chromosomes from an untreated patient with acute myelogenous leukemia
Cancer Genet. Cytogenet. 16, 109–116, 1985

Lushbaugh, C., Humason, G., and Clapp, N.
Histology of colon cancer in *Saguinus oedipus oedipus*
Dig. Dis. Sci. (in press)

Lushbaugh, C., Humason, G., and Clapp, N.
Histology of colitis: *Saguinus oedipus oedipus* and other marmosets
Dig. Dis. Sci. (in press)

Malone, B., Lee, T-c., and Snyder, F.
Inactivation of platelet activating factor by rabbit platelets: Lyso-platelet activating factor as a key intermediate with phosphatidylcholine as the source of arachidonic acid in its conversion to a tetraenoic acylated product
J. Biol. Chem. 260, 1531–1534, 1985

Polednak, A. P., and Hollis, D. R.
Mortality and causes of death among workers exposed to phosgene in 1943–45
Toxicol. Ind. Health 1, 137–151, 1985

Ricks, R. C.
Emergency response to hazardous materials accidents
J. Aeromed. Health Care (July-August), 14–15, 1985

Robinson, M., Blank, M. L., and Snyder, F.
Acylation of lysophospholipids by rabbit alveolar macrophages:
Specificities of CoA-dependent and CoA-independent reactions
J. Biol. Chem. 260, 7889–7895, 1985

Robinson, M., and Snyder, F.
Metabolism of platelet activating factor by rat alveolar macrophages: Lyso-PAF as an obligatory intermediate in the formation of alkylarachidonoyl glycerophosphocholine species
Biochim. Biophys. Acta (in press)

Russell, R. G., Brian, D. A., Lenhard, A., Potgieter, L. N. D., Gillespie, D., and Clapp, N. K.
Coronavirus-like particles and campylobacter in marmosets with diarrhea and colitis
Dig. Dis. Sci. (in press)

Snyder, F., Blank, M. L., Lee, T-c., Malone, B., Robinson, M., and Woodard, D.
Alkylacetylglycerols versus "acyl"-PAF as precursors in PAF biosynthesis and the role of arachidonic acid
Pharmacol. Res. Commun. (in press)

Som, P., Oster, Z. H., Yamamoto, K., Meinken, G. E., Srivastava, S. C., Yonekura, Y., Ebner, S. A., Atkins, H. L., Brill, A. B., Fawwaz, R. A., Alderson, P. O., Coffey, J., Carlton, E., and Hübner, K. F.
Some factors affecting the cerebral and extracerebral accumulation of N-isopropyl-*p*-iodo-amphetamine (IAMP)
Int. J. Nucl. Med. Biol. 12, 185–196, 1985

Surles, J. R., Wykle, R . L., O'Flaherty, J. T., Salzer, W. L., Thomas, M. J., Snyder, F., and Piantadosi, C.
Facile synthesis of platelet-activating factor and racemic analogues containing unsaturation in the *sn*-1-alkyl chain
J. Med. Chem. 28, 73–78, 1985

Tan, E. L., and Snyder, F.
Metabolism of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) by capillary endothelial cells isolated from rat epididymal adipose tissue
Thromb. Res. 38, 713–717, 1985

Tardif, S. D.
Status of the cotton-top tamarin (*Saguinus oedipus*) in captivity
Primate Conserv. (in press)

Tardif, S. D.
Histological evidence for age-related differences in ovarian function in tamarins (*Saguinus* sp.)
Biol. Reprod. (in press)

Urso, P., and Gengozian, N.
Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after *in utero* exposure to benzo[a]pyrene
J. Toxicol. Environ. Health 14, 569–584, 1984

Washburn, L. C., Blair, L. D., Byrd, B. L., and Sun T. T.
Comparison of Ga-68 EDTA, [1–^{11}C]α-aminoisobutyric acid, and [^{99m}Tc]sodium pertechnetate in an experimental blood-brain barrier lesion
Int. J. Nucl. Med. Biol. (in press)

Washburn, L. C., Sun, T. T., Byrd, B. L., and Callahan, A. P.
Resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan by high-performance liquid chromatography

1028688

J. Labelled Compd. Radiopharm. 22, 135–142, 1985

Washburn, L. C., Sun, T. T., Crook, J. E., Byrd, B. L., Carlton, J. E., and Steplewski, Z. S.
^{90}Y-Labeled monoclonal antibodies for cancer therapy
Int. J. Nucl. Med. Biol. (in press)

Zukowska-Grojec, Z., Blank, M. L., Snyder, F., and Feuerstein, G.
The adrenergic system and the cardiovascular effects of platelet activating factor (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) in SHR and WKY rats
Clin. Res. Hypertens.—Theory and Practice A7, 1015–1031, 1985
1015–1031, 1985

Chapters, Review Articles, and Other Publications

Beck, W. L., and Hodges, H. D.
Radioactive materials: Safe handling
In *Encyclopedia of Materials Science and Engineering*, Pergamon Press, New York (in press)

Burr, W. W., Jr.
Human experience and epidemiology
In *Biokinetics and Analysis of Uranium in Man, Proceedings of U. S. Uranium Registry Conference, 1984*, Hanford Environmental Health Foundation, Richland, Washington (in press)

Clapp, N. K., Henke, M. A., Holloway, E. C., and Tankersley, W. G.
Carcinoma of the colon in the cotton-top tamarin: A radiographic study
Original publication: *J. Am. Vet. Med. Assoc.* 183, 1328–1330, 1983, *Yearb. Cancer* (in press)

Clapp, N. K., Lushbaugh, C. C., Humason, G. L., Gangaware, B. L., and Henke, M. A.
The cotton-top tamarin as an animal model of colorectal cancer metastasis
In *The Biology and Treatment of Colorectal Cancer Metastasis*, (A. Mastromarino, ed.), Martinus Nijhoff Publishing, Hingham, Massachusetts (in press)

Clapp, N. K., Lushbaugh, C. C., Humason, G. L., Gangaware, B. L., and Henke, M. A.
The marmoset as a model of ulcerative colitis and colon cancer
In *Carcinoma of the Large Bowel*, (Ingalls, J. F., and Mastromarino, A., eds.), pp. 247–261, Alan R. Liss, New York, 1985
Prog. Clin. Biol. Res. 186, 247–261, 1985

Coffey, J. L., Watson, E. E., Hübner, K. F., and Stabin, M. G.
Radiopharmaceutical absorbed dose considerations
In *Clinical Aspects of Nuclear Medicine Science* (Hladik, W. B. III, Saha, G. B., and Stady, K. T., eds.), Williams and Wilkins Corp., Baltimore (in press)

Cragle, D. L., Hollis, D. R., Shy, C. M., and Newport, T. H.
A retrospective cohort mortality study among workers occupationally exposed to metallic nickel powder at the Oak Ridge Gaseous Diffusion Plant
In *Nickel in the Human Environment*, (Sunderman, F. W., Jr., Aitio, A., Berlin, A., Bishop, C., Buringh, E., Davis, W., Gounar, M., Jacquignon, P. C., Mastromatteo, E., Rigaut, J. P., Rosenfeld, C., Saracci, R., and Sors, A., eds.), IARC Scientific Publication 53, pp. 57-63, International Agency for Research on Cancer, Lyon, France, 1984

Cragle, D. L., Shy, C. M., Struba, R. J., and Siff, E. J.
A case-control study of colon cancer and water chlorination in North Carolina
In *Proceedings of the 5th Conference on Water Chlorination: Environmental Impact and Health Effects, 1984*, Williamsburg, Virginia, 5, 153–159, 1984

Crook, J. E., and Mroczkowski, P. J.
Red cell Na-K cotransport: Dose-response effects of furosemide inhibition in black and white hypertensives
In *Diuretics: Chemistry, Pharmacology and Clinical Applications* (*Proceedings of the First International Conference, 1984*, Miami Beach, Florida), (Puschett, J. B., ed.), pp. 453–455, Elsevier Science Publishing Co., New York, 1984

Crook, J. E., and Mroczkowski, P. J. [Letter to the Editor]
Red cell cotransport activity and sodium content in black men
Hypertension 7, 652, 1985

DuFrain, R. J.
Probing sister chromatid exchange mechanisms with halogenated pyrimidines
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 41–58, Plenum Publishing Corporation, New York, 1984

Erickson, B. H.
Effects of ionizing radiation on mammalian spermatogenesis: A model for chemical effects
In *Reproductive Toxicology*, (Dixon, R. L., ed.), Raven Press, New York (in press)

Flanders, W. D., Cragle, D. L., and McClanahan, J. W.
Evaluation on Nonworker Residents as a Comparison Group in Occupational Studies of Oak Ridge Workers
Oak Ridge Associated Universities, Oak Ridge, Tennessee, ORAU–241, 1985

Frome, E. L.
Regression methods for binomial and Poisson distributed data
In *Proceedings of the AAPM First Midyear Topical Symposium, Multiple Regression Analysis: Applications in the Health Sciences, 1984*, Mobile, Alabama (in press)

Frome, E. L., and McLain, R.
A macro for fitting nonlinear models to Poisson distributed data
In *Proceedings of the Eighth Annual SAS Users Group International Conference*, pp. 883–888, Hollywood, Florida, 1985

Fry, R. J. M., and Fry, S. A.
Effects on humans of exposure to radioactive materials: late somatic effects

1028689

In *Encyclopedia of Materials Science and Engineering*, Pergamon Press, New York (in press)

Fry, S. A., Lushbaugh, C. C., Shy, C. M., Cragle, D. L., Checkoway, H., Blum, S., Carpenter, A. V., Dupree, E. A., Frome, E. L., Groer, P. G., and Wilson, J.
The U.S. Department of Energy health and mortality study: the Oak Ridge studies
In *Epidemiological Studies of Some Populations Exposed to Ionizing Radiation*, (Weeks, J. L., Compiler), pp. 18–40, Atomic Energy of Canada Limited, Pinawa, Manitoba, AECL-8360, 1985

Groer, P. G.
Bayesian estimates for the rate of Three-Mile Island type releases
In *Low-Probability High-Consequence Risk Analysis*, (Waller, R. A., and Covello, V. T., eds), pp. 127–136, Plenum Publishing Corporation, New York, 1984

Hammond, B. F., and Martin, P. G.
Calcium-cadmium effects on bone density and strength of the femur from multiparous rats
Nutr. Res. Suppl. (in press)

Hodges, H. D. [Letter to the Editor]
A new speciality for the nuclear medicine technologists
J. Nucl. Med. Technol. 12, 87, 1984

Hübner, K. F., Littlefield, L. G., and DuFrain, R. J.
Biologische Indikatoren fur ionisierende Strahlen
Muench. Med. Wochenschr. (in press)

Lee, T-c., Blank, M. L., Fitzgerald, V., Malone, B., and Snyder, F.
Biosynthesis of intermediates in PAF metabolic pathway: Characterization of 1-alkyl-2-acetyl-*sn*-glycerol kinase in rabbit platelets
In *New Horizons in Platelet Activating Factor Research*, (Lee, M. L., and Winslow, C. M., eds.), John Wiley & Sons Limited, Chichester, England (in press)

Lee, T-c., and Snyder, F.
Function, metabolism, and regulation of platelet activating factor and related ether lipids
In *Phospholipids and Cellular Regulation*, (Kuo, J. F., ed.), CRC Press, Inc., Boca Raton, Florida (in press)

Littlefield, L. G., Colyer, S. P., Sayer, A. M., and DuFrain, R. J.
Persistence of SCE-inducing lesions after G_0 exposure of human lymphocytes to differing classes of DNA-damaging chemicals
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 663–676, Plenum Publishing Corporation, New York, 1984

Lushbaugh, C. C., Brown, D. G., and Frome, E. L.
Longevity of irradiated burros
In *Proceedings, Twenty-Second Hanford Life Sciences Symposium, 1983*, Richland, Washington (in press)

Lushbaugh, C. C., Hübner, K. F., and Fry, S. A.
Effects of high doses of radiation
In *Proceedings of the Eighth Life Sciences Symposium, Radiation and Its Effects*, 1980, Los Alamos, New Mexico (in press)

Lushbaugh, C. C., Hübner, K. F., Fry, S. A., and Beck, W. L.
Effects on humans of exposure to radioactive materials: acute effects
In *Encyclopedia of Materials Science and Engineering*, Pergamon Press, New York (in press)

McFee, A. F., and Sherrill, M. N.
Accumulation of SCEs in lymphocytes during chronic ingestion of a mutagen
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 585–594, Plenum Publishing Corporation, New York, 1984

Ricks, R. C.
Response to a spill of radioactive material
In *Encyclopedia of Materials Science and Engineering*, Pergamon Press, New York (in press)

Snyder, F.
Chemical and biochemical aspects of "platelet activating factor," A novel class of acetylated ether-linked choline-phospholipids
Med. Res. Rev. 5, 107–140, 1984

Snyder, F.
Metabolism, regulation, and function of ether-linked lipids
In *Biochemistry of Lipids and Membranes*, (Vance, D., and Vance, J., eds.), pp. 271–298, The Benjamin/Cummings Publishing Company, Menlo Park, California, 1985

Snyder, F.
The significance of dual pathways for the biosynthesis of platelet activating factor: Alkylacetylglycerols and lyso-PAF as immediate precursors
In *New Horizons in Platelet Activating Factor Research*, (Lee, M. L., and Winslow, C. M., eds.), John Wiley & Sons Limited, Chichester, England (in press)

Snyder, F., Blank, M. L., Lee, T-c., Woodard, D. S., and Malone, B.
Metabolism of platelet activating factor: the role of alkylacetylglycerols in PAF biosynthesis
Adv. Inflammation Res. (in press)

Snyder, F., Hoffman, D., and Record, M.
The selective cytotoxicity of ether-linked phospholipid analogs of platelet activating factor toward tumor cells: Their biochemical mechanism of action at the cell membrane
In *Aktuelle Onkologie, Zuckschwerdt Verlag*, Munich, Federal Republic of Germany (in press)

Snyder, F., Lee, T-c., Blank, M., Malone, B., Woodard, D., and Robinson, M.
Platelet activating factor (PAF): alternate pathways of biosynthesis and the mechanism of activation
Adv. Prostaglandin Thromboxane Leukotriene Res. (in press)

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Snyder, F., Lee, T-c., and Wykle, R. L.
Ether-linked glycerolipids and their bioactive species: enzymes and metabolic regulation
In *The Enzymes of Biological Membranes*, (Martonosi, A. N., ed.), Vol. 2, pp. 1–58, Plenum Publishing Corporation, New York, 1985

Published Abstracts of Papers Presented at Meetings

Boland, C. R., and Clapp, N. K.
Oncodevelopmental mucins in the colons of New World monkeys with ulcerative colitis and cancer
Gastroenterology 88, 330, 1985

Clapp, N. K., Henke, M. A., Lushbaugh, C. C., Humason, G. L., McArthur, A. H.
Preliminary observations of demographic factors affecting occurrence of marmoset colitis
Gastroenterology 88, 1350, 1985

Clapp, N. K., McArthur, A. H., Carson, R. L., Henke, M. A., and Peck, O. W.
Endoscopy of the colon in tamarins and marmosets
Lab. Anim. Sci. (in press)

Clapp, N. K., and Tardif, S. D.
The successful rearing of cotton-top tamarins using colony-born breeders
Lab. Anim. Sci. 34, 504, 1984

Crook, J. E., and Mroczkowski, P. J.
Correlative analysis of arginine vasopressin (AVP) plasma concentrations with red cell Na-K cotransport activity, Na-K pump activity and intracellular Na concentration in hypertensive subjects
Regul. Pept. Suppl. 4, 227, 1985

Dobson, L., Straume, T., Carrano, A., Minkler, J., Deaven, L., Littlefield, G., and Awa, A.
Cytogenetic effectiveness of neutrons from replica of Hiroshima Bomb: Preliminary results from an interlaboratory study
In *Proceedings of the Fourth International Conference on Environmental Mutagens*, Stockholm, Sweden, 1985 (in press)

DuFrain, R. J., and Batson, J. S.
SCE evaluation in lymphocytes from chimeric tamarins
Mamm. Chromosome Newslett. 25, 10, 1984

DuFrain, R. J., Linkous, S. L., and Jennings, C. J.
In vivo SCE analysis in streptonigrin (NSC-45383) treated neonatal rabbits using BrdUrd, IdUred and CldUrd tablets
Environ. Mutagen. 7, 53, 1985

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action
Biometrics (in press)

Littlefield, L. G., Hübner, K. F., Joiner, E. E., and DuFrain, R. J.
Cytogenetic techniques in radiation dose assessment: Evaluations in 10 persons exposed to ^{60}Co gamma radiation in the 1984 Mexican accident
Environ. Mutagen. 7, 6, 1985

Littlefield, L. G., Sayer, A., Colyer, S., Joiner, E. E., Outlaw, J., and Dry, L.
Persistent radiation-induced chromosome lesions in lymphocytes of the Y-12 accident survivors: Evaluations of 25 years post-exposure
Mamm. Chromosome Newslett. 25, 18, 1984

McFee, A. F.
Failure of the estrous cycle or early pregnancy to influence SCE numbers in swine lymphocytes
J. Dairy Sci. (in press)

McFee, A. F., Lowe, K. W., and DuFrain, R. J.
The radiomimetic character of streptonigrin evaluated by a standardized mouse bone marrow assay
Environ. Mutagen. 7, 53, 1985

Robinson, M., Blank, M., and Snyder, F.
Selective acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (alkyllyso-GPC) with arachidonate by a CoA-independent transacylation in rabbit alveolar macrophages
Fed. Proc. 44, 1608, 1985

Snyder, F., Blank, M. L., Lee, T-c., and Malone, B.
Biological activities and metabolism of an antihypertensive acetylated ether-lined phospholipid (platelet activating factor, PAF)
J. Am. Oil Chem. Soc. 62, 614, 1985

Snyder, F., Woodard, D., Malone, B., and Lee, T-c.
The enzymatic synthesis of alkylacetylglycerols and their subsequent conversion to platelet activating factor
In *Abstracts, 13th International Congress of Biochemistry*, Amsterdam, p. 565, (TH-479), 1985, Amsterdam, 1985

Tardif, S. D., and Clapp., N. K.
Reproductive longevity in tamarin females (*Saguinus sp.*)
Lab. Anim. Sci. 34, 504, 1984

Tardif, S., Lenhard, A., Carson, R., and McArthur, A.
Hand-rearing of infant *Saguinus oedipus* with subsequent introduction into social groups
Am. J. Primatol. 8, 368–369, 1985

Washburn, L. C., Lingen, J. K., Sun, T. T., and Byrd, B. L.
Synthesis and tumor uptake studies of ^{14}C-labeled α-aminoisobutyric acid analogs
J. Labelled Compd. Radiopharm. 21, 1238–1239, 1984

Woodard, D. S., Lee, T-c., and Snyder, F.
Platelet activating factor (PAF) biosynthesis via a specific cholinephosphotransferase in rat kidney medulla microsomes
Fed. Proc. 44, 2607, 1985

1028691

Miscellaneous Papers or Talks Presented at National and International Scientific Conferences

Crook, J. E., Carlton, J. E., and Holloway, E. C.
Evaluation of copper-64 (Cu-64), a positron emitter, as a new myocardial imaging agent.
XII InterAmerican Congress of Cardiology, 1985, Vancouver, British Columbia, Canada

Lee, T-c.
Hormonal induction and developmental modulation of acyl-CoA reductase and akyldihydroxyacetonephosphate synthase in mouse preputial glands.
Peroxisomes and their metabolites in cellular functions
Joint UNESCO-IUB Workshop, 1985 Zeist, The Netherlands

Littlefield, L. G.
Biological methods for estimating human exposure to ionizing radiation.
International Seminar on the Analysis, Evaluation, Control and Prevention of Environmental Risks, 1985, Mexico City, Mexico

Littlefield, L. G.
Mexican Accident: Methodology and results of cytogenetic evaluations in five of the Juarez Yonke Fenix Junkyard Workers and in five persons who lived on Aldama Street.
Instituto de Investigaciones Biomedicas at the Universidad Nacional, 1985, Mexico City, Mexico

Snyder, F.
Platelet activating factor (PAF), alternate pathways of biosynthesis, the mechanism of inactivation, and the reacylation of lyso-PAF with arachidonate
Kyoto Conference on Prostaglandins, 1984, Kyoto, Japan

Snyder, F.
Alkylacetylglycerols versus lyso-PAF as precursors in PAF biosynthesis and the role of arachidonic acid in PAF metabolism
Leucotrienes & PAF-Acether 85, 1985, Paris

Snyder, F. and Lee, T-c.
The enzymatic synthesis of alkylacetylglycerols and their subsequent conversion to platelet activating factor
13th International Congress of Biochemistry, 1985, Amsterdam, The Netherlands

Snyder, F.
Overview of ether lipid field
Gordon Research Conference, Lipid Metabolism, 1985, Meriden, New Hampshire

Snyder, F.
Recent developments in PAF metabolism
Gordon Research Conference, Phagocytes, 1985, Andover, New Hampshire

Snyder, F.
Biochemistry of a novel group of ether-linked phospholipid mediators (platelet activating factor)
The Second Princeton Liposome Conference, Lipids in Cell Biology, 1985, Princeton, New Jersey

summaries of published papers

Reprints of the following published papers are available. Send requests to one of the authors or the chairman's office, Medical and Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117.

Albert, D. H. and Snyder, F.
Release of arachidonic acid from 1-alkyl-2-acyl-*sn*-glycero-3-phosphocholine, a precursor of platelet-activating factor, in rat alveolar macrophages
Biochim. Biophys. Acta 796, 92–101, 1984

Platelet activating factor and the bioactive metabolites of arachidonic acid are secreted by alveolar macrophages in response to stimulation by phagocytic agents or calcium ionophore. We have previously shown a deacylation-acetylation sequence in the formation of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (PAF) from alkylacyl-(long chain)-GPC [Albert, D. H. and Snyder, F. (1983) *J. Biol. Chem.* 258, 97–102]. This sequence may be an important source of 20:4 during inflammatory reactions since, in alveolar macrophages, the ether lipid precursor of PAF represents 35% of the choline glycerophospholipids and has a much higher content (35%) of 20:4 in the *sn*-2 position than does diacyl-GPC (17%). Alveolar macrophages prelabeled with ^{14}C-labeled fatty acids (16:0, 18:1, 18:2 and 20:4) and [1-^{3}H]alkyllyso-GPC were used to study the release of fatty acids from ether-linked and diacyl phospholipids. Each of these fatty acids was incorporated primarily into the choline glycerophospholipids of alveolar macrophages. The release of 20:4 from macrophage phospholipids was increased by treatment of the labeled cells within with calcium ionophore A23187 (2μM) or zymosan (1 mg/ml), whereas the release of 16:0, 18:1 and 18:2 was not increased above control levels by either stimuli. Although more of the labeled 20:4 is released from the diacyl-GPC (50% of the total released), substantial amounts (44%) of 20:4 are derived from alkylacyl-GPC after incubating the stimulated cells for 60 min. The loss of 20:4 continued from the diacyl species throughout the incubation period studied, whereas a slower net release of 20:4 lost from the alkylacyl-GPC fraction was evident after 2 h. We conclude that the deacylation-reacylation cycle is an important aspect of the metabolism of 20:4 and alkylacyl-GPC during inflammatory stimulation of alveolar macrophages and that the deacylation of this ether-linked phospholipid (which is the first step in the formation of PAF) is responsible for a significant amount of the 20:4 released.

Blank, M. L., Cress, E. A., Robinson, M., and Snyder, F.
Metabolism of unique diarachidonoyl and linoleoylarachidonoyl species of ethanolamine and choline phosphoglycerides in rat testes
Biochim. Biophys. Acta 833, 366–371, 1985

Selected molecular species of rat testicular 1,2-diradyl-*sn*-glycero-3-phosphocholines and 1,2-diradyl-*sn*-glycero-3-phosphoethanolamines were quantitated as their diradylglycerobenzoate derivatives using a recently developed high-performance liquid chromatographic method. Increased amounts of docosapentaenoic acid were found in glycerophospholipids containing ether moieties compared with the diacyl phospholipids (e.g.,

1028692

docosapentaenoate-containing species comprised >80% of the alkylacyl subclass of the ethanolamine phospholipids as opposed to 29.3% of the diacyl subclass). Within 2 h after intratesticular injections of [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid, the 20:4–20:4 and 18:2–20:4 molecular species of the diacyl subclass of both the choline and ethanolamine glycerophosphatides had the highest specific radioactivities. These unique molecular species (20:4–20:4 and 18:2–20:4) also exhibited the largest percentage decrease in specific radioactivity 24 h after the intratesticular injections of [^{3}H]arachidonic acid, which indicates these two species possess a high metabolic turnover. Two of the arachidonate-containing molecular species (18:1–20:4 and 18:0–20:4) in the ethanolamine plasmalogens showed only a small decrease in specific radioactivity, whereas a third species (16:0-20:4) actually had a 44% increase in specific radioactivity 24 h after the intratesticular injections of [^{3}H]arachidonate. These data indicate that the 20:4–20:4, 18:2–20:4, and 18:1–20:4 species of phosphatidylcholine and/or phosphatidylethanolamine are most rapidly labeled after administration of [^{3}H]arachidonic acid and that they appear to serve as the source of the [^{3}H]arachidonate that is ultimately transferred to ethanolamine plasmalogens.

Blank, M. L., Lee, T-c., Cress, E. A., Malone, B., Fitzgerald, V., and Snyder, F.
Conversion of 1-alkyl-2-acetyl-*sn*-glycerols to platelet activating factor and related phospholipids by rabbit platelets
Biochem. Biophys. Res. Commun. 124, 156–163, 1984

The metabolic pathway for 1-alkyl-2-acetyl-*sn*-glycerols, a recently discovered biologically active neutral lipid class, was elucidated in experiments conducted with rabbit platelets. The total lipid extract obtained from platelets incubated with 1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycerols or 1-alkyl-2-[^{3}H]acetyl-*sn*-glycerols contained at least six metabolic products. The six metabolites, identified on the basis of chemical and enzymatic reactions combined with thin-layer or high-performance liquid chromatographic analyses, corresponded to 1-alkyl-*sn*-glycerols, 1-alkyl-2-acetyl-*sn*-glycero-3-phosphates, 1-alkyl-2-acyl(long-chain)-*sn*-glycero-3-phosphoethanolamines, 1-alkyl-2-acetyl-*sn*-1-alkyl-2-acyl (long-chain)-*sn*-glycero-3-phosphocholines, and 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines (platelet activating factor). These results indicate that the metabolic pathway for alkylacetylglycerols involves reaction steps catalyzed by the following enzymatic activities: choline- and ethanolaminephosphotransferases, acetylhydrolase, an acyltransferase, and a phosphotransferase. The step responsible for the biosynthesis of platelet activating factor would appear to be the most important reaction in this pathway and this product could explain the hypotensive activities previously described for alkylacetyl-(or propionyl)-glycerols. Of particular interest was the preference exhibited for the utilization of the 1-hexadecyl-2-acetyl-*sn*-glycerol species in the formation of platelet activating factor.

Cabot, M. C.
Hydrolysis of novel diacylglycerol analogs and phorbol diesters by serum lipase
Biochim. Biophys. Acta 833, 330–335, 1984

Rat serum, active in the hydrolysis of the tumor-promoting phorbol diester, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), was examined with regard to lipid interferences of [^{3}H]TPA hydrolysis and enzyme substrate specificity. The enzymatic hydrolysis of TPA could be enhanced 8-fold, over crude serum, by using a lipid-free acetone powder of rat serum. Addition of lipid to the lipid-free acetone powder produced potent inhibition of TPA hydrolysis. The inclusion of multilamallar liposomes resulted in similar inhibition, and isolation of liposomes by high-speed centrifugation showed that 95% of the radiolabeled TPA was associated with the fatty pellet. Substrate specificity studies demonstrated that the serum activity hydrolyzes the long-chain ester of TPA and the long-chain primary acyl group of diacylglycerols. TPA was hydrolyzed at approximately twice the rate of dioleoylglycerol; however, the most reactive substrates were those synthetic analogs of diacylglycerol containing a short-chain ester group at the *sn*-2 position. Palmitic acid was liberated from [1-^{14}C]palmitoyl-2-acetyl-*sn*-glycerol and [1-^{14}C]palmitoyl-2-butyryl-*sn*-glycerol at 120- and 33-times the rate of TPA hydrolysis, respectively. Lipase resistant 1-hexadecyl-2-[^{3}H]acetylglycerol was also used as substrate, but the *sn*-2 ester moiety showed poor lability. The diacylglycerol analogs are new lipase substrates and, in view of their similarities to the fatty acyl portion of TPA, it is thought that these compounds could serve as protein kinase C activators.

Cabot, M. C.
Tumor promoting phorbol diesters: substrates for diacylglycerol lipase
Biochem. Biophys. Res. Commun. 123, 170–177, 1984

Enzyme activity in rat serum was examined utilizing the potent tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and various glycerolipids as substrates. The serum activity was specific for hydrolysis of the long chain tetradecanoate moiety of TPA, hydrolyzed mono- and diacylglycerols, but was not effective against triacylglycerols, cholesterylesters, or phospholipids. Heating the enzyme preparation at 56°C for 1 min was dually effective in reducing the hydrolysis of both TPA and dioleoylglycerol by 83–86% of control levels. The potent diacylglycerol lipase inhibitor, RHC 80267, inhibited the hydrolysis of TPA in the 0.2–1.0μ M range and was also a potent blocker of monoacyl- and diacylglycerol hydrolysis. In substrate competition studies, exogenous unlabeled TPA was added to the [^{14}C]dioleoylglycerol-containing reaction mixture, however, this produced an approximate 3-fold stimulation of [^{14}C]dioleoylglycerol hydrolysis. Although we have not established whether the hydrolysis of TPA and diacylglycerol is the work of one enzyme, the effectiveness of the specific lipase inhibitor, RHC 80267, demonstrates that diacylglycerol lipase can utilize TPA as substrate, a finding never before documented. This point is of interest in light of the theory that phorbol esters act by mimicry of the natural lipid mediator, diacylglycerols.

1028693

Cabot, M. C., and Jaken, S.
Structural and chemical specificity for diradylglycerols for protein kinase C activation
Biochem. Biophys. Res. Commun. 125, 163–169, 1984

The structural and chemical specificity of diacylglycerols, lipid components of the quaternary complex for protein kinase C activation, have been evaluated. The ether-linked analogs of the diacyl lipids, either dialkyl or alkyl acyl, were not effective activators of protein kinase C and thus had little influence on reducing the Ca^{++} requirement of the enzyme. Ester-linked compounds such as 1-palmitoyl-*sn*-2-butyrylglycerol were as effective as dioleoylglycerol in stimulating protein phosphorylation. Increasing the carbon number at the *sn*-2 position from two to four resulted in enhanced enzymatic activity, suggesting that the chain length at the secondary hydroxyl is also of importance. These data clearly establish the necessity of the *sn*-1 carbonyl group of ester-linked glycerolipids in the protein kinase C activation complex.

Checkoway, H., Mathew, R. M., Shy, C. M., Watson, J. E., Jr., Tankersley, W. G., Wolf, S. H., Smith, J. C., and Fry, S. A.
Radiation, work experience, and cause specific mortality among workers at an energy research laboratory
Br. J. Ind. Med. 42, 525–533, 1985

A retrospective cohort mortality study was conducted among 8375 white male employees who had worked at the Oak Ridge National Laboratory for at least one month between 1943 and 1972. This plant has been the site of energy related research, including uranium and plutonium reactor technology and radioisotope production. Radiation doses, primarily from gamma rays, were generally low; the median cumulative exposure for workers was 0.16 rems. Historical follow up was conducted for the years 1943–77 and ascertainment of vital status was achieved for 92.3% of the cohort. Standardised mortality ratios (SMRs) were computed to contrast the subjects' cause specific mortality experience with that of the United States white male population. The observed number of 966 deaths from all causes was 73% of the number expected. Mortality deficits were also seen for arteriosclerotic heart disease (SMR = 0.75, 344 observed) and all cancers (SMR = 0.78, 194 observed). These results are indicative of the healthy worker effect and the favourable influence on health of the cohort's relatively high socioeconomic status. Non-statistically significant raised SMRs were seen for all leukaemias (SMR = 1.49, 16 observed), cancer of the prostate (SMR = 1.16, 14 observed), and Hodgkin's disease (SMR = 1.10, 5 observed). Internal comparisons of mortality (standardised rate ratios, SRRs) were made between subgroups of the cohort according to radiation dose level and duration of employment in various job categories. No consistent gradients of cause specific mortality were detected for radiation exposure. Leukaemia mortality was highest among workers with ⩾10 years employment in engineering (SRR = 2.40) and maintenance (SRR = 3.12) jobs. The association of leukaemia with employment in engineering was unexpected; maintenance jobs entail potential exposures to radiation and to a wide range of organic chemicals, metals, and other substances.

Cragle, D. L., Hollis, D. R., Qualters, J. R., Tankersley, W. G., and Fry, S. A.
A mortality study of men exposed to elemental mercury
J. Occup. Med. 26, 817–821, 1984

A cohort of 2,133 white males who were exposed to elemental mercury vapors between 1953 and 1963 was followed up through the end of 1978. Death certificates were obtained for 371 of the 378 workers who were reported by the Social Security Administration to be deceased. The mortality experience of this group was compared with the age-adjusted mortality experience of the U.S. white male population. Mortality has not been studied previously in assessing the long-term health effects of mercury exposure. Standardized mortality ratios (SMRs) were calculated for a comparable unexposed worker population to determine the mortality patterns among workers at the same plant who were not involved in the mercury process. Statistically significant excesses of deaths from cancer of the lung (SMR = 1.34; 71 observed, 52.9 expected) and cancer of the brain and other CNS tissues (SMR = 2.30; 13 observed, 5.65 expected) were observed among the plant workers who were not involved in the mercury process. An excess of deaths from cancer of the lung was also observed among the mercury workers (SMR = 1.34; 42 observed, 31.36 expected), although the elevation of this SMR was not statistically significant. Since excesses of lung cancer were evident in both groups of workers, it is unlikely that they are related to the mercury exposure and more probable that they are due to some other factor present in the plant or to some life-style factor prevalent among the plant workers. Exposure to mercury vapors at this plant was not related to any excess of deaths from diseases or cancers of organs determined to be target organs for mercury (liver, lung, brain and CNS, and kidney). No excesses were found when level of exposure and length of exposure were considered.

Daugherty, J. P., and Clapp, N. K.
Association of nitrosamine-derived radioactivity with nuclear and mitochondrial DNA in mice
GANN: Jpn. J. Cancer Res. 76, 197–201, 1985

In order to better understand the carcinogenic process, a comparative study was undertaken of the association of nitrosamine-derived radioactivity with nuclear and mitochondrial DNA isolated from tumor-susceptible and non-tumor-susceptible tissues of two strains of mice with different susceptibilities to tumor induction by dimethylnitrosamine (DMN) or diethylnitrosamine (DEN). A single dose of either [^{14}C]DMN or [^{14}C]DEN was administered by intragastric intubation to young adult male RFM or BALB/c mice. The DNA isolated from the mitochondrial fraction of tumor-susceptible tissues bound several times (10 to 90) more nitrosamine than the nuclear DNA. These results suggest that mitochondrial DNA may be a target in the carcinogenic process induced by nitrosamine compounds.

1028694

DuFrain, R. J.
Probing sister chromatid exchange mechanisms with halogenated pyrimidines
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 41–58, Plenum Publishing Corporation, New York, 1984

This communication describes the use of 6 different halogenated pyrimidine analogues, bromodeoxyuridine (BrdUrd), chlorodeoxyuridine (CldUrd), iododeoxyuridine (IdUrd), bromodeoxycytidine (BrdCyd), chlorodeoxycytidine (ClcCyd), and iododeoxycytidine (IdCyd), to achieve sister chromatid differentiation (SCD) and evaluate sister chromatid exchange (SCE) formation in mitogen-stimulated human lymphocytes. Also included are a description of an in vivo experiment with BrdUrd, ClcUrd, and IdUrd; a discussion of pyrimidine metabolism effects on SCEs; and the presentation of an update on the "conformation hypothesis" for SCE formation. This hypothesis revision includes a model that centers on the idea that the sum of the conformational alterations of the DNA polymerase-DNA template complex at replication is the controlling factor in SCE formation.

DuFrain, R. J.
Is cancer of the colon familial in cotton-top tamarins?
Cancer Genet. Cytogenet. 14, 83–87, 1985

Ten cases of spontaneous adenocarcinoma of the colon were observed in first-generation *Saguinus oedipus oedipus* born and raised under defined colony conditions. In this series of 10 cases, 3 animals had a wild parent (caught and imported) that also died of cancer of the colon and 4 of the remaining 7 were two unrelated sets of siblings (one a set of fraternal twins). This somewhat unexpected familial association or clustering of these malignancies suggests the possibility of a significant hereditary component to the development of cancer in these tamarins. The histopathology of the tumors in *S. o. oedipus* is compatible with a genetic influence, as it is strikingly similar to a dominantly inherited mucoid adenocarcinoma of the colon in humans.

Feuerstein, G., Lux, W. E., Jr., Ezra, D., Hayes, E. C., Snyder, F., and Faden, A. I.
Thyrotropin-releasing hormone blocks the hypotensive effects of platelet-activating factor in the unanesthetized guniea pig
J. Cardiovasc. Pharmacol. 7, 335–340, 1985

Platelet-activating factor (PAF) and leukotrienes, newly described classes of vasoactive lipids, may play a role in anaphylaxis. It has recently been suggested that the vasoconstrictor effects of PAF in isolated rat lung are related to release of leukotrienes C_4 and D_4. Thyrotropin-releasing hormone (TRH), a tripeptide, has potent anithypotensive activity in experimental shock, including that resulting from either leukotriene D_4 administration or antigen-induced anaphylaxis. We utilized an unanesthetized guinea pig model to study the relationships among PAF, leukotrienes, and TRH and their potential interactions on the cardiovascular system. PAF (1 nmol/600 g body weight i.v.) produced profound hypotension which was completely blocked by TRH (2 mg/kg i.v.). Nafazatrom or FPL 55712, a presumed receptor antagonist of leukotrienes, was ineffective, whereas U-60257, a leukotriene synthesis inhibitor, displayed incomplete blockade. Moreover, leukotriene-like immunoreactivity in plasma did not increase following PAF administration. Thus, hypotension produced by PAF does not appear to result secondarily from release of cysteinyl leukotrienes. Moreover, the ability of TRH to block the hypotensive effects of PAF may partially account for its beneficial effects in experimental anaphylaxis and provides further rationale for the therapeutic evaluation of this peptide in anaphylactic shock.

Feuerstein, G., Lux, W. E., Jr., Snyder, F., Ezra, D., and Faden, A. I.
Hypotension produced by platelet-activating factor is reversed by thyrotropin-releasing hormone.
Circ. Shock 13, 255–260, 1984.

Platelet-activating factor (PAF), a vasoactive phospholipid implicated in anaphylactic reactions, causes severe hypotension in experimental animals that is highly resistant to pharmacological therapy. In the present studies, we showed that PAF (1 nmol/600 g body weight, IV) produced profound hypotension in unanesthetized guinea pigs that was promptly and completely reversed by thyrotropin-releasing hormone (TRH) (2 mg/kg, IV) or by the synthetic TRH analog MK771 (2 mg/kg, IV). TRH also reversed this hypotension when administered intracerebroventricularly (ICV) at a dose (0.02 mg/kg) that was systemically ineffective. The opiate receptor antagonist naloxone (5 mg/kg) was less effective than TRH in reversing the cardiovascular consequences of PAF administration. These data suggest that TRH reverses PAF-induced shock through central receptor-mediated mechanisms. This therapeutic action of TRH may partially account for the beneficial cardiovascular effects of this peptide in anaphylactic shock.

Flanders, W. D., Cragle, D. L., and McClanahan, J. W.
Evaluation of Nonworker Residents as a Comparison Group in Occupational Studies of Oak Ridge Workers
Oak Ridge Associated Universities, Oak Ridge, Tennessee, ORAU–241, 1985

Residents of a thirteen-county region surrounding Oak Ridge, Tennessee, have been evaluated as a comparison group for studies of possible health effects associated with employment at three Oak Ridge facilities. Several problems constitute a case against their use as a comparison group. First, a substantial amount of time and effort would be required to gather the information necessary to use them as a comparison group. Second, bias may result from an inability to extrapolate census data accurately. Finally, Oak Ridge workers and other residents probably have different socioeconomic statuses and possibly different social, cultural, and ethnic backgrounds so that confounding is likely.

Frome, E. L., and Checkoway, H.
Epidemiologic programs for computers and calculators
Use of Poisson regression models in estimating incidence rates and ratios
Am. J. Epidemiol. 121, 309–323, 1985

Summarizing relative risk estimates across strata of a covariate is commonly done in comparative epidemiologic studies of incidence or mortality. Conventional Mantel-Haenszel and rate standardization techniques used for this purpose are strictly suitable

only when there is no interaction between relative risk and the covariate, and tests for interaction typically are limited to examination for departures from linearity. Poisson regression modeling offers an alternative technique which can be used for summarizing relative risk and for evaluating complex interactions with covariates. A more general application of Poisson regression is its utility in modeling disease rates according to postulated etiologic mechanisms of exposures or according to disease expression characteristics in the population. The applications of Poisson regression analysis to problems of summarizing relative risk and disease rate modeling are illustrated with examples of cancer incidence and mortality data, including an example of a nonlinear model predicted by the multistage theory of carcinogenesis.

Groer, P. G.
Bayesian estimates for the rate of Three-Mile Island type releases
In *Low-Probability High-Consequence Risk Analysis*, (Waller, R. A., and Covello, V. T., eds), pp. 127–136, Plenum Publishing Corporation, New York, 1984

The Bayesian approach described in this paper uses two kinds of information to estimate the rate of Three Mile Island (TMI) type releases: prior information contained in the prior distribution for the rate and experimental information from the operating experience with pressurized water reactors (PWR). Two prior distributions, but the same operating experience -- expressed as total reactor years -- are used to obtain the posterior distributions for the rate. One prior distribution is uniform over a finite range, the other one is the lognormal prior for PWR-9 releases given in the Rasmussen (WASH-1400) report. The probability of occurrence of a PWR-9 release by a certain total operating experience (reactor years) is calculated for uniform priors with difference ranges and for the lognormal prior for a PWR-9 release. The information defined as entropy—contained in the lognormal prior is compared with the information in the posterior distribution bases on the uniform prior. It is found that the lognormal PWR-9 prior contains considerably more information than the posterior resulting from the operating experience and a uniform prior. An alternate, conceptually more satisfying method to measure information learned through experience and expected sample information is sketched briefly. The same PWR operating experience up to the end of 1981 (651 reactor years) produces a post-posterior based on the lognormal PWR-9 prior with a mode at a rate of 2.8×10^{-4} per reactor year and a post-posterior stemming from the uniform prior with a modal rate of 1.5×10^{-3} per reactor year. Remarks on how Bayesian techniques should be used to estimate rates of radiation-induced cancers after accidental radioactive releases from reactors conclude the paper.

Lee, T-c.
Biosynthesis of platelet activating factor: substrate specificity of 1-acetyl-2-lyso-*sn*-glycero-3-phosphocholine:acyl-CoA acetyltransferase in rat spleen microsomes
J. Biol. Chem. 260, 10952–10955, 1985

The substrate requirements and specificity of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine (alkyllyso-GPC):acetyl-CoA acetyltransferase were investigated. The following findings were observed: (1) When the ether bond of alkyllyso-GPC is substituted with an ester linkage, the resulting compound, palmitoyllyso-GPC, can serve as a substrate, albeit at a reduced rate (50%). In addition, palmitoyllyso-GPC is a competitive inhibitor in the reaction with respect to concentration dependence of alkyllyso-GPC and a noncompetitive inhibitor when the concentrations of acetyl-CoA are varied. (2) Octadecyllyso-GPC is acetylated at a slightly higher rate than hexadecyllyso-GPC and unsaturated alkyllyso-GPC is a preferable substrate to its saturated counterpart. (3) The homologous series of short chain acyl-CoAs demonstrate an inverse relationship of chain length with the values of their apparent K_m and V_{max}, e.g., the longer the acyl-CoA chain, the smaller the values of V_{max} and apparent K_m. (4) The effect of polar head group modification of alkyllyso-GPC on the acetyltransferase activity is related to the degree of methylation of the amine group. The choline base analog gives the highest enzyme activity, and the ethanolamine derivative is the least active, while N',N'-dimethylethanolamine and monomethylethanolamine analogs are the substrates with intermediate activities. These results on substrate selectivity of acetyltransferase correlate with the known structural requirements essential for the biological activities elicited by platelet activating factor and thus suggest that the acetyltransferase may be important in governing the chemical structure of platelet activating factor synthesized in vivo.

Lenihan, D. J. Greenberg, N. and Lee, T-c.
Involvement of platelet activating factor in physiological stress in the lizard, *Anolis carolinensis*
Comp. Biochem. Physiol. 81C, 81–86, 1985

1. Platelet activating factor (PAF) and 1-*O*-alkyl-linked choline-containing phosphoglycerides have been identified in the blood of the lizard, *Anolis carolinensis*. 2. The level of PAF in the blood of chronically stressed lizards is 250% higher than in that of controls; the blood contains sufficient 1-*O*-alkyl-linked choline-containing phosphoglycerides to support the synthesis of PAF. 3. Incorporation of PAF is higher in the liver than in the blood; control animals incorporated more PAF than chronically stressed animals. 4. "Lyso"-PAF is the major metabolite in the blood; whereas, in the liver both "lyso"-PAF and "acyl"-PAF are predominant products. 5. 1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine: acetylhydrolase activity in the chronically stressed or corticosterone-implanted lizards is increased 2- to 3-fold over the control. 6. Involvement of PAF in the physiological response to psychological stress is indicted.

Littlefield, L. G., Colyer, S. P., Sayer, A. M., and DuFrain, R. J.
Persistence of SCE-inducing lesions after G_0 exposure of human lymphocytes to differing classes of DNA-damaging chemicals
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 663–676, Plenum Publishing Corporation, New York, 1984

We conducted studies to determine whether cycling human lymphocytes are equally efficient in repairing sister chromatid exchange (SCE)-producing lesions

1028696

induced by differing classes of DNA-damaging chemicals. Lymphocytes were pulse-treated during G_0 with mitomycin C (MMC), N,N',N"-triethylenethiophosphoramide (ThioTEPA), ethylmethanesulfonate (EMS), or *cis*-diamminedichloroplatinum (*cis*-DDP). Bromodeoxyuridine (BrdUrd) was added to the 72 hr cultures at 0 hr or at 48 hr after phytohemmagglutinin stimulation. The concentrations of chemicals employed induced a greater than 2-fold increase in SCEs in second-division metaphases from lymphocytes cultured in the presence of BrdUrd for the entire 72 hr. The analysis of SCEs in uniformly harlequinized metaphses from G_0-treated lymphocytes cultured in BrdUrd for the terminal 24 hr showed no increase above baseline after exposure to MMC, and intermediate increases above baseline after exposures to ThioTEPA and *cis*-DDP. However, after G_0 treatment with EMS, the observed SCE frequency was consistent with that expected had all DNA lesions persisted and continued to give rise to SCEs during 3 cell cycles. These findings suggest that cycling human lymphocytes are not equally efficient in eliminating SCE-producing lesions after exposure to differing classes of DNA-damaging chemicals.

Littlefield, L. G., Joiner, E. E., and Sayer, A. M.
Premature separation of centromeres in marrow chromosomes from an untreated patient with acute myelogenous leukemia
Cancer Genet. Cytogenet. 16, 109–116, 1985

Cytogenetic studies in an untreated patient with acute myelogenous leukemia revealed that greater than 60% of his marrow metaphases had pronounced anomalies in the centromere region. In the least affected chromosomes, the centromere appeared as a nonstaining hole, whereas in the most affected chromosomes, it appeared that the centromere segment had prematurely migrated at right angles to the telomeric portions. The population of mitoses exhibiting premature separation of the centromeres was reduced to virtually none during periods of drug induced remission. Our findings suggest that cytogenetic changes in malignant myeloid cells may be characterized by defects in centromere separation, as well as by alteration in karyotype.

Malone, B., Lee, T-c., and Snyder, F.
Inactivation of platelet activating factor by rabbit platelets: Lyso-platelet activating factor as a key intermediate with phosphatidylcholine as the source of arachidonic acid in its conversion to the tetraenoic acylated product
J. Biol. Chem. 260, 1531–1534, 1985

[^{3}H]PAF (platelet activating factor or 1-alkyl-2-acetyl-GPC) is converted to 1-alkyl-2-lyso-GPC and 1-alkyl-2-acyl-GPC by rabbit platelets (GPC is *sn*-glycero-3-phosphocholine). The deacetylation reaction does not involve the transfer of the acetate of PAF to any other lipid class, and added exogenous lyso-PAF readily mixes with the cellular pool of the [^{3}H]lyso-PAF intermediate formed from [^{3}H]PAF. [^{3}H]1-Alkyl-2-acyl-GPC produced during the inactivation of [^{3}H]PAF contained primarily the tetraenoic acyl species (approximately 80% of the ^{3}H in this fraction). The source of the arachidonic acid used for the reacylation of the lyso-PAF intermediate is the diacyl species, phosphatidylcholine.

McFee, A. F., and Sherrill, M. N.
Accumulation of SCEs in lymphocytes during chronic ingestion of a mutagen
In *Sister Chromatid Exchanges*, (Tice, R. R., and Hollaender, A., eds.), pp. 585–594, Plenum Publishing Corporation, New York, 1984

Studies in 2 large-animal species have shown that continued ingestion of a mutagen increases sister chromatid exchanges (SCE) in circulating lymphocytes at rates directly related to the accumulated dose but differing between species. Groups of 3 pigs received daily oral doses of 0, 1.25, or 2.50 mg/kg body weight of 7,12-dimethylbenzanthracene (DMBA) for 160 consecutive da; similar groups of sheep received 0, 0.625, or 1.25 mg/kg daily for up to 114 da. Lymphocytes taken at 3-wk intervals from pigs and every 2 wk from sheep were cultured, and 25 randomly selected second-division metaphases from each culture were scored for SCE. Swine lymphocytes had elevated numbers of SCE at the earliest sampling times after DMBA feeding was begun. Sister chromatid exchanges continued to increase gradually among pigs consuming 1.25 mg/kg/da for 160 da whereas those on 2.5 mg/kg tended to plateau at approximately 2.5 times their pretreatment level after some 80 da. Sheep treated with comparable levels of DMBA (1.25 mg/kg) exhibited a much more rapid rise in SCE than pigs; within 40 da their SCEs were at 4 times the control level and animals were exhibiting toxicity symptoms. Low-dose sheep also showed a rapid initial increase but plateaued at about 2.5 times the control level beyond 40 da.

Polednak, A. P., and Hollis, D. R.
Mortality and causes of death among workers exposed to phosgene in 1943–45
Toxicol. Ind. Health 1, 137–151, 1985

Mortality and causes of death from death certificates were analyzed among workers exposed to phosgene while working at a uranium-processing plant in Tennessee in 1943–45. Standardized mortality ratios (SMRs) were calculated by using death rates for U.S. white males. As of 1979, SMRs for all causes and for various selected causes were similar in 694 male chemical workers chronically exposed to low levels of phosgene in 1943–45 and in 9280 male controls who worked at the same plant. SMRs for diseases of the respiratory system were 107 (14 observed vs. 13.07 expected) in the chemical workers and 119 (292 observed vs. 245.75 expected) in the controls. In a group of 106 males who were acutely exposed to high levels of phosgene, there were 41 deaths observed vs. 33.87 expected (SMR = 121; 95% confidence limits = 86 and 165). One death, occurring within 24 hours of exposure, was from pulmonary edema due to phosgene poisoning (coded to "accidental causes"). Five deaths were coded to diseases of the respiratory system (SMR = 266; 95% CL = 86 and 622); in 2 of these 5 deaths, "bronchitis" due to phosgene exposure had been reported in 1945. Among 91 female workers with acute high-level phosgene exposure, frequencies of symptoms and early health effects (pneumonitis and bronchitis) differed from those reported for the 106 male cases; preliminary data on vital status of these females are too incomplete for analysis, and further follow-up is needed.

1028697

Robinson, M., Blank, M. L., and Snyder, F.
Acylation of lysophospholipids by rabbit alveolar macrophages: Specificities of CoA-dependent and CoA-independent reactions
J. Biol. Chem. 260, 7889–7895, 1985

Intact alveolar macrophages were found to acylate alkyl- and acyl-lysophospholipids with a high selectivity for arachidonate. A specific mechanism appears responsible for the incorporation of arachidonate into lysophospholipids in intact cells since the kinetic pattern for the formation of the 20:4 species was different from all other species. This specificity was investigated in more detail by examining the enzymatic acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine by macrophage membranes; in the absence of CoA, ATP, and Mg^{2+}, this lysophospholipid was acylated with a high preference for arachidonate that was independent of added free fatty acids. The addition of CoA alone increased the rate of acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine, mainly due to an increase in the formation of species other than those containing arachidonate. When CoA, ATP, and Mg^{2+} were present, the macrophage membranes catalyzed the acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine without preference for arachidonate. A different apparent K_m and V_{max} was observed for reactions involving each co-factor condition. We conclude that the acylation of alkyl- and acyl-lysophospholipids by rabbit alveolar macrophages occurs by three separate mechanisms; a CoA-independent transacylation, a CoA-dependent transacylation (reverse reaction catalyzed by acyl-CoA acyltransferase), and an acyl-CoA dependent acylation. The CoA-independent transacylation reaction is unique in that it is specific for arachidonate and accounts for the selective acylation of alkyl- and acyl-lysophospholipids by arachidonate in membrane preparations of alveolar macrophages. This reaction appears to be extremely important in the remodeling of phospholipid molecular species and the mobilization of arachidonate into ether-linked lipids. The transfer of arachidonate to 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine also is of importance in the final inactivation step for platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine), whereby 1-alkyl-2-arachidonoyl-*sn*-glycerol-3-phosphocholine (a stored precursor of both platelet activating factor and arachidonic acid metabolites) is formed.

Som, P., Oster, Z. H., Yamamoto, K., Meinken, G. E., Srivastava, S. C., Yonekura, Y., Ebner, S. A., Atkins, H. L., Brill, A. B., Fawwaz, R. A., Alderson, P. O., Coffey, J., Carlton, E., and Hübner, K. F.
Some factors affecting the cerebral and extracerebral accumulation of N-isopropyl-*p*-iodo-amphetamine (IAMP)
Int. J. Nucl. Med. Biol. 12, 185–196, 1985

The whole body distribution of IAMP was studied in mice, rats, rabbits, dogs and marmosets by tissue counting, scintigraphy and quantitative macroautoradiography. Identical distribution patterns of IAMP were observed in these species showing high concentration in the brain, lungs, eyes, liver and kidneys. Following the initial distribution, IAMP was released from the lungs and accumulated in the blood and liver. In the cerebrum the activity decreased with time whereas it progressively increased in the eyes and juxtamedullary region of kidneys.
The effect of ACTH, propranolol and metopyrone (metyrapone, USP) on the distribution of IAMP was studied in normal mice. The distribution of IAMP in Dahl salt-sensitive hypertensive rats was compared to Dahl salt-sensitive normotensive rats. Blocking doses of potassium iodide (KI) in marmosets and rats did not affect the distribution pattern of IAMP. Carrier amphetamine in doses of up to 1.5 mg/kg also did not significantly alter the biodistribution of IAMP. When higher doses (up to 8.0 mg/kg) were given, the activity in the gut, lungs, and liver decreased but there was no effect on the uptake in the brain, kidneys, eyes, adrenals, muscle and spleen. This suggests a higher ratio of saturable to nonsaturable binding sites in the latter organs.

Surles, J. R., Wykle, R. L., O'Flaherty, J. T., Salzer, W. L., Thomas, M. J., Snyder, F., and Piantadosi, C.
Facile synthesis of platelet-activating factor and racemic analogues containing unsaturation in the *sn*-1-alkyl chain
J. Med. Chem. 28, 73–78, 1985

Platelet-activating factor, 1 (PAF, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine), and octadecyl-PAF were synthesized chemically as the racemates. The *sn*-1-*O*-alkyl isomers were isolated after treatment of the racemates with phospholipase A_2 and subsequent reacetylation of the 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholines released. Analogues of PAF containing unsaturated alkyl moieties at the *sn*-1 position (2,4,5) were synthesized by utilizing the methoxyethoxymethyl protecting group as a novel method for preparing unsaturated alkyl lipids. This procedure provides a facile means for preparing unsaturated ether phospholipids of defined structure that may be tritiated to high radiospecific activity for metabolic studies. Unsaturation in the alkyl chain had minimal effect on the bioactivites examined in this study.

Urso, P., and Gengozian, N.
Subnormal expression of cell-mediated and humoral immune responses in progeny disposed toward a high incidence of tumors after in utero exposure to benzo[a]pyrene
J. Toxicol. Environ. Health 14, 569–584, 1984

Pregnant mice were exposed to 150 μg benzo[a]pyrene (BaP) per gram of body weight during fetogenesis (d11-17 of gestation) and the progeny were assayed for humoral and cell mediated immune responses at different time intervals after birth. Immature offspring (1-4 wk) were severely suppressed in their ability to produce antibody-(plaque-) forming cells (PFC) against sheep red blood cells (SRBC) and in the ability of their lymphocytes to undergo a mixed lymphocyte response (MRL). Lymphocytes from these progeny showed a moderate to weak capacity to inhabit production of colony-forming units (CFU) in host spleens following transfer with semiallogeneic bone marrow (BM) cells into lethally X-irradiated recipients syngeneic to the BM (in vivo graft-versus-host response, GVHR). A severe and sustained suppression in the MLR and the PFC response occurred from the fifth month up to 18 mo. The in vivo GVHR, also subnormal later in life, was not as severely suppressed as the other two parameters. Tumor incidence in the BP-

1028698

exposed progeny was 8- to 10-fold higher than in those encountering corn oil alone from 18 to 24 mo of age. These data show that in utero exposure to the chemical carcinogen BaP alters development of components needed for establishing competent humoral and cell-mediated functions of the immune apparatus and leads to severe and sustained postnatal suppression of the defense mechanism. The immunodeficiency exhibited, particularly in the T-cell compartment (MLR, GVHR), before and during the increase in tumor frequency, may provide a favorable environment for the growth of nascent neoplasms induced by BaP.

Washburn, L. C., Sun, T. T., Byrd, B. L., and Callahan, A. P.
Resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan by high-performance liquid chromatography
J. Labelled Compd. Radiopharm. 22, 135–142, 1985

High-performance liquid chromatographic (HPLC) resolution of [^{11}C]DL-leucine and [^{11}C]DL-tryptophan, using modifications of a previously developed technique for resolution of [^{11}C]DL-valine, was used to produce [^{11}C]L-leucine and [^{11}C]L-tryptophan. The technique employs commercially available reverse phase HPLC columns and chiral mobile phases containing cupric acetate and L-proline.

Zukowska-Grojec, Z., Blank, M. L., Snyder, F., and Feuerstein, G.
The adrenergic system and the cardiovascular effects of platelet activating factor (1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) in SHR and WKY rats
Clin. Res. Hypertens.- Theory and Practice A7, 1015–1031, 1985

1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (1-hexadecyl-2-acetyl-GPC, platelet activating factor, PAF) was previously shown to produce profound hypotension and sympathetic activation in conscious rats. To determine the role of the sympatho-adrenomedullary system in the cardiovascular responses elicited by 1-hexadecyl-2-acetyl-GPC, the vasoactive phospholipid was administered (l nmol/300 g) to a) intact, b) bilaterally demedullated, and c) propranolol- (a β-adrenoceptor blocker) treated SHR and WKY rats. The hypotensive response to 1-hexadecyl-2-acetyl-GPC was prolonged in demedullated or propranolol-pretreated WKY rats and in propranolol-treated SHR rats. The extreme tachycardia produced by 1-hexadecyl-2-acetyl-GPC in both the WKY and SHR rats was abolished by propranolol pretreatment. Pressor responses to norepinephrine during the 1-hexadecyl-2-acetyl-GPC-induced hypotension in propranolol-pretreated rats were suppressed in both the normotensive and SHR rats. Plasma acetylhydrolase activity, which inactivates PAF, was higher in hypertensive (SHR) rats or demedullated WKY rats than in the normotensive (WKY) rats. These results show that the tachycardia evoked by 1-hexadecyl-2-acetyl-GPC is mediated solely by sympathetic activation and the β-adrenergic receptors and further indicate the major role of the sympathetic system and β-adrenoceptors in recuperation from 1-hexadecyl-2-acetyl-GPC-induced shock. The data also suggest that acetylhydrolase in serum is an important regulatory enzyme for controlling PAF levels in the vascular compartment.

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Training Programs

Training programs at the division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduates, university faculty, and high school vocational trainees. Special short-term courses dealing with radiation health problems are available for biomedical personnel.

Graduate students participating in Ph.D. training programs have come from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama (Birmingham), and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals; the advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by the Oak Ridge National Laboratory, University Programs Division at ORAU (sponsored by DOE), and individual research grants.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows have been available from a variety of sources: the U.S. Department of Energy, Damon Runyon-Walter Winchell Cancer Fund, the Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National

1028700

Institutes of Health and American Cancer Society Grants, the World Health Organization (International Agency for Research on Cancer), the Fogarty International Center (U.S. Public Health Service), and divisional funds.

Summer research opportunities make it possible for faculty and advanced undergraduate students from colleges and universities throughout the United States to participate in any of the division's research programs. The DOE-sponsored summer program is administered through the University Programs Division at ORAU.

Short-term courses in the management of medical problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of ten REAC/TS courses were given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at ORAU universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

The following individuals participated in the training programs at the division during the past year:

1. **Predoctoral students**

   Arvind Carpenter* (Epidemiology) University of Alabama

   Elizabeth Dupree Ellis* (Epidemiology) University of North Carolina

   Dabney Johnson (Biochemistry) University of Tennessee

   Zigrida Smith, (Biochemistry) University of Tennessee

1028701

Jefferson Surles† (Biochemistry) University of North Carolina

Donna Woodard* (Biochemistry) University of Tennessee

2. **Postdoctoral fellows**

Michel Record (Biochemistry)

Mitchell E. Robinson (Biochemistry)

3. **Visiting scientists**

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Yen-Wan Hung (Radiopharmaceuticals) Livingstone College, Salisbury, North Carolina

Velma Richardson (Cytogenetics) Tuskegee University, Tuskegee, Alabama

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William V. Elbert (Radiopharmaceuticals) Clemson University, Clemson, South Carolina

Joseph S. Kalinowski (Biochemistry) Penn State University, State College, Pennsylvania

Phylissa S. Miller (Epidemiology) University of Tennessee, Knoxville

5. **High school vocational education students**

Vicki Brown (Biochemistry) Anderson County High School

Wendy Packan (Radiopharmaceuticals) Oak Ridge High School

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* Thesis research being completed at the division.
†Portion of thesis research being done at the division.

1028702

non-doe funded research

W. Burr
Biomedical research support grant. National Institutes of Health, Division of Research Resources, $13,036* (1 April 1984 to 31 March 1985; current year of continuing award)

W. Burr
Evaluation of devices and techniques to determine previous radiation exposure. National Cancer Institute, $261,220* (2 September 1985 through 1 September 1986)

N. Clapp
Operation of a marmoset colony for cancer research. National Cancer Institute, $260,000*‡ (1 June 1985 to 31 May 1986; first year of 3-year award)

N. Clapp
Support of marmoset colony. National Foundation for Ileitis and Colitis, $25,000‡§ (1 July 1985 to 30 June 1986; first year of 3-year award)

N. Clapp
Collaborative studies of colitis and colon cancer in tamarins. Harbor-UCLA School of Medicine, $47,970‡§ (1 September 1985 to 31 August 1986; first year of 5-year award)

J. Crook
A 14-week double-blind placebo controlled study of long acting nifedipine in essential hypertension. Pfizer Pharmaceuticals, $61,261* (1 July 1984 to 31 July 1985)

J. Crook
Comparative study of the effects of nitrendipine and hydrochlorthiazide in hypertensive patients. Miles Pharmaceuticals, $51,177* (1 December 1984 to 31 August 1985)

G. Eisele
Actinide metabolism studies. Nevada Operations Office/U. S. Department of Energy,‡ $147,000* (1 October 1985 to 30 September 1986; eighth year of 8-year award)

G. Eisele
Food chain transport of synfuels. U. S. Environmental Protection Agency-U. S. Department of Energy Interagency Agreement, $67,674* (1 October 1984 to 30 September 1986; current year of continuing award)

L. G. Littlefield
Studies of radiation-induced chromosome damage in humans. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $193,757* (9 September 1985 to 29 September 1986; current year of continuing award)

A. McFee
Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $274,158* (30 September 1985 to 29 September 1986; second year of 2-year award)

M. Record
Topography of membrane-bound enzymes, precursors, and products related to platelet activating factor (PAF) in tumor cells with high metastatic capacity. Fogarty International Center (U.S. Public Health Service), $25,500† (1 September 1985 to 31 August 1986)

M. Record
Topographical mapping of ether-linked lipids and related enzymes in tumors and other cells. World Health Organization (International Agency for Research on Cancer), $21,410† (1 October 1984 to 31 August 1985)

R. Ricks
Development and implementation of radiological training courses for health physics and medical personnel. Federal Emergency Management Agency, $124,370* (30 September 1985 through September 20, 1986)

R. Ricks
Reactor status considerations in emergency decision making. Federal Emergency Management Agency, $98,000* (1 June 1985 through September 30, 1986)

F. Snyder
Ether lipids in cancer: enzyme mechanisms and inhibitors. National Cancer Institute, $110,527† (30 September 1985 to 29 September 1986; a 1-year award)

F. Snyder
Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells. American Cancer Society, $92,584* (1 January 1985 to 31 December 1985; second year of 2-year award)

F. Snyder
A new class of biologically active phospholipids. National Heart, Lung, and Blood Institute, $71,818† (1 April 1985 to 31 March 1986; fifth year of 5-year award)

S. Tardif
Determinants of reproductive competence in tamarins. National Institutes of Health, Division of Research Resources, $42,745† (5 September 1985 to 4 September 1986; second year of 3-year award)

L. C. Washburn
Radiolabeled monoclonal antibodies for cancer therapy. National Cancer Institute, $84,518† (1 September 1985 to 31 August 1986; first year of 3-year award)

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*Total costs, i.e., direct plus overhead (current year)
†Direct costs (current year)
‡Not under DOE prime contract
§These collaborative awards are reimbursable to the NCI contract

1028703

N. K. Clapp

Genetic epidemiology and immunologic parameters in tamarin colitis and colon cancer. Harbor-UCLA School of Medicine (Los Angeles), Department of Medicine, G. Petersen; Division of Gastroenterology, W. Snape, S. Targan.

Fecal bile acid analysis in marmosets. Beth Israel Medical Center (New York), Lipid Research Laboratory, B. Cohen.

In vitro tetraploidy in skin fibroblasts of colon cancer tamarins. Cornell Medical College (New York), Laboratory of Cell Biology, B. S. Danes.

Genetic segregation and familial cancer relationships in colon cancer tamarins. Creighton University (Omaha), Preventive Medicine and Public Health, W. Kimberling and H. Lynch.

Coronaviruses in colitis and colon cancer. University of Tennessee (Knoxville), College of Veterinary Medicine, D. Brian.

Ontogenetic skeletal changes in *Saguinus fuscicollis* and *Saguinus oedipus*. University of Tennessee (Knoxville), Department of Anthropology, W. Bass.

Carcinoembryonic antigens in colon tumors. Harvard Medical School (Boston), Mallory Gastroenterology Laboratory, P. Thomas.

Lectin binding in colitis and colon cancer in cotton-top tamarins. VA Medical Center (Ann Arbor), Gastroenterology Section, C. R. Boland.

Culture of tamarin colon cancer cells. Baylor College of Medicine (Houston), Department of Pharmacology, M. Brattain; Massachusetts General Hospital (Boston), Gastrointestinal Department, D. Podolsky

Fecal toxins in tamarin colon disease. University of Tennessee Memorial Research Center (Knoxville), J. Fuhr.

Idiopathic ulcerative colitis in marmosets. University of Nevada, School of Medicine (Reno), O. Peck; The Ohio State University (Columbus), Physiology Department, J. Wood.

In vitro culture of tamarin colonic tumors. Oak Ridge National Laboratory, Biology Division, E. Perkins.

CEA and LDH_k in colon-cancer bearing tamarins. Roswell Park Memorial Institute (Buffalo), Surgical Developmental Oncology Department, N. Petrelli.

Heavy metal toxicity in tamarins. University of Tennessee (Knoxville), School of Veterinary Medicine, W. Farkas.

Effect of human GM-CSF in marmosets. UCLA School of Medicine (Los Angeles), Division of Hematology/Oncology, D. Golde.

Response of marmosets to pulmonary toxicity. Oak Ridge National Laboratory, Biology Division, F. Tryka, H. Witschi.

J. E. Crook

Production of positron-emitting radionuclides using the Tandem van de Graaff accelerator. Oak Ridge National Laboratory, Physics Division, P. D. Miller, Jr.

Development of software for quantitative positron tomography. The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, G. Smith; Vanderbilt University (Nashville), Department of Biomedical Engineering, P. H. King.

^{11}C-Amino acids/positron ECT for *pancreatic studies*. Brookhaven National Laboratory, Medical Department, A. B. Brill.

Clinical investigation of newer antihypertensive agents. The University of Tennessee Center for the Health Sciences (Knoxville), Department of Medicine, A. Beasley, and Department of Family Practice, G. Shocklett.

Synthesis of labeled compounds using organoborane techniques. University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka.

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp.

Use of radionuclides in animal models of human disease. The University of Tennessee Center for the Health Sciences (Knoxville), Department of Radiology, K. F. Hübner.

R. J. DuFrain

Species comparisons in cytogenetic assays. Oak Ridge National Laboratory, Biology Division, R. J. Preston.

Statistical analysis and Poisson regression studies in biological dosimetry. Oak Ridge National Laboratory, Computer Science Division, E. L. Frome.

S. A. Fry

Morbidity among workers exposed to mercury at the Oak Ridge Y-12 plant. University of Michigan (Ann Arbor), Department of Environmental and Industrial Health, L. Fine.

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Radiation Epidemiology Branch (Bethesda), S. Machado and R. Kleinerman.

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons. Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straume, A. Carrano, and J. Minkler; Los Alamos National Laboratory (Los Alamos), L. Devan; Radiation Effects Research Foundation (Hiroshima), A. Awa.

Coordinated research program on Routine Use of Chromosomal Aberration Analysis in Occupationally Exposed Radiation Workers. International Atomic Energy Agency (Vienna), A. Bianco.

Cytogenetic follow-up studies of a survivor exposed to ^{241}Am. Hanford Environmental Health Foundation (Washington), B. D. Breitenstein.

Human sperm cytogenetics (assessment of radiation-induced chromosomal aberrations after accidental exposure). Lawrence Livermore National Laboratory, B. Brandriff.

1028704

The effect of methyl acetimidate on cellular proliferation and chromosome structure. Tuskegee University (Alabama), Department of Biology, V. Richardson.

C. C. Lushbaugh

United States Transuranium Registry. Hanford Environmental Health Foundation, B. D. Breitenstein.

C. C. Lushbaugh and N. K. Clapp

Marmoset T and B cellular immunology. Meharry Medical College (Nashville), Department of Microbiology, P. Patel.

C. C. Lushbaugh and S. A. Fry

Epidemiology of human carcinogenesis. University of Alabama (Birmingham), Department of Epidemiology, W. D. Flanders.

Comprehensive epidemiology of DOE atomic workers. University of North Carolina (Chapel Hill), Department of Epidemiology, C. M. Shy.

Study of mortality among former Manhattan Engineer District sites at Linde Corporation and the Electro Metallurgical Laboratory. Union Carbide Corporation, Corporate Epidemiology Department, (Danbury, Connecticut), S. G. Austin and T. A. Lincoln.

C. C. Lushbaugh and L. C. Washburn

Diethylenetriaminepentaacetate (DTPA) project. Department of Energy contractors, 44 coinvestigators.

A. F. McFee

In vivo mouse assay for chemically induced SCE and chromosomal aberrations. Brookhaven National Laboratory, R. Tice.

F. Snyder

Biological effects of unique phospholipid analogs. California State University (Northridge), Department of Chemistry, J. Hajdu.

Mass spectrometry of unique lipids. Oak Ridge National Laboratory, Analytical Chemistry Division, G. L. Glish.

Nuclear magnetic resonance studies of unique lipids. Oak Ridge National Laboratory, Chemistry Division, B. M. Benjamin.

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Piantadosi.

Effects of a new group of antihypertensive phospholipids on cardiovascular function. University of Tennessee (Knoxville), Department of Psychology, J. E. Lawler.

The role of intracellular transfer proteins in the cellular movement of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (a hypotensive and platelet aggregating phospholipid). Western Carolina University (Cullowhee), Department of Biology, R. H. Lumb.

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein.

Selective cytotoxic activity of alkyllysophospholipids in tumor cells. Technische Universitat Munchen (West Germany), Division of Oncology, W. E. Berdel.

Pulmonary properties of an antihypertensive phospholipid. University of British Columbia (Vancouver), Pulmonary Research Laboratory, R. R. Schellenberg.

S. D. Tardif

Reproductive aging in tamarin females. University of Wisconsin (Madison), Department of Psychology, C. Snowdon and T. Ziegler.

Demographic analyses and computerized colony management. Southwest Foundation for Biomedical Research (San Antonio), Department of Genetics, B. Dyke and T. Gage.

Skeletal age changes in tamarins. Southwest Texas State University (San Marcos), Department of Sociology and Anthropology, D. Glassman.

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. The Wistar Institute (Philadelphia), Z. S. Steplewski.

Production of ^{11}C- and ^{18}F-labeled radiopharmaceuticals. Brookhaven National Laboratory, Chemistry Department, J. S. Fowler.

Short-lived alpha-emitting radionuclides for labeling monoclonal antibodies. Oak Ridge National Laboratory, Chemical Technology Division, J. E. Bigelow.

1028705

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1028706

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1028707

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1028708

1985/1986

1028709

Oak Ridge Associated Universities

Medical and Health Sciences Division Research Report 1985/1986







1028710

The Medical and Health Sciences Division conducts research on biomedical problems involving the respiratory, vascular, pancreatic, and gastrointestinal systems. Emphasis is on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Research programs in the division are focused on the following areas: (a) preclinical and clinical development of new positron radiopharmaceutical agents for in vivo diagnosis of diseases in humans, (b) biochemical aspects of cellular lipid mediators and membrane lipids in disease processes, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), (e) food chain transport studies of synfuels and actinides, and (f) interdisciplinary investigations of the marmoset as a primate model for spontaneous colon cancer and other human diseases, particularly those associated with energy-related toxicants. Predoctoral and postdoctoral training is provided in each research program area. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a treatment and decontamination facility and a demonstration and training site.

Oak Ridge Associated Universities is a private, not-for-profit association of 49 colleges and universities. It is a managing and operating contractor of the U.S. Department of Energy, conducting research and educational programs in the areas of energy, health, and the environment for DOE, ORAU's member institutions, other colleges and universities, and other private and governmental organizations.

ORAU-281, April 1987

This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

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Printed in the United States of America
Available from
National Technical Information Service
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1028711

Oak Ridge Associated Universities

Medical and Health Sciences Division Research Report 1985/1986

1028712

Highlights

In lipid biochemistry studies, MHSD researchers characterized the complete enzymatic reaction steps for the de novo synthesis of "platelet activating factor" (PAF), a unique class of phospholipids that possesses biological properties implicated in a variety of diseases. Whereas a remodeling pathway for the synthesis of PAF is reponsible for increased PAF in cells exposed to pharmacological stimuli, the de novo route appears to be the physiological source of [illegible]

In a study of the phospholipids that make up cell membranes, the biochemists observed that phospholipid species with two polyunsaturated chains incorporate arachidonic acid at a rate much greater than other such species, indicating that they have a specific functional role in membranes.



MHSD nuclear medicine specialists have successfully radiolabeled three monoclonal antibodies that may be useful for cancer diagnosis and therapy. Plans call for using these monoclonal antibodies in studies with tumor-bearing mice to assess the dose required for future radioimmunotherapeutic trials in cancer patients.

The nuclear medicine group also demonstrated early uptake of carbon-14 labeled (alpha)-amino acids by pheochromocytomas, hypertension-producing tumors of the adrenal medulla. The ability to locate the tumors makes surgical removal possible.

In studies to define mechanisms involved in chromosome aberration induction, MHSD cytogeneticists determined that a radioprotective chemical that scavenges hydroxyl radicals is highly effective in reducing the amount of chromosome breakage observed following exposure of human cells to X-radiation.



In collaboration with the Clinical Nuclear Medicine Program, cytogenetics staff members successfully established in tissue culture an apparent continuous cell-line derived from a rat pheochromocytoma tumor. These cells will be used in in vitro studies designed to parallel ongoing evaluations in tumor-bearing rats.



As part of the Health and Mortality Study of DOE Workers, researchers at the Center for Epidemiologic Research completed studies of the mortality of workers at the Oak Ridge Y-12 and Savannah River Plants and at the former uranium processing site at Linde Corporation. They also completed a case-control study of the occurrence of cancer of the brain and central nervous system at the Oak Ridge Y-12 Plant and Oak Ridge National Laboratory.

1028713

At the request of the White House Office of Science and Technology Policy and the Office of Management and Budget, MHSD staff that support the Committee on Interagency Radiation Research and Policy Coordination facilitated the review by member agencies of the EPA draft for "Radiation Protection Guidance to Federal Agencies for Occupational Exposure" that if approved will replace guidelines established in 1960. The CIRRPC staff produced three other reports on federal radiation issues, ra[illegible] protection and health effects, and a[illegible] participation in international radiation activities.



In studies of the development of colon cancer in cotton-top tamarins as a model of human carcinogenesis, researchers at the Marmoset Research Center identified oncodevelopmental mucins in both colitis and colon cancer in [illegible]rins that are similar to those fo[illegible] in humans with the same diseases.

In its training courses, REAC/TS provided instruction on medical management of radiation accidents to 178 people and initiated a new course, "Basic Concepts of Radiation for Attorneys," that was attended by 71 attorneys.



At the request of the U.S. State Department, REAC/TS staff reported to U.S. embassies in Moscow, Warsaw, and Bucharest following the Chernobyl nuclear reactor accident to provide consultation to the embassy staffs. The staff also assisted in the evaluation of people suspected of having uranium contamination following an accident in Gore, Oklahoma.

A new program was initiated to provide technical assistance to the medical director of the DOE Contractor Occupational Medicine Program of the Office of Environment, Safety, and Health. MHSD's new Occupational Medicine Program will provide expertise for biennial assessments of all DOE contractor occupational medical departments and will provide assistance on a variety of occupational medical problems.

A Center of Excellence for studies of human reliability programs was established by DOE in MHSD. In the area of alcohol and drug abuse, the center has assembled an extensive data base of existing literature. The center will develop psychologic assessment methodology, provide technical assistance, and design a training program to assist DOE in developing a human reliability program.



1028714

Preface

The unifying feature of research programs at the Medical and Health Sciences Division is their strong orientation toward solving problems related to human diseases. Cancer and radiation research represent the major emphasis, but some projects also cover areas that contribute to our understanding of respiratory, cardiovascular, inflammatory, and gastrointestinal disorders.

Both the cover and photographs of the research report for this year highlight several ongoing collaborative p s between different research units of our division. Details of these collaborative efforts are discussed in the section of this booklet that describes the scopes of current research programs. Not all levels of interactions among our diverse programs or those from earlier years are depicted, but we believe the specific projects illustrated in this report typify the cooperative spirit among division scientists and exemplify the interdisciplinary nature of our research efforts. The continuing strong financial support from DOE and other agencies clearly reflects the scientific strengths of the multifaceted research programs that make up the Medical and Health Sciences Division.

W. W. Burr, Jr.
Chairman

Fred Snyder
A iate Chairman

Contents

Scope of Research Programs 1

- **Biochemistry** 1
- **Preclinical and Clinical Nuclear Medicine** 4
- **Cytogenetics** 7
- **Large Animal Radiobiology** 10
- **Marmoset Research** 11
- **Radiation Emergency Assistance Center/Training Site** 12
- **Epidemiology** 14
- **Committee on Interagency Radiation Research and Policy Coordination** 16
- **Occupational Medicine** 17

Publications 18

- **Full-length Journal Articles** 18
- **Chapters, Review Articles, and Other Publications** 19
- **Published Abstracts of Papers Presented at Meetings** 21
- **Miscellaneous Papers or Talks Presented at National and International Scientific Conferences** 22

Summaries of Published Papers 24

Major Awards, Honors, and Appointments 28

Training Programs 29

Non-DOE-Funded Research 31

Collaborative Research 32

Medical and Health Sciences Division Staff 34

Administration and Committees 36

1028715



As part of the REAC/TS project to coordinate national use of DTPA, the Biochemistry Program and the Preclinical Nuclear Medicine Program collaborate in research on new, highly lipophilic chelating agents.

Scope of Research Programs

Biochemistry

Program Director: Fred Snyder

The Biochemistry Program consists of three major areas of research: (1) cellular phospholipid mediators that include platelet activating factor (PAF) and related ether-linked acetylated glycerolipids, (2) selective antitumor ether-linked phospholipid analogs, and (3) the role of phospholipids in cell membranes.

The focus of this program is on the metabolism, regulation, and function of the ether-linked and diacyl types of glycerolipids in mammalian cells. Emphasis is on studies of the mode of action of the biologically active phospholipids and the mechanisms of the reactions catalyzed by enzymes involved in their biosynthesis and catabolism. The approach ranges from molecular to in vivo studies; enzyme preparations, subcellular organelles, membrane vesicles, cultured cells, and animal models are utilized in these investigations.

Practical aims of this program are to establish the role of phospholipids and other glycerolipids in health and in the pathogenesis of cancer, hypertension, inflammation, and respiratory diseases.

Cellular Phospholipid Mediators

The chemical structure of a tissue factor that possessed hypotensive and platelet aggregating activities was elucidated as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine by our laboratory and

1028716

two other independent groups of investigators in the fall of 1979. This unique
of phospholipids (generally
.red to as "platelet activating factor," or "PAF") possesses multifunctional biological properties with far-reaching effects that are implicated in a variety of diseases such as inflammation, allergies, hypertension, shock, and asthma. It is also thought that the PAF type mediators are required for normal physiological functions at the cellular level.

Soon after the structural studies that identified PAF were published, the enzymatic steps (Fig. 1, Rxs VI, VII, and XI) were described by our laboratory for the biosynthesis of PAF via an acetyltransferase (remodeling route) or a dithiothreitol (DTT)-insensitive cholinephosphotransferase (de novo pathway) and for the inactivation of PAF via an acetylhydrolase. During the past year we characterized the complete enzymatic reaction steps for the de novo synthesis of PAF.

Only two pathways are known to exist for the biosynthesis of PAF. The most extensively investigated reactions in PAF biosynthesis have been those associated with the remodeling pathway that converts alkylacylglycero-phosphocholines, via a lyso intermediate, to PAF (Fig. 1; Rxs V and VI) through the combined actions of a phospholipase A_2 and an acetyltransferase, since it is this route that forms PAF in inflammatory and allergic responses. For example, acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase is stimulated three- to tenfold by different stimuli (e.g., *ionophore* A23187, zymosan, thrombin) in a variety of cells.

The de novo route for PAF biosynthesis involves the conversion of 1-alkyl-2-lyso-*sn*-glycero-3-P to PAF through a stepwise reaction sequence of acetylation (Fig. 1; Rx IX), dephosphorylation (Fig. 1; Rx X), and the transfer of phosphocholine from CDP-choline to 1-alkyl-2-acetyl-*sn*-glycerols (Fig. 1; Rx XI) by a DTT-insensitive cholinephosphotransferase. The last step in the de novo pathway for PAF biosynthesis does not appear to be affected by agents that stimulate the enzymes in the remodeling route. These findings indicate the remodeling pathway is solely

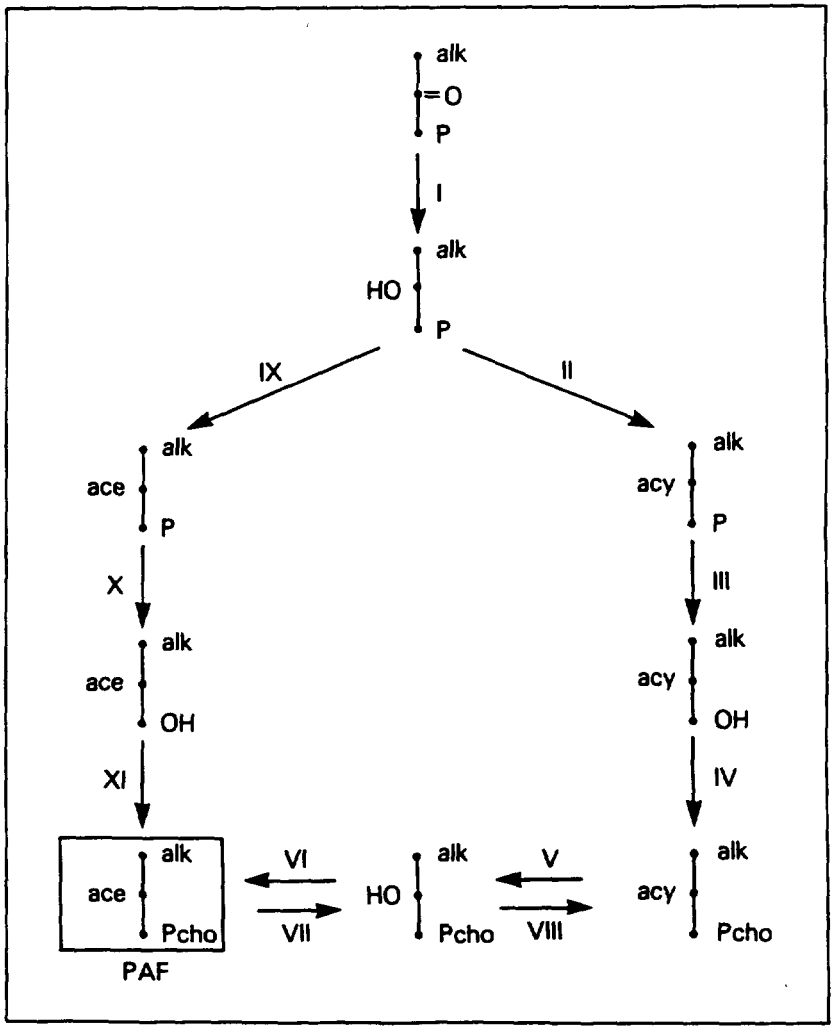


Figure 1. Two different pathways for the biosynthesis of PAF. The de novo pathway, which appears to be the physiological route
'e biosynthesis of the PAF mediator,
/es Reactions IX, X, and XI. The remodeling pathway ("the PAF cycle") for PAF biosynthesis is thought to be the major route involved in inflammatory reactions (Reactions V and VI). The specific reaction steps indicated by Roman numerals are catalyzed by the following enzymes: I. NADPH:alkyldihydroxyacetone-P oxidoreductase (EC 1.1.1.100); II. acyl-CoA:1-akyl-2-lyso-*sn*-glycero-3-P acyltransferase (EC 2.3.1.63); III. 1-alkyl-2-acyl-*sn*-glycero-P phosphohydrolase; IV. 1-alkyl-2-acyl-*sn*-glycerol:CDP-choline cholinephosphotransferase (EC 2.7.8.2); V. phospholipase A_2; VI. acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine acetyltransferase (EC 2.3.1.67); VII. 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase (EC 3.1.1.48); VIII. donor phospholipid (20:4):1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine transacylase; IX. acetyl-CoA:1-alkyl-2-lyso-*sn*-glycero-3-P acetyltransferase; X. 1-alkyl-2-acetyl-*sn*-glycero-3-P phosphohydrolase; and XI. CDP-choline:1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase (EC 2.7.8.16). Symbols used in this illustration include alk for alkyl, acy for acyl, ace for acetyl, and cho for choline.

1028717

responsible for the increased biosynthesis of PAF in cells exposed to pharmacological stimuli (e.g., inflammatory, a[illegible]; and anaphylactic reactions), whereas the de novo route serves as the physiological source of PAF.

Our most recent results have demonstrated that the acetyltransferase (Fig. 1; Rx IX) and cholinephosphotransferase (Fig. 1; Rx XI) involved in de novo synthesis of PAF are distinctly separate enzyme activities from the acetyltransferase (Fig. 1; Rx VI) and cholinephosphotransferase (Fig. 1; Rx IV) in the remodeling pathway for PAF synthesis. These conclusions have been reached on the basis of studies of temperature sensitivities, substrate specificities, responses of the enzyme activities to a variety of agents (e.g., thiol groups, detergents, ethanol), and differences in the tissue distribution of these enzyme activities. Our results demonstrate that many of the enzymes involved in PAF metabolism are highly specific and therefore are potentially good candidates f[illegible] development of inhibitors and an[illegible]odies that could be useful probes for studies of PAF regulation and/or the diagnosis and treatment of PAF-related diseases.

Selective Antitumor Ether-Linked Phospholipid Analogs

Several unnatural alkyl phospholipids have been shown to be highly cytotoxic toward certain neoplastic cells when compared to normal cells. The chemical structure of this type of antineoplastic phospholipid is closely related to the group of novel bioactive phospholipid cellular mediators known as PAF.

Two unnatural alkyl phospholipid analogs that have elicited the most potent selective cytotoxic responses in tumor cells are octadecyl-2-acetamide-glycerophosphocholine and hexadecyl-2-methoxy-glycerophosphocholine. N[illegible]e PAF, on the other hand, p[illegible]ses relatively little cellular toxicity. Structural features required for the selective antineoplastic activity of the unnatural phospholipids are (a) an ether-linked alkyl moiety at the 1-position, (b) an apparently nonmetabolizable group at the 2-position, and (c) a quaternary phosphobase substituent at the 3-position of the *sn*-glycerol moiety.

Our current findings indicate that the low alkyl cleavage enzyme activity found in tumor cells does not explain the antineoplastic action of the methoxy or acetamide phospholipid analogs. To begin with, the alkyl cleavage enzyme has been shown to have a low activity in normal human neutrophils and Madin-Darby canine kidney cells, as well as HL-60 leukemic cells, even though they respond differently to the cytotoxic effects of the antitumor phospholipid analogs. In addition, octadecyl-2-methoxy-glycerophosphocholine is not a substrate for the cleavage enzyme, and morphological data indicate that only a relatively small amount of the cytotoxic phospholipid penetrates the interior of cells where the alkyl cleavage enzyme is located.

A particularly interesting observation has been that the human leukemic cells, which are highly sensitive to the cytotoxic analogs, show a preferential sequestering of the antineoplastic methoxy analog where it appears to be concentrated at the cell surface. Through analyses of subcellular membrane fractions isolated by ultracentrifugation in a Percoll gradient, we have also recently documented that tritiated octadecyl-2-methoxy-glycerophosphocholine was located in the plasma membrane of HL-60 cells. Enrichment of the methoxy phospholipid analog in the surface membranes of the cell could result in the inhibition of vital cell functions that could lead to cell death by affecting lipid metabolic enzymes involved in the assembly of membranes, protein kinase activities, and/or the inhibition of the transport of required nutrients across the plasma membrane.

The Role of Phospholipids in Cell Membranes

Phospholipids that comprise cell membranes represent a heterogeneous population of molecular species, each being defined by the chemical nature of the polar head group, aliphatic linkages (ester versus ethers), and the type of aliphatic chains (chain length and unsaturation) present. Precise control of membrane lipid composition appears to be critically important for cellular function since profound morphological and biochemical changes can occur in cells even after only small modifications in the content of specific phospholipids.

Last year we reported the presence and high turnover of 18:2-20:4 and 20:4-20:4 molecular species of diacylglycerophosphocholines and diacylglycerophosphoethanolamines in rat testes. Recent experiments have also documented the presence of unusual polyunsaturated molecular species in the phospholipids of rat erythrocyte membranes. These unique molecular structures of phospholipids in erythrocytes consist of arachidonic acid paired with linoleic, docosahexaenoic, or arachidonic acids. Of particular interest is our observation that the phospholipid species with two polyunsaturated chains in rat erythrocytes incorporate arachidonic acid at a rate much greater than those of other arachidonic acid-containing species. The apparent high metabolic activity of these unique phospholipids with two polyunsaturated moieties suggests that they have a specific functional role in membranes.

Ether-linked lipids are important

We are attempting to explain the activity of several unnatural alkyl phospholipids that have been shown to be highly cytotoxic toward certain neoplastic cells, such as cancerous tumor cells.

1028718

constituents of most cells but their levels can vary greatly according to cell types and classes. Both the alkyl and alk-1-enyl ether groupings exist primarily as analogs of phosphatidylcholine, phosphatidylethanolamine, and triacylglycerols. The ether-linked phospholipids are associated with membranes and it is known that they can influence membrane fluidity. We have been especially interested in the specificity of enzymatic acylation of lyso ether-linked phospholipids since the fatty acid inserted at the *sn*-2 position can play an important role in the various enzymatic functional activities associated with membranes, such as the desaturase involved in plasmalogen biosynthesis.

In experiments with both intact Madin-Darby canine kidney cells and cell-free membrane preparations from these cells, we were able to show that 1-[^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphoethanolamine is preferentially acylated with polyunsaturated fatty a[illegible] the order 20:4>20:5>20:3>22:6. Our data suggest that this type of acylation occurs in the absence of cofactors and probably proceeds by a CoA-independent transacylation mechanism that involves diacyl phospholipids as the donor of the polyunsaturated acyl moiety.

Such a pathway has been described earlier by us and others for the selective transfer of polyunsaturated acyl groups (especially arachidonic acid) to lyso-phospholipid acceptor molecules. Desaturation of the alkyl chain to an alk-1-enyl group (plasmalogens) also exhibits specificity, with the desaturase having preference for the 16:0–22:6 species. Thus, our results from this investigation indicate that there is a high selectivity in the final acylation and desaturation steps of alkyl lipids in the biosynthetic pathway for plasmalogens, a phospholipid that is a significant constituent of [illegible]thanolamine phospholipid fraction [illegible]any cells.

Preclinical and Clinical Nuclear Medicine

Program Director: James Crook

The major objectives of these related research programs are to develop new radiopharmaceutical agents and to carry out phase I and II testing of such materials in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of potential agents for radiodiagnosis and radiotherapy, tests of these substances in animals for effectiveness and toxicity, and clinical efficacy trials in humans according to U.S. Food and Drug Administration rules and guidelines.

Monoclonal Antibodies

A major research effort is aimed at development of radiolabeled monoclonal antibodies for cancer diagnosis and therapy. The National Cancer Institute has funded a study entitled "Radiolabeled Monoclonal Antibodies for Cancer Therapy," which is currently in its second year of support. Monoclonal antibodies to tumor-associated antigens have the ability to specifically direct radionuclides and cytotoxic drugs to malignant sites.

Radiolabeled monoclonal antibodies have elicited great scientific interest in recent years. Monoclonal antibodies have been labeled with radionuclides of iodine and various gamma-, beta-, and alpha-emitting metal ion radionuclides. Although still often used, monoclonal antibodies labeled with radioiodine have an unfortunate tendency to deiodinate in vivo, thus limiting their usefulness.

Labeling monoclonal antibodies with metal ion radionuclides requires use of the bifunctional chelate technique, in which a chelating agent is covalently coupled under mild conditions to the monoclonal antibody molecule. Several bifunctional chelate techniques have been developed, and we are currently investigating three such methods. Two involve coupling the commonly used chelating agent, diethylenetriaminepentaacetic acid (DTPA), to free amino groups of monoclonal antibodies via an amide linkage using either the cyclic DTPA anhydride or an active DTPA ester of N-hydroxysuccinimide. The other entails coupling 1-(p-isothiocyanatobenzyl)DTPA to free amino groups of monoclonal antibodies by a thiourea linkage.

Currently two monoclonal antibodies with specificity for human colorectal carcinoma, CO17-1A and BR55-2, and a monoclonal antimelanoma antibody, ME-361, are under investigation. These monoclonal antibodies were all developed and supplied by our collaborator, Dr. Zenon Steplewski, at the Wistar Institute in Philadelphia, Pennsylvania. We have successfully radiolabeled these three DTPA-conjugated monoclonal antibodies with gamma-emitting indium-111, beta-emitting yttrium-90, and alpha-emitting einsteinium-253 and fermium-255. The tumor uptakes and general tissue distributions of the radiolabeled monoclonal antibodies in tumor-bearing nude mice still need to be optimized, but preliminary results have been encouraging. Plans are to use the optimized tissue distribution data to make calculations of the radiation dosimetry of the radiolabeled monoclonal antibodies, which will be used to assess the dose required for tumor growth/regression studies in tumor-bearing nude mice and for future radioimmunotherapeutic trials in cancer patients.

Current techniques for detection of spontaneous tamarin colorectal carcinoma are not adequately sensitive for consistent early diagnosis. Therefore, we are attempting, in collaboration with Dr. Neal Clapp of ORAU's Marmoset Research Center and Dr. Zenon Steplewski of the Wistar Institute, to develop a radiolabeled monoclonal antibody with sufficient specificity for this tumor to permit early diagnosis through scintigraphic imaging. Because monoclonal antibody BR55-2, which has specificity for human colorectal carcinoma, was shown by Dr. Steplewski to have cross reactivity toward the tamarin tumor, this monoclonal antibody was

1028719

radiolabeled with both indium-111 and iodine-131, and imaging studies were carried out in tamarins bearing spontaneous colorectal carcinomas. However, no definitive statement could be made regarding tumor localization of the radiolabeled antibody, apparently due to the weak affinity of BR55-2 for the tamarin tumor.

We now plan to use standard hybridoma production and screening techniques to develop a monoclonal antibody with specificity for spontaneous tamarin colorectal carcinoma, which will then be radiolabeled and tested to determine its efficacy for early diagnosis of the disease. A grant application was recently submitted to the National Cancer Institute to obtain support for this work. Development of an improved method for early diagnosis of spontaneous colorectal carcinoma would greatly increase the value of this nonhuman primate model as a tool for research into the etiology and therapy of human colorectal carcinoma.

Through collaboration with Dr. Lucas Lawless, a DOE Faculty Research Participant from Fordham University in New York, work has begun on a method for detection of cancer cells circulating in the peripheral blood of tamarins bearing spontaneous colorectal carcinomas. Tamarin peripheral lymphocytes, along with circulating cancer cells, were separated, washed, and incubated with the monoclonal antibody BR55-2. The cells were washed, incubated with fluorochrome-conjugated goat anti-mouse antibody, washed again, and examined using fluorescence microscopy. The results to date have been inconclusive, thought again to be largely due to the weak affinity of BR55-2 for the tamarin colorectal carcinoma. The development described above of a monoclonal antibody directed specifically against this tumor is expected to aid in this research.

Also in collaboration with Dr. Lawless we have extracted the immunoglobulin fraction, containing tumor-associated antibodies, from the serum of tamarins bearing spontaneous-tamarin colorectal carcinomas. This immunoglobulin mixture was then coupled with a fluorochrome and purified. The fluorochrome-labeled tumor-associated antibodies thus produced will be studied as a direct fluorescent stain for tamarin colorectal carcinoma cells.



The Preclinical and Clinical Nuclear Medicine Programs are attempting in collaboration with the Marmoset Research Center to develop a radiolabeled monoclonal antibody with sufficient specificity for tamarin colorectal carcinoma to permit early diagnosis through scintigraphic imaging.

In other studies we have obtained from Los Alamos National Laboratory a limited quantity of titanium-44 ($T_{1/2}$ = 47 yr), the parent of positron-emitting scandium-44 ($T_{1/2}$ = 3.9 hr). Work has been initiated on setting up

1028720

and testing a titanium-44/scandium-44 generator, which will make scandium- routinely available for a variety of potential radiopharmaceutical applications, including radiolabeling of monoclonal antibodies for positron emission tomographic radioimmunodiagnosis of cancer.

Positron Emission Tomography

Emphasis continues to be placed on the use of positron emission tomography (PET) to produce high-resolution, three-dimensional images as well as to act as a noninvasive probe of biochemical function. Thus, it can be used specifically to detect metabolic alterations associated with disease processes. The Clinical Nuclear Medicine Program uses PET to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications in cardiovascular and neoplastic diseases, with emphasis on a more quantitative evaluation of the in vivo distribution of such radiopharmaceuticals.

Through the work of Dr. Gary Smith, a consultant from the University of Tennessee Medical Center at Knoxville, and Michael Keefe, a DOE Graduate Student Research Participant, the facilities of the Clinical Nuclear Medicine Program have been improved through the addition and implementation of new software interactive programs for ORAU's ECAT II™ positron tomographic scanner. These new programs will improve quantitative imaging with resultant better interpretation of regions of interest, especially in the thorax.

Continuation of clinical studies with ^{11}C-labeled D- and L-tryptophan and D- and L-valine for pancreatic imaging and with ^{11}C-labeled 1-aminocyclobutanecarboxylic acid (ACBC) for tumor imaging, which have been carried out in recent years in collaboration with the Medical and Chemistry Departments of Brookhaven National Laboratory (BNL), has been held in abeyance due to the lack of suitable whole-body imaging instrumentation at BNL. But the prospects for reactivation of this research appear good because of two PET-related projects in the Oak Ridge-Knoxville area, both of which ORAU has supported.

(1) A small Oak Ridge-based corporation, ORBIT, Inc., headed by Dr. John Neiler, has obtained phase II support from the DOE's Small Business Innovation Research Program to construct a small, proton-only, low-energy (8 MeV) cyclotron and to test its utility as a source of clinically important radionuclides for PET (carbon-11, nitrogen-13, oxygen-15, and fluorine-18). It is expected this cyclotron will be placed in Oak Ridge, at least for the period of time required to test the machine, and that it may become available as a source of radionuclides for MHSD's Preclinical and Clinical Nuclear Medicine Programs.

(2) The University of Tennessee Medical Center at Knoxville's Department of Radiology has obtained funding for a Medical Radiologic Imaging Center, which is currently under construction. This center will include state-of-the-art equipment and expertise in PET, magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT), as well as such more common imaging modalities as ultrasound and X-ray computed tomography (CT). DOE has encouraged the formation of a "center of excellence," through which existing DOE-supported radiopharmaceutical/nuclear medicine programs at ORAU Medical and Health Sciences Division, ORNL Health and Safety Research Division, and the University of Tennessee Department of Chemistry would lend their support and expertise to the new imaging center to the extent that such collaboration would mutually benefit the participating institutions and the advancement of their research programs. The PET portion of the new center is expected to become operational in the spring of 1987.

In our ongoing PET research, we have shown with the ^{14}C-labeled compound that the unnatural, fluorine-containing amino acid DL-α-monofluoromethylalanine is taken up by tumor tissue with a specificity similar to that of ACBC. Labeling DL-α-monofluoromethylalanine with fluorine-18 would provide a tumor-imaging agent labeled with a positron emitter that has a longer half-life than carbon-11 (110 min vs 20.4 min). Through collaboration with Dr. John L. Hubbard, a DOE Faculty Research Participant from Marshall University in Huntington, West Virginia, we are attempting to develop a synthetic route to this ^{18}F-labeled amino acid.

Pheochromocytomas are hypertension-producing tumors of the adrenal medulla. Since surgical removal is possible, this represents a potentially curable form of hypertension. Thus, detection and early surgical treatment are desirable. The ^{14}C-labeled alicyclic α-amino acids, ACBC, 1-aminocyclopentanecarboxylic acid (ACPC), and 1-aminocyclohexanecarboxylic acid (ACHC), were administered to hypertensive New England Deaconess Hospital rats bearing subcutaneously implanted pheochromocytomas. Early uptake was demonstrated by the tumor for all three α-amino acids. However, unlike previous studies performed for assessment of tumor uptake, in which ACHC was the least effective amino acid, in this study ACHC showed greater affinity for the pheochromocytomas than ACPC.

A major research effort is aimed at development of radiolabeled monoclonal antibodies for cancer diagnosis and therapy.

1028721

Experiments relative to an apparent sex preference of the pheochromocytom ve continued. At present, work to est..sh the tumor in cell culture has progressed largely through our collaborative efforts with MHSD's Cytogenetics Program. The New England Deaconess Hospital rat model of hypertension also has afforded us an opportunity to continue to investigate the role of the red cell Na^+-K^+ cotransport system and how its function may be altered in a high catecholamine model of high blood pressure. Clinically pertinent investigations of the erythrocyte cotransport of Na^+ and K^+ are also ongoing. Recent clinical studies of the efficacy of two new calcium channel blockers in controlling mild to moderate hypertension, in collaboration with the University of Tennessee Medical Center at Knoxville, have been completed.

A patent for copper-64 citrate, a positron emitting compound, as a new myocardial imaging agent was applied for ba· ' on experimental work performed in .. A cooperative study with Computer Technology and Imaging, Inc. (CTI) of Knoxville has afforded the opportunity to compare canine myocardial images obtained using ORAU's ECAT II™ and CTI's state-of-the-art ECAT III™ and ECAT Model 931 instruments. Collaboration has also been initiated with Dr. R. E. Gompf of the University of Tennessee's College of Veterinary Medicine to study the role of copper-64 citrate in the diagnosis of mitral valve disease, a type of acquired heart disease in which the size of the left ventricle is increased. Plans to extend these observations to clinical studies are under way.

A clinical study using gallium-68 EDTA and PET to quantitatively assess changes in the blood-brain barrier (BBB) in patients with dementia was carried out in collaboration with Drs. Karl Hübner and Gary Smith of the U sity of Tennessee Medical Center at Knoxville. This study showed no differences between the BBB transfer constant or intra-vascular volume for various clinical types of dementia (Alzheimer's disease, multi-infarct dementia, and depressive dementia) and normal brain.

Through the work of Andrew Hinck, a DOE Undergraduate Student Research Participant, we initiated a project aimed at development of ^{68}Ga-labeled radiopharmaceuticals with potential for imaging regional brain perfusion. A series of N-alkyl derivatives of ethylenediaminetetraacetic acid (EDTA) was synthesized in which the alkyl group varied in lipophilicity. Such N-substituted EDTA derivatives have excellent chelating properties and produce neutral complexes with trivalent cations such as Ga^{+3}. Although animal studies still have to be carried out, it is possible, because of their neutrality, that complexes made from derivatives with highly lipophilic alkyl groups might cross the BBB and distribute within the brain in a manner consistent with use of the ^{68}Ga-labeled agent for PET imaging of regional brain perfusion.

Another research effort is directed toward the development of radiopharmaceuticals for PET studies of pulmonary function. One of the major metabolic activities of the lungs involves regulation of various biogenic amines. Lung endothelial cells sequester ^{11}C-labeled straight-chain primary aliphatic amines very selectively. However, the rapid metabolic loss of the label makes it difficult to accurately quantitate the pulmonary uptake. Our work in this area is currently focused on development of an ^{18}F-labeled ω-substituted primary aliphatic amine. The presence of a radiofluorine substituent in the ω-position should result in a much delayed loss of radiolabel compared to our previous work with amines labeled in the α-position with carbon-11. Therefore, we are attempting to develop a synthesis for ^{18}F-labeled 7-fluoroheptylamine and 12-fluorododecylamine.

Chelation Therapy

Chelation therapy with DTPA drugs is the method of choice for decorporation of internally deposited actinide contaminants. As part of MHSD's project to coordinate national use of DTPA (see REAC/TS section), the Preclinical Nuclear Medicine and Biochemistry Programs collaborate in research on new, highly lipophilic chelating agents. Current research involves a study in rats of the decorporation efficacy and pharmacological tolerance of calcium-DTPA encapsulated in a new type of liposome, called "large unilamellar vesicles (LUV)."

Cytogenetics

Program Director:
L. Gayle Littlefield

The Cytogenetics Program conducts basic research studies to define mechanisms involved in the induction, repair, and persistence of chromosome damage induced by radiation and DNA-damaging chemicals and applies cytogenetic techniques as a biological indicator of dose in suspected radiation overexposures. Experimental studies employing in vitro test systems, systematic evaluations in exposed populations, and studies in animals as surrogates for humans are used to define and quantify adverse cytogenetic consequences that might be expected to result from human exposure to genotoxic agents.

Studies in Exposed Persons

Studies continue in selected populations having recent or previous radiation exposures. In response to requests for assistance, during last year we provided cytogenetic dosimetry estimates for nine persons referred to REAC/TS. The analysis of radiation-induced chromosome aberrations in cultured lymphocytes from seven of these individuals provided evidence that their unexplained high film-badge readings were spurious or that a suspected overexposure did not result in their receiving a measureable whole-body dose. In two other cases which involved significant overexposures, cytogenetic dosimetry evaluations

1028722

verified physical estimates of their dose.

In collaboration with scientists at the ıtion Epidemiology Branch of the National Cancer Institute, we continued basic research studies aimed at determining the applicability of cytogenetic systems for dose estimation in persons having partial-body radiation exposure many years previously. During the five-year period during which this population study has been supported by an NCI-DOE Interagency Agreement, detailed cytogenetic evaluations have been completed in approximately 575 control and irradiated persons selected by NCI from various cohorts having medical exposures 20–40 years ago. This large cytogenetic data base now includes quantitative information on the frequencies of baseline and radiation-induced chromosome aberrations in over 115,000 metaphases from lymphocyte cultures from these subjects.

Previously we completed cytogenetic evaluations on 146 persons who received multiple fluoroscopic examinations during the course of treatment for diseases of the lung. Data have been encoded for statistical evaluations to determine whether the frequency of lymphocytes bearing radiation-induced chromosome aberrations can still be correlated with radiation dose in these persons having fractionated low-dose radiation to the thorax upwards of 30 years earlier. Similar cytogenetic evaluations are in progress in approximately 200 adults who received radiation therapy for enlarged thymus during infancy.

In Vitro Dose Response Studies

The Cytogenetics Program also continued participation in an International Cooperative Program on the Routine Use of Chromosome Aberration Analysis in Occupationally Exposed Radiation Workers, sponsored by the International Atomic Energy Agency (IAEA). In a recent collaborative study, scientists at the National Radiological Protection Board (NRPB) in Great Britain exposed human blood to various doses of X or gamma radiation under conditions designed to mimic acute whole-body or partial-body exposures. Replicate samples of irradiated blood were shipped by air to cytogenetics laboratories, including our own, in ten countries for lymphocyte culture, evaluation of radiation-induced chromosome aberrations, and estimation of radiation dose. Preliminary results and interlaboratory comparisons of data were presented and discussed at an IAEA meeting which was held at the University of Kyoto in Japan, and detailed statistical evaluations are currently in progress at the NRPB.

We continued basic research studies aimed at defining mechanisms involved in chromosome aberration induction in irradiated lymphocytes.

Mechanisms Involved in Aberration Induction

Basic research studies are under way to define mechanisms involved in chromosome aberration induction in irradiated human lymphocytes. It is well-known that ionizing radiation induces structural alterations in biologically important macromolecules, such as deoxyribonucleic acid (DNA), via both "direct" and "indirect" mechanisms. Direct effects are caused by prompt ionizations along the DNA molecule which may result in breakage of chemical bonds or other alterations of DNA structure or conformation. Similar adverse effects may be generated "indirectly" through the formation and subsequent interaction with DNA of chemically reactive intermediaries, called "free radicals."

In ongoing studies we are attempting to define the role of these highly reactive radical species in producing DNA lesions that are involved in the formation of chromosome aberrations. As one approach to this problem, we have evaluated radiation-induced chromosome aberrations in human lymphocytes exposed to radiation in the presence of a radioprotective chemical that is an effective scavenger of hydroxyl (OH) radicals.

In experiments completed to date we have observed that the frequency of radiation-induced chromosome aberrations varies inversely with concentration of scavenger added to culture medium, that "saturated" concentrations of scavenger in culture medium protect against the induction or expression of greater than 60% of the radiation-induced chromosome aberrations, and that similar levels of protection are observed when radiation is administered during different stages of the cell cycle. One interpretation of these experimental findings is that greater than 60% of the chromosome damage incurred following radiation exposure is mediated through the interaction of OH radicals with DNA.

Studies in Animal Models

This year we began new collaborative studies with staff scientists in the Clinical Nuclear Medicine Program. Research activities in this program currently include experimental studies in New England Deaconess Hospital rats, which develop spontaneous pheochromocytomas and which will accept pheochromocytoma tumor implants. We have recently been successful in establishing in tissue culture an apparent continuous cell-line from a biopsy of actively proliferating tumor cells excised from a male rat. These pheochromocytoma-derived cells, which to date have been passaged greater than 17 times, display epitheloid morphology, have an average doubling time of about 24–27 hr, and exhibit a hyperdiploid chromo-

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some complement. Studies are under way to determine whether the cells are tumorigenic in nude mice and to fully characterize the chromosome complement of the cell-line which will be used in a series of in vitro studies designed to parallel ongoing research studies in tumor-bearing rats.

We continue to use experimental animal models in studies designed to provide information on the basic sequelae of damage at the cellular and chromosomal levels. Mammalian cytogenetics also has useful applications for the study of chemical effects. Under the terms of a contract with the National Toxicology Program, we have been examining variables and evaluating the effectiveness of an in vivo cytogenetic system for assessing the genotoxicity of chemicals. A considerable battery of in vitro assays is available and has been used to measure the carcinogen/mutagen potency of chemicals. However, none of these have proven very predictive of a chemical's in vivo action. These, while other systems may be useful for preliminary screening purposes, the final definitive evaluation of a chemical needs to be conducted in an in vivo assay system; cytogenetic measurements in mouse bone marrow cells provide convenient endpoints for such assays (Fig. 2).

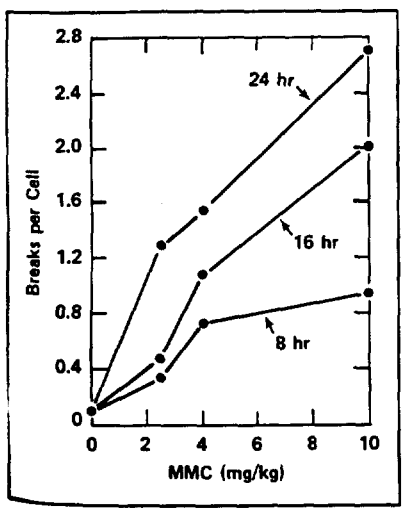
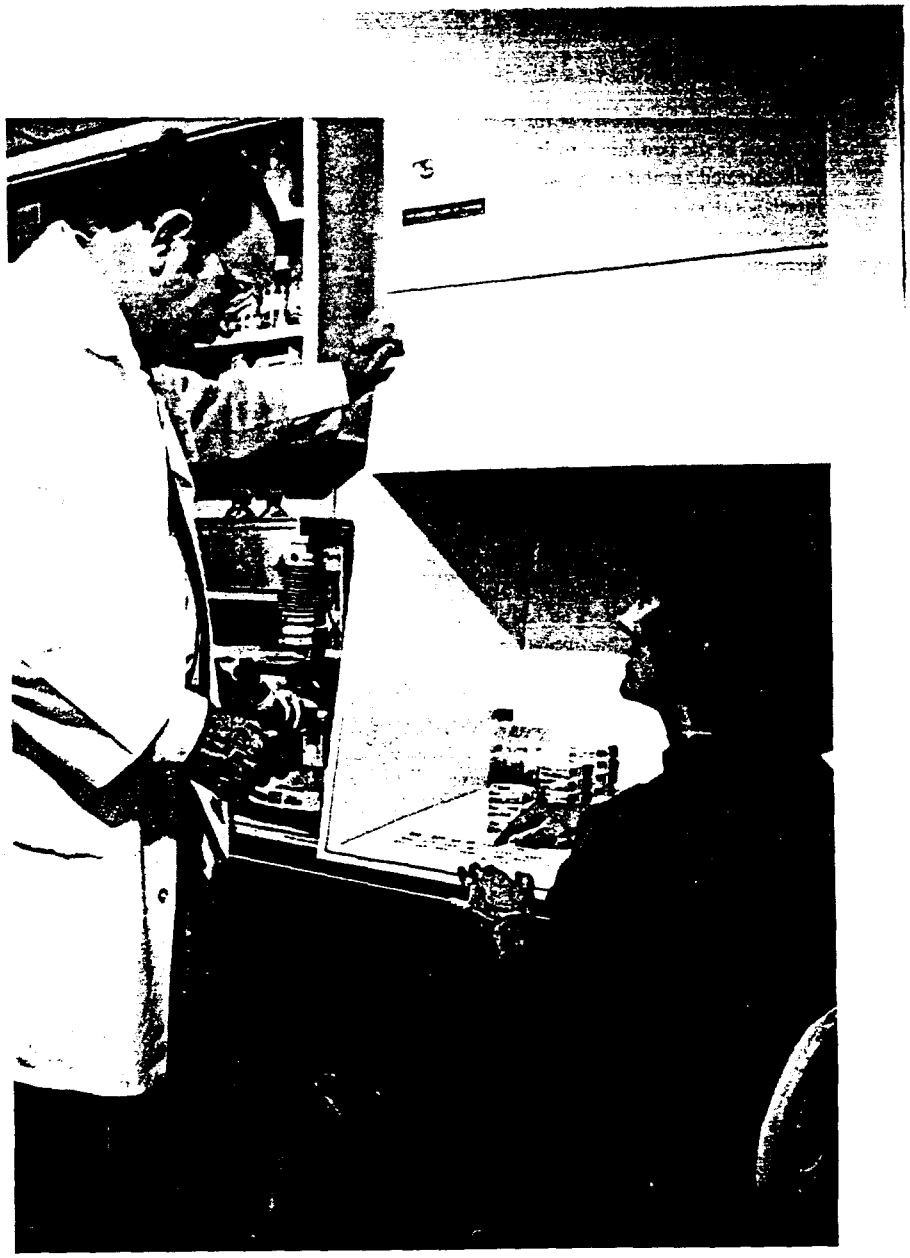


Figure 2. Mitomycin C induced aberrations in mouse bone marrow of mice killed at various posttreatment times, an illustration of the effect of sampling time as a variable in the mouse bone marrow cytogenetics assay. Sampling at times later than the commonly used 8 hours after treatment results in significantly increased sensitivity of the assay for detecting clastogenic chemicals.



This year the Cytogenetics Program began new collaborative studies with staff scientists in the Clinical Nuclear Medicine Program that include experimental work with New England Deaconess Hospital rats, which develop spontaneous pheochromocytomas and which accept pheochromocytoma tumor implants.

In a cooperative study conducted in conjunction with Brookhaven National Laboratory, we have identified a series of potential variables in the mouse in vivo cytogenetic assay procedure and have systematically evaluated their possible contribution to the variability inherent in the system. On the basis of these results we have developed a standardized protocol for evaluating the genotoxic effects of chemicals in the intact animal based on the induction of chromosome aberrations and sister chromatid exchanges, as well as on interference with cell division kinetics.

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The qualitative similarity of the results obtained in the two laboratories has demonstrated an ability to minimize interlaboratory variability, which is a major contributor to the uncertainty surrounding many quantitative tests.

While the primary emphasis of this program is currently on the testing of chemicals, we are continuing to investigate some of the areas dealing with basic factors influencing the system. We are presently acquiring a very large quantity of data on the mitotic index and average generation time among cells of both treated and control animals. These data will eventually permit us to evaluate the possible relationship between a chemical's effect on cell proliferation and its induction of chromosome aberrations and sister chromatid exchanges.

Large Animal Radiobiology

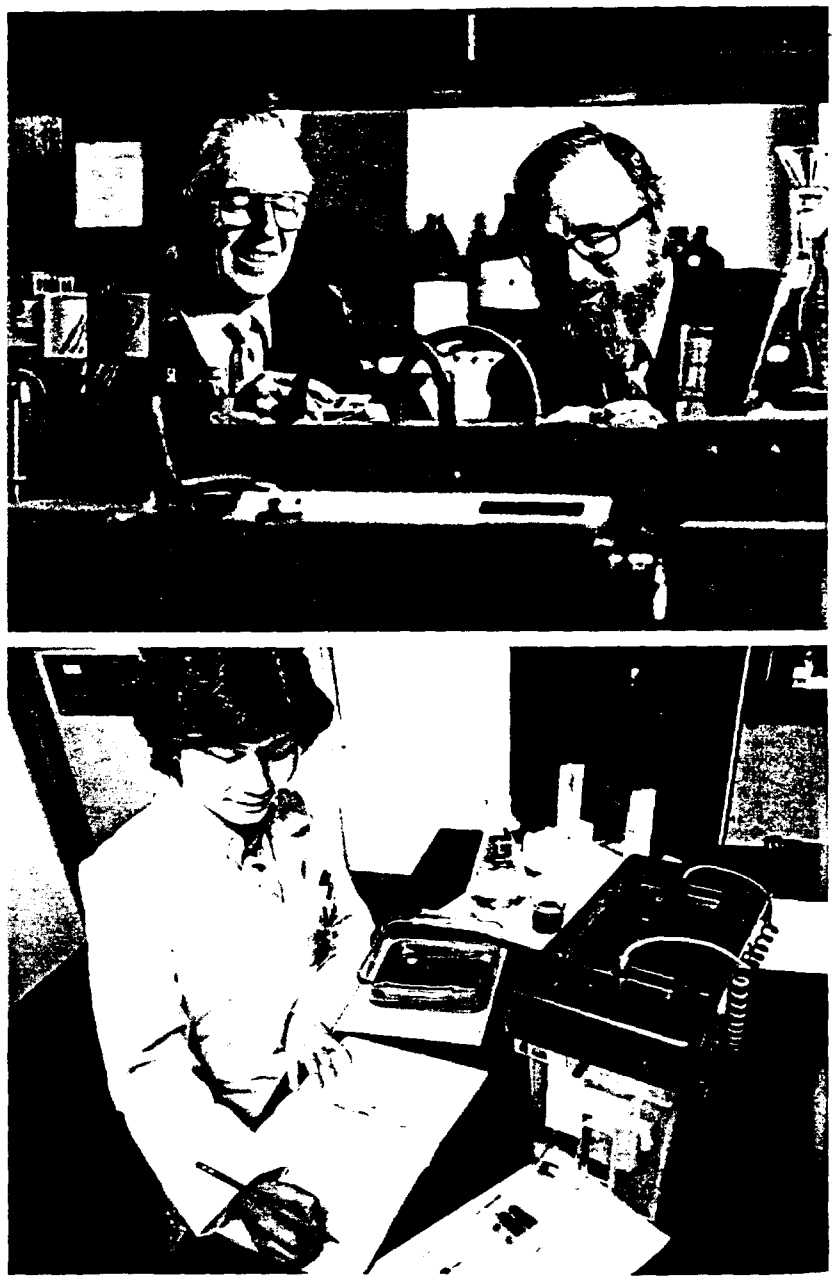
Program Director: G. R. Eisele

Measurement of strontium-90 in excreta has been used as an index of internal deposition for many years. However, the interpretation of urine bioassay results is an indirect technique that requires assumptions of the metabolism of the radionuclide.

A new program could result in a more accurate method of assessing the internal deposition of strontium-90, a radioactive nuclide.

A new research program funded by DOE through Battelle Northwest Laboratories will explore the direct measurement of ^{90}Sr in bone by detection of bremsstrahlung in vivo and could result in a more accurate method of assessment of the internal deposition of ^{90}Sr. The same procedures will be extended to neptunium with in vivo detection and quantification procedures.

This study is part of DOE's internal dosimetry evaluation and upgrade program. Oak Ridge National Laboratory will assist us with the bremsstrahlung in vivo evaluations. Collaboration is also expected with Lawrence Livermore National Laboratory and others, such as Argonne National Laboratory and Battelle. We anticipate this to be a three-year program.



The Marmoset and Preclinical Nuclear Medicine Programs collaborate in efforts to find a method for detecting cancer cells circulating in the peripheral blood of tamarins bearing spontaneous colorectal carcinomas and to use purified fluorochrome-labeled tumor-associated antibodies as a direct fluorescent stain for tamarin colorectal carcinoma cells.

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M· noset Research

Program Director: Neal K. Clapp

The Medical and Health Sciences Division continues under a National Cancer Institute contract to breed and raise tamarins and marmosets for cancer research. Our objectives are (1) to maintain a breeding colony of *Saguinus oedipus* and to produce a maximum number of healthy animals at minimum cost, (2) to provide facilities for experimental studies of oncogenic substances in tamarins, and (3) to provide professional and technical support for others to safely perform experimental protocols approved and requested by the National Cancer Institute. Additional marmoset colony support was obtained from the National Foundation for Ileitis and Colitis.

Proceedings of a marmoset workshop at ORAU, cosponsored by ORAU; N· National Institute for Arthritis, D.· ive Diseases, and Kidney; Division of Research Resources; and the National Foundation for Ileitis and Colitis in April 1984, was published this fiscal year (*Digestive Diseases and Sciences*, December 1985 Supplement). This publication of the workshop proceedings has broadened awareness of the ORAU marmoset colony and has generated several collaborative research efforts.

Colon Cancer Studies

ORAU marmoset research personnel are studying tamarin and marmoset colonic tissues by endoscopy and biopsy to determine the pathogenesis of colitis and any relationship to the development of colon cancer in cotton-top tamarins. We conduct this study in collaboration with gastroenterologists at the University of Nevada School of Medicine and C· 'tate University College of Medici·. ·revious observations from tissues of necropsied animals identified a strong correlation between colon cancer and colitis although many animals do not have acute colitis at death. In collaboration with University of Michigan gastroenterologists, oncodevelopmental mucins that are very similar to those found in humans with the same diseases have been identified in both colitis and colon cancer in tamarins.

As part of the ongoing research in the Preclinical and Clinical Nuclear Medicine Program, we have collaborated in an effort to detect spontaneous tamarin colorectal carcinoma: the nuclear medicine staff is attempting develop monoclonal antibodies labeled with radioiodine with sufficient specificity for this tumor to permit early diagnosis through scintigraphic imaging. Also included in this collaboration is an effort to find a method for detecting cancer cells circulating in the peripheral blood of tamarins bearing spontaneous colorectal carcinomas and to use purified fluorochrome-labeled tumor-associated antibodies as a direct fluorescent stain for tamarin colorectal carcinoma cells. (See Preclinical and Clinical Nuclear Medicine Section.)

In a collaborative effort with Dr. Daniel Podolsky, Massachusetts General Hospital and Harvard University, we are establishing primary cell cultures from a tamarin colorectal carcinoma and are now maintaining them at ORAU. Once they have grown to adequate numbers for characterization and utilization, these cells will be used for various studies, such as raising specific monoclonal antibodies and determining their immunogenic characteristics and carcinogenicity in nude mice.

Breeding and Reproduction

Reproductive efficiency of the cotton-top tamarin colony continues at a high level, although primarily colony-born animals are now the more common breeding pairs.

During the year we conducted research on the behavioral and endocrine determinants of reproductive competence in the cotton-top tamarin through a grant from the Division of Research Resources of NIH. The initial objective was to define the importance of the social aspects of rearing infant siblings on the subsequent maternal behavior of females. Combined studies of behavior and the relation of demographic parameters to reproductive performance have enhanced our ability to select competent breeders. Demographic analyses have also been used to identify other areas for future research into tamarin reproductive function.

We are studying tamarin and marmoset colonic tissues to determine the pathogenesis of colitis and any relationship to the development of colon cancer in cotton-top tamarins.

We are examining reproductive aging in female tamarins to determine whether these species display endocrine, ovarian, or fertility changes similar to menopausal women. Preliminary results indicate that a significant decline in ovarian follicular development and reduced fertility occurs with age in tamarin females. Collaborative studies with the University of Wisconsin are providing longitudinal data on endocrine function in aging tamarin females. Results indicate that the hormone profile of the aged tamarin is different from those observed in breeding females. It appears that the aged tamarin has greater variation in ovarian hormone levels and a greater chance of being acyclic.

Efforts were initiated in 1984 to form an international studbook for the endangered cotton-top tamarin which was published in January 1987. The studbook is crucial to the formation of sound conservation and research planning for this species.

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Other Collaboration

Collaborative research projects with external investigators are designed to characterize the biology, biochemistry, physiology, nutritional aspects, and behavioral characteristics of the marmoset as an experimental animal and to relate these findings to disease processes. These investigations involve the following studies: T and B cell immunology, cell culture of tamarin colon cancers, presence of oncogenes, in vitro tetraploidy in skin fibroblasts as a marker of cancer susceptibility, genetic epidemiology and familial relationships in tamarin colon cancer, and idiopathic ulcerative colitis in colon cancer susceptibility and expression. Other projects are investigating the effect of fecal mutagens and fecal microflora, coronaviruses, lectin binding, fecal toxins, serum enzymes, and fecal enzymes in colonic diseases of marmosets.

A collaborative research relationship has been developed with the Harbor-UCLA School of Medicine, which has established an NIH-funded center for the study of inflammatory bowel disease. The marmoset model of inflammatory bowel disease is being used there to investigate such diverse parameters as genetic epidemiology, slow-moving proteases, leukotrienes, and the presence of oncogenes in colonic disease processes that are observed and followed clinically by ORAU personnel. By comparing the onset of colon carcinoma in colony-born cotton-top tamarins from families that have colon cancer in their histories and families that have no cancer in their histories, we have determined that this unique species' susceptibility appears to be panspecific rather than familial in contrast with observations in humans where a significant percentage of colonic neoplasms are familial.

We are now initiating collaborations with the Laboratory of Comparative Carcinogenesis at Frederick Cancer Research Facility in the National Cancer Institute. Concerted efforts are under way to study changes in the fecal mutagens and microbial flora in relation to mutagenicity and carcinogenesis.

Radiation Emergency Assistance Center/Training Site (REAC/TS)

Program Director: Robert Ricks

The Radiation Emergency Assistance Center/Training Site (REAC/TS) continued to provide support for medical management of radiation accidents. REAC/TS support includes on-site emergency medical assistance and consultative services. REAC/TS continued to work closely with the World Health Organization (WHO) in the role of Western Hemisphere Collaboration Center.

Medical Assistance

REAC/TS continued to provide 24-hour direct or indirect assistance for medical management of radiation accidents. We had 63 calls for assistance in FY86, including 7 accidents, 47 incidents (nonaccidents), and 9 assistance requests by a foreign country.

REAC/TS provided direct assistance to the U.S. State Department following the accident at Chernobyl. Embassy personnel at U.S. facilities in Moscow, Warsaw, and Bucharest were evaluated for radioactive contamination and otherwise informed regarding the potential health impact of the Chernobyl reactor accident.

In Oak Ridge, REAC/TS provided whole-body counting (primarily for I-131 contamination) for U.S. citizens in UF-6 release at Gore, Oklahoma. In addition, cytogenetic dosimetric evaluations were conducted on persons suspected of accidental exposure to ionizing radiation.

Clinical use of DTPA continued to be coordinated by REAC/TS with evaluation (by whole-body counting) of individuals undergoing DTPA chelation therapy for known or suspected internal contamination with plutonium-239 or americium-241. Results of such whole-body counting evaluations are used to determine the efficacy of therapy and to determine a need for continued use of DTPA in persons internally contaminated. The DTPA Registry now contains information on over 500 persons who have received at least one dose of calcium or zinc DTPA following known or suspected internal contamination with transuranics.

Training

During the past year, REAC/TS continued to offer courses of instruction in medical management of radiation accidents to medical, paramedical, and health physics personnel. Participants included 62 physicians, 60 nurses, 39 health physicists, and 17 others for a total of 178 individuals. Since 1976, a total of 88 training courses have been offered at REAC/TS with 1,674 health professionals trained.

In FY86, we expanded our training activities to include attorneys. A pilot training course entitled "Basic Concepts of Radiation for Attorneys" was attended by 74 people (71 attorneys, 1 health physicist, and 2 others). The course was highly successful and will be offered on a regular basis in the future.

We had 63 calls for assistance in our continuing effort to provide support for medical management of radiation accidents.

the Soviet Union and Eastern Bloc countries following the Chernobyl core inventory atmospheric release. REAC/TS also assisted in evaluation of persons suspected of having internal uranium contamination following the

1028727

REAC/TS course graduates continue to comprise a broad base of occupational backgrounds and locales within the United States and 17 foreign countries. In addition, we continue to participate in formal training endeavors sponsored by government, private industry, medical/health physics societies, hospitals, and other various professional groups.

REAC/TS continued its close association with the Federal Emergency Management Agency (FEMA). In FY86, we assisted in conducting FEMA programs at FEMA'S Emergency Management Institute, Emmitsburg, Maryland, through teaching and development of course materials. We also assisted FEMA in evaluating and updating their existing courses in radiological training. In FY87, FEMA will continue to provide funding support for conducting REAC/TS training courses in radiation accident management. Also, in FY87, REAC/TS will assist FEMA in conducting a national teleconference entitled "Handling the Radiation Accident Victim in the Hospital Emergency Department." This teleconference will be broadcast through the Emergency Education Network (EENET).



In response to requests for assistance, the cytogenetics staff provided dosimetry estimates for nine persons referred to REAC/TS during the last year.

Registries

Through the REAC/TS Registry system, long-term follow-up of persons involved in earlier radiation accidents continues. The REAC/TS Registry at present contains information on 835 events (accidents or incidents) involving over 141,000 persons. The registry has identified 285 serious radiation accidents worldwide involving 136,254 persons and 60 acute fatalities. The number of persons in the REAC/TS Registry database grew considerably due to the Chernobyl reactor accident.

Through the DOE ≥ 5 Rem Study Registry, follow-up of persons exceeding the occupational exposure limit continues for the purpose of establishing trends in morbidity and mortality. The DTPA user registry has been expanded to include 55 facilities with 46 coinvestigator physicians reporting annually on 574 persons who have received at least one dose of DTPA chelate for actual or suspected internal contamination. Information on foreign radiation accidents continues to be added to the registry system through contact with physicians and health physicists in various foreign countries and through close liaison with the WHO Collaboration Center in Paris.

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'demiology

Program Director: Shirley A. Fry

The research objective of this multidisciplinary program is to examine the effects on morbidity and mortality of chronic exposure to low levels of ionizing radiations from external and/or internal sources alone or in the presence of chemical toxicants, particularly uranium and other toxic metals that potentially may confound or modify the effects of exposure to radiation among workers in the nuclear industry. A related objective is to evaluate the health and mortality experience of workers to identify untoward effects, if any, of their employment at some time since 1942 at selected facilities of the U.S. Department of Energy (DOE) and its predecessor agencies (Manhattan Engineer District [MED], Atomic Energy Commission [AEC], Energy Research and elopment Administration [ERDA]) their contractors.

Other programmatic objectives include (1) development and application of improved statistical methods, including probabilistic models, for the analysis of epidemiologic data, (2) critical evaluation of the methods and results of studies of health effects among other populations exposed to ionizing radiations, occupationally or otherwise, and to other industrial toxicants, (3) advancement of methodologies in the areas of data collection and records management and ccupational hazards assessment, and (4) development and application of improved computer systems to ensure the high quality and validity of the data bases and to facilitate the processing, manipulation, and analysis of large volumes of data in support of ongoing programmatic activities.

These objectives are being pursued as part of the overall Health and Mortality Study of DOE Workers that has an estimated total population of 600,000 present and former workers. The part of the Health and Mortality Study assigned to ORAU includes about 200,000 workers of all race and sex groups—males, females, whites, nonwhites—at DOE's research and development and production facilities operated by Martin Marietta Energy Systems, Inc., in Oak Ridge, Tennessee, Paducah, Kentucky, and at the Gaseous Diffusion Plant in Portsmouth, Ohio; at the Fernald Feed Materials Plant (FFMP) in Fernald, Ohio, operated by Westinghouse Corporation; and at the Savannah River Plant (SRP) in Aiken, South Carolina, operated by E. I. du Pont de Nemours and Company. Other subpopulations in the ORAU component of the Health and Mortality Study are populations of workers who were employed at former MED uranium processing and refining facilities in upstate New York and St. Louis, Missouri, and a population of about 3,000 workers reported to have received ≥ 5 rem in a year while employed at any DOE or contractor facility since 1947.

The objective is to examine the effects of chronic exposure to low levels of ionizing radiation alone or in the presence of toxic chemicals that may confound or modify the effects of radiation exposure.

In these studies we have the collaboration of the School of Public Health of the University of North Carolina, Chapel Hill, the Epidemiology Department of the University of Alabama at Birmingham, and the Department of Biostatistics and Epidemiology of the University of Cincinnati. For practical reasons, our studies currently focus on the white male workers among these facilities' populations. However, follow-up of vital status is maintained for all race and sex groups in anticipation of studying these groups in the future.

Completed Studies

During the past year, we completed a final analysis of mortality among workers employed between 1947 and 1974 at the Oak Ridge Y-12 Plant using U.S., state, and internal comparison populations. The effects of exposure to alpha radiation from inhaled uranium dust were of particular interest in this study, for which radiation doses to the lungs were estimated for individual workers from their radiation monitoring data.

We also completed a preliminary analysis of mortality for workers employed between 1953 and 1974 at the Savannah River Plant. Evaluation is continuing of this cohort's mortality experience taking into account occupational exposure to radiation.

In collaboration with Union Carbide Corporation's corporate epidemiology group, an analysis was completed of all and specific causes of mortality, including selected site and type specific cancers, among former workers at the MED uranium processing site at Linde Corporation. In a completed case-control study, 89 cases of primary cancer of the brain and central nervous system identified among workers hired at the Oak Ridge Y-12 Plant and Oak Ridge National Laboratory between 1943 and 1979 were compared with individually matched controls from the same plants, with respect to their occupational exposures to radiation, selected chemicals, and to nonoccupational risk factors.

Published reports on these several studies are anticipated in 1987.

Ongoing Studies

A study is continuing of age-specific mortality among all workers employed between 1943 and 1947 at any of DOE's facilities in Oak Ridge. This study utilizes the Mortality Analysis System (MAS) developed by our biostatistics and computer staffs to expedite the evaluation, using external and internal comparison populations, of the

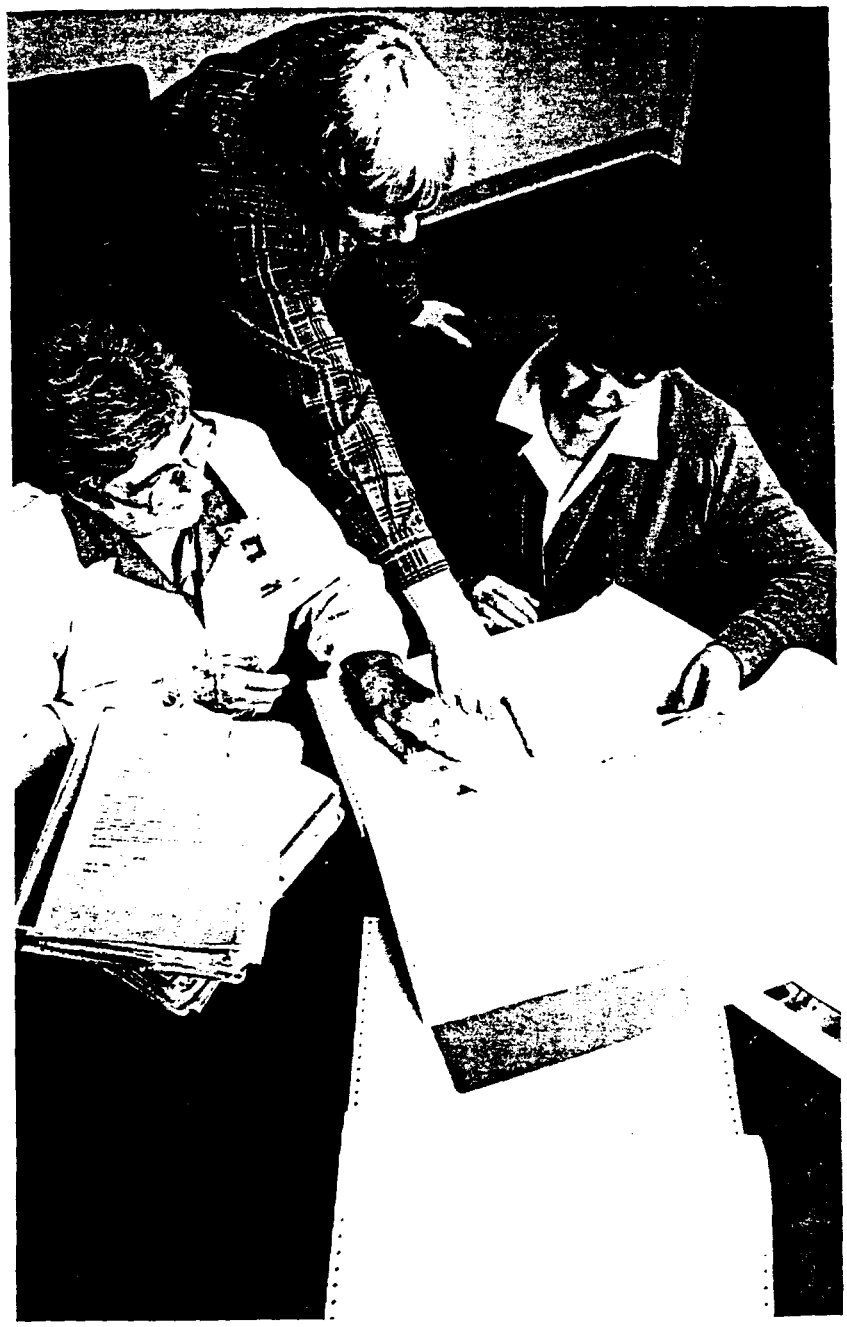
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effects on mortality rates of different
l of factors of interest (in the
c at analysis these include socio-economic status, birth year, duration of employment, length of follow-up, and potential for occupational exposure to radiation).

Other analyses currently in progress include (1) mortality among white males employed at the Oak Ridge Gaseous Diffusion Plant and Mallinckrodt Chemical Works, (2) lung cancer among workers employed between 1943 and 1947 at the Y-12 Plant, Oak Ridge, and (3) mortality and morbidity among workers at the Fernald Feed Materials Plant and among those who received ⩾ 5 rem during any year of employment from 1947 at any DOE or contractor site.

In this latter study, a computer-based follow-up telephone interview program is in progress to obtain morbidity and other relevant medical, occupational, and social factors from members of the rem cohort and their individually matched comparison subjects to determine if there are increased incidences of selected diseases among the cohort and, if so, if they are related to occupational exposure to radiation. Also to be determined in this study is whether morbidity is a more sensitive measure of the human health effects of radiation. To date, we have completed interviews with study subjects at seven facilities with an overall participation rate of ⩾ 65%.

Data preparation continues for planned analyses of mortality among workers at the Paducah and Portsmouth Gaseous Diffusion Plants, and among cohorts of welders and chemical operators at all Oak Ridge plants. Mortality and morbidity data for FFMP workers are being updated in preparation for analyses taking into account exposure to uranium as estimated from area and personnel monitoring data. Indices of tential exposure to uranium assigned y knowledgable plant personnel, and used in an earlier analysis, were found to be in good general agreement with the levels of exposure estimated from the monitoring data. Work continues to develop statistical and probabilistic methodologies for analyses that include time dependent variables.



Staff at the Center for Epidemiology help maintain and update the REAC/TS registries on radiation accidents.

Other Support

Our research staff is providing ongoing support to the International Nickel Speciation Steering Committee, to task groups of the National Cancer Institute (NCI) and the National Council on Radiation Protection and Measurements, and to the Committee on Interagency Radiation Research and Policy Coordination and the Biological

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Effects of Internally Deposited Alpha-Emitting Radionuclides (BEIR IV).

:hnical support in locating death data ıas been provided to the Cancer Epidemiology Branch, NCI, under an interagency agreement with DOE.

We continue to operate the Death Certificate Retrieval Office (DCRO) in support of the ORAU and other DOE epidemiology groups, primarily those at Los Alamos and Hanford who are conducting studies of other DOE worker populations as part of the Health and Mortality Study. Data on vital status and deaths among DOE employees are retrieved primarily through the Social Security Administration and the vital statistics departments of the states. Other sources of these data include the National Death Index, state death indexes, state motor vehicle records, the Civil Service Commission, the Veterans' Administration, and other large retirement systems. The DCRO staff has a consultative role in the implementation

ł operation of the recently established .nessee State Cancer Registry, which will be a valuable source of cancer incidence data for prospective studies of workers at the Oak Ridge plants.

A long-term goal of the epidemiology program is to merge all employee rosters and associated exposure and other relevant data where it is epidemiologically feasible. This data base could provide (1) a sufficient cumulative population radiation dose and total years of follow-up necessary for a meaningful assessment of risks associated with exposure to low levels of ionizing radiation, (2) a basis for the continued surveillance of DOE workers' health experience, and (3) a resource contributing to the resolution of medicolegal arguments and the establishment of regulatory standards for health protection. The data base also provides a retrospective basis for a registry of radiation workers in the United States.

Committee on Interagency Radiation Research and Policy Coordination

Program Director:
Anthony H. Ewing

The White House Office of Science and Technology Policy (OSTP) created the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) in 1984, chartering it under the Federal Coordinating Council for Science, Engineering, and Technology. CIRRPC coordinates radiation matters between federal agencies, evaluates radiation research, and provides advice on the formulation of radiation policy. Eighteen federal agencies have designated policy representatives to CIRRPC, and, of these, fourteen agencies have designated senior scientists as members of CIRRPC's Science Panel.

ORAU provides the necessary personnel and facilities to serve as a technical assistance office to handle technical, administrative, and logistical functions for CIRRPC. ORAU/CIRRPC staff assist CIRRPC by analyzing and drafting technical documents, providing guidance on radiation-related issues, managing technical assistance projects contracted to other organizations, determining consultant needs and arranging for such services, convening meetings and seminars to address radiation issues of concern to the federal agencies, participating in subpanel deliberations and assisting with CIRRPC reports from their inception to publication. ORAU/CIRRPC staff also manage all the logistics for CIRRPC meetings, including announcements, agendas, and minutes. They also maintain the official CIRRPC records and a state-of-the-art automated communication network among the members.

Currently five science subpanels in CIRRPC are addressing issues of concern to multiple federal agencies: indoor radon, the radioepidemiological tables, the scientific basis of radiation protection standards, high linear-energy transfer radiation, and the research agenda for radiofrequency health effects. Two policy subpanels are studying the issue of metrication of radiation units and the impact of the radioepidemiological tables.

At the direction of CIRRPC, ORAU has contracted with the National Academy of Sciences, National Research Council, to conduct a comprehensive review and analysis of existing knowledge on the biological effects of ionizing radiation and to publish a detailed report summarizing these findings. When completed, the study of "Effects on Populations of Exposures to Low Levels of Ionizing Radiation" (BEIR V) will update and extend the 1980 BEIR III report by including among its new findings those made following the reassessment of the dosimetry for the survivors of the atomic weapons detonated during World War II and a summary of findings in the BEIR IV report on the effects of internally deposited alpha-emitting radionuclides. The findings are expected to be in a form suitable for use in making health risk assessments, assigning share values for radiation as a causative disease agent, and formulating radiation exposure control decisions. Like previous BEIR reports, it is expected that BEIR V will be both an important scientific document used in developing U.S. policy on radiation protection and

We assist the Committee on Interagency Radiation Research and Policy Coordination as it coordinates radiation matters between agencies, evaluates radiation research, and provides advice on radiation policy.

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a much referenced social document used, for example, in the U.S. court system. The report is expected to be completed : end of 1988.

ı hrough a contract between ORAU and the National Committee on Radiation Protection and Measurements, CIRRPC is funding an assessment of the radiation exposure to the U.S. population from both man-made and natural sources.

In addition, a task force coordinated by ORAU has been initiated to develop a compendium of fact sheets containing legal and technical information on Federal radiation protection standards and guides, particularly those that protect the public. The compendium will be a reference document that can be used by CIRRPC to consider any further actions necessary to enhance coordination of radiation protection within the federal government. The perceived lack of such coordination has been cited by a number of CIRRPC member agencies.

During this second year of operation, ' ıember agencies have called upon ι ıRPC to assist in coordinating science and policy issues and to provide a vehicle to accomplish multiagency tasks. At the request of OSTP and the Office of Management and Budget (OMB), the draft "Radiation Protection Guidance to Federal Agencies for Occupational Exposure" was distributed to CIRRPC member agencies for review, and ORAU coordinated the response. If approved, this document will replace those portions of the present federal guidance published in 1960 applicable to occupational exposures.

On May 8, 1986, we conducted a joint briefing at OSTP for the President's Acting Science Advisor and CIRRPC members on programs related to indoor radon. The information exchange enhanced the potential for coordination and cooperation among the ongoing programs on this issue of n>ı·ıonal concern.

.bassador-at-Large Richard Kenney, Department of State, requested and received a briefing on CIRRPC activities and possible assistance in coordination of U.S. Government response to any request from the International Atomic Energy Agencies in connection with the reactor accident at Chernobyl in the Soviet Union.

During the year we produced three major publications. The effort initiated in CIRRPC's first year to define those radiation issues of sufficient importance and scope to require interagency attention resulted in "Report on Identification of Federal Radiation Issues." Increased public attention to the potential health hazards associated with elevated levels of naturally occurring radioactive radon gas in indoor air prompted a report which identifies the major technical issues concerning indoor radon, "Radon Protection and Health Effects," CIRRPC Science Panel Report No. 4.

In addition, member agencies of CIRRPC are considering what role the committee might play in assuring that U.S. participation in international organizations is consistent with U.S. radiation policy and in encouraging cooperation in research and information exchange. As an initial step in attaining their objective, the CIRRPC member agencies provided information on points of contact within the agency having liaison roles on radiation matters with international organizations, staff attendance at international meetings on radiation matters, and a listing of international organizations which routinely invite agency representatives to participate in such meetings. An initial compilation was contained in the CIRRPC report, "Member Agency Participation in International Radiation Activities."

Occupational Medicine

Program Director: T. A. Lincoln

To be of technical assistance to the medical director for the DOE Contractor Occupational Medicine Program of the Office of Environment, Safety & Health (ES&H), a strong staff that is experienced in occupational medicine, laboratory (pathology) medicine, legal medicine, and psychology has been organized in MHSD. This group provides expertise for biennial assessments of all DOE contractor occupational medical departments nationwide.

A management data base is being developed to enable prompt retrieval of information on the content, staffing, and scope of these programs. Assistance is provided to ES&H and DOE contractors on a variety of other occupational medical problems. An overview and management program for medical follow-up of former workers who had been potentially exposed to beryllium during their employment with DOE contractors is also being developed.

We provide assistance to the DOE's Office of Environment, Safety & Health and DOE contractors on a variety of occupational medical problems.

Technical reviews and workshops are conducted by the Occupational Medicine Program on selected topics to identify and help implement needed research and management. Occupational Medicine also contributes to the MHSD's ability to assist the DOE General Counsel and other legal agencies of the federal government with medical case reviews, medicolegal depositions, and expert testimony. We also provide back-up support for the REAC/TS program.

During the past year a Center of Excellence for studies of human reliability programs was established by DOE in the medical division and coordinated by the Occupational Medicine Program. This center is carrying out major responsibilities concerning alcohol and drug abuse. The development of psychologic assessment methodology, provision of other technical assistance as needed, and the design of a training program are being provided to DOE in its effort to develop a Human Reliability Program.

1028732

Publications

Full-length Journal Articles

Blank, M. L., Lee, T-c., Cress, E. A., Fitzgerald, V., and Snyder, F.
Plasmalogen biosynthesis in Madin-Darby canine kidney cells: Selectivity in the acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphoethanolamine and the subsequent desaturation step
Arch. Biochem. Biophys. (in press)

Blank, M. L., Spector, A. A., Kaduce, T. L., Lee, T-c., and Snyder, F.
Metabolism of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) and 1-alkyl-2-acetyl-*sn*-glycerol by human endothelial cells
Biochim. Biophys. Acta 876, 373–378, 1986

Blank, M. L., Spector, A. A., Kaduce, T. L., and Snyder, F.
[illegible]sition and incorporation of [^{3}H]arachidonic acid into molecular species of phospholipid classes by cultured human endothelial cells
Biochim. Biophys. Acta 877, 211–215, 1986

Boland, C. R., and Clapp, N. K.
Oncodevelopmental mucins in the colons of new world monkeys with ulcerative colitis: The association between inflammation and neoplasia
Gastroenterology (in press)

Brown, A. J., Glish, G. L., McBay, E. H., and Snyder, F.
Alkyldihydroxyacetonephosphate synthase mechanism: ^{18}O studies of the fatty acid release from acyldihydroxyacetone phosphate
Biochemistry 24, 8012–8016, 1985

Carpenter, A. V., Flanders, W. D., Frome, E. L., Crawford-Brown, D. J., and Fry, S. A.
Central nervous system cancers and radiation exposure: A case-control study among workers at two nuclear facilities
J. Occup. Med. (in press)

Clapp, N. K., Lushbaugh, C. C., Humason, G. L., Gangaware, B. L., and Henke, M. A.
Natural history and pathology of colon cancer in *Saguinus oedipus oedipus*
Dig. Dis. Sci. 30, 107S–113S, 1985

Clapp, N. K., McArthur, A. H., Carson, R. L., Henke, M. A., Peck, O. C., and Wood, J. D.
[illegible]ization and biopsy of the colon in tamarins and marmosets by endoscopy: A promising technique
Lab. Anim. Sci. (in press)

Clapp, N. K., and Tardif, S. D.
Marmoset husbandry and nutrition
Dig. Dis. Sci. 30, 17S–23S, 1985

Clapp, N. K., and Tardif, S. D.
Assessing the current status of cotton top tamarins: Collaboration between conservationists and biomedical researchers
Lab. Primate Newslett. (in press)

Conti, C. J., Clapp, N., Klein-Szanto, A. J. P., Nesnow, S., and Slaga, T. J.
Survival curves and incidence of neoplastic and non-neoplastic disease in SENCAR mice
Carcinogenesis 6, 1649–1652, 1985

Dupree, E. A., Cragle, D. L., McLain, R. W., Crawford-Brown, D. J., and Teta, M. J.
Mortality among workers at a uranium processing facility: Linde Air Products Company Ceramics Plant, 1943–1949
Scand. J. Work Environ. Health (in press)

Eisele, G. R.
A perspective on gossypol ingestion in swine
Vet. Human Toxicol. 28, 118–122, 1986

Eisele, G. R.
Metabolism and effects of organic compounds in animals
Vet. Human Toxicol. 27, 417–421, 1985

Eisele, G. R.
Distribution of indole in tissues of dairy cattle, swine, and laying pullets
Bull. Environ. Contam. Toxicol. 37,246–252, 1986

Eisele, G. R., and Erickson, B. H.
Gonadal retention and localization of ^{241}Am in orally exposed swine
Health Phys. 49, 1276–1279, 1985

Eisele, G. R., Tsai, S. C., Payne, J., Kyle, B., and Urso, I.
The distribution of orally administered ^{14}C-aniline HCl in tissues of dairy cattle, swine and laying pullets
J. Anim. Sci. 61, 1492–1497, 1985

Fagan, T. C., Nelson, E. B., Lasseter, K. C., Crook, J., Morledge, J., and Conrad, K. A.
Once- and twice-daily nitrendipine in patients with hypertension and noninsulin-dependent diabetes
Pharmacotherapy 6, 128–136, 1986

Frome, E. L. and DuFrain, R. J.
Maximum likelihood estimation for cytogenetic dose-response curves
Biometrics 42, 73–84, 1986

Hammond, B. F., and Martin, P. G.
Calcium-cadmium effects on bone density and strength of the femur from multiparous rats
Nutr. Res. Suppl. 1, 511–514, 1985

Hoffman, D. R., Hoffman, L. H., and Snyder, F.
Cytotoxicity and metabolism of alkyl phospholipid analogues in neoplastic cells
Cancer Res. (in press)

Hubbard, J. W., Cox, R. H., Lawler, J. E., Blank, M. L., and Snyder, F.
Cardiac output and peripheral vascular resistance during intravenous infusion of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious normotensive and spontaneously hypertensive rats
Res. Commun. Chem. Pathol. Pharmacol. 49, 459–462, 1985

Klein-Szanto, A. J. P., Conti, C. J., Aldaz, C. M., Clapp, N., Nesnow, S., and Slaga, T. J.
Effects of chronic topical application of 12-O-tetradecanoylphorbol-13-acetate on the skin and internal organs of SENCAR mice
Environ. Health Perspect. 68, 75–80, 1986

1028733

Lee, T-c., Kirk, P., and Snyder, F.
Testosterone induction of microsomal acyl-CoA reductase and a cytosolic regulatory protein in mouse preputial glands
I n. *Biophys Acta* 875, 129–132, 1986

Lee, T-c., Malone, B., and Snyder, F.
A new de novo pathway for the formation of 1-alkyl-2-acetyl-*sn*-glycerols, precursors of platelet activating factor
Biochemical characterization of 1-alkyl-2-lyso-*sn*-glycero-3-P:acetyl-CoA acetyltransferase in rat spleen
J. Biol. Chem. 261, 5373–5377, 1986

Littlefield, L. G., and Joiner, E. E.
Analysis of chromosome aberrations in lymphocytes of long-term heavy smokers
Mutat. Res. 170, 145–150, 1986

Lushbaugh, C. C., Fry, S. A., Ricks, R. C., Hübner, K. F., and Burr, W. W.
Historical update of past and recent skin damage radiation accidents
Brit. J. Radiol. (in press)

Lushbaugh, C., Humason, G., and Clapp, N.
Histology of colitis: *Saguinus oedipus oedipus* and other marmosets
Dig. Dis. Sci. 30, 45S–51S, 1985

Lushbaugh, C., Humason, G., and Clapp, N.
Histology of colon cancer in *Saguinus oedipus oedipus*
Dig. Dis. Sci. 30, 119S–125S, 1985

Pearce, N.
Time-related factors as potential confounders or effect modifiers in studies based on an occupational cohort
S *J. Work Environ. Health* 12, 97–107, 1986

R. .son, M., Blank, M. L., and Snyder, F.
Highly unsaturated phospholipid molecular species of rat erythrocyte membranes: Selective incorporation of arachidonic acid into phosphoglycerides containing polyunsaturation in both acyl chains
Arch. Biochim. Biophys. (in press)

Robinson, M., and Snyder, F.
Metabolism of platelet activating factor by rat alveolar macrophages: Lyso-PAF as an obligatory intermediate in the formation of alkylarachidonoyl glycerophosphocholine species
Biochim. Biophys. Acta 837, 52–56, 1985

Russell, R. G., Brian, D. A., Lenhard, A., Potgieter, L. N. D., Gillespie, D., and Clapp, N. K.
Coronavirus-like particles and campylobacter in marmosets with diarrhea and colitis
Dig. Dis. Sci. 30, 72S–77S, 1985

Snyder, F., Blank, M. L., Johnson, D., Lee, T-c., Malone, B., Robinson, M., and Woodard, D.
Alkylacetylglycerols versus lyso-PAF as precursors in PAF biosynthesis and the role of arachidonic acid in PAF metabolism
Pharmacol. Res. Commun. (in press)

Tardif, S. D.
Histologic evidence for age-related differences in ovarian function in tamarins (*Saguinus* sp.; primates)
Biol. Reprod. 33, 993–1000, 1985

T *, S. D., Carson, R. L., and Clapp, N. K.
L .ng performance of captive-born cotton-top tamarin (*Saguinus oedipus*) females: Proposed explanations for colony differences
Am. J. Primatol. (in press)

Tardif, S. D., Carson, R. L., and Gangaware, B. L.
Comparison of infant care in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus* *oedipus*)
Am. J. Primatol. 11, 103–110, 1986

Urso, P., Gengozian, N., Rossi, R. M., and Johnson, R. A.
Suppression of humoral and cell-mediated immune responses in vitro by benzo(a)pyrene
J. Immunopharmacol. 8, 223–241, 1986

Washburn, L. C., Blair, L. D., Byrd, B. L., and Sun, T. T.
Comparison of ^{68}Ga EDTA, [1-^{11}C]α-aminoisobutytic acid, and [^{99m}Tc]sodium pertechnetate in an experimental blood-brain barrier lesion
Int. J. Nucl. Med. Biol. 12, 267–269, 1985

Washburn, L. C., Hwa Sun, T. T., Crook, J. E., Byrd, B. L., Carlton, J. E., and Steplewski, Z. S.
^{90}Y-Labeled monoclonal antibodies for cancer therapy
Nucl. Med. Biol. (Int. J. Radiat. Appl. Instrum. Part B) 13, 453–456, 1986

Chapters, Review Articles, and Other Publications

Beck, W. L., and Hodges, H. D.
Radioactive materials: Safe handling
In *Encyclopedia of Materials Science and Engineering*, pp. 4044–4046, Pergamon Press Ltd., Oxford, England, 1986

Blank, M. L., Byrd, B. L., Cress, E. A., Washburn, L. C., and Snyder, F. S.
Use of liposomal DTPA in heavy metal detoxification
In *Liposomes as Drug Carriers: Recent Trends and Progress* (Gregoriadis, G., ed.), John Wiley & Sons, Ltd., Chichester, England (in press)

Blank, M. L., Robinson, M., and Snyder, F.
Chromatographic analyses of ether-linked lipids involved in PAF metabolism
In *Platelet Activating Factor*, (Snyder, F., ed.), Plenum Publishing Corporation, New York (in press)

Burr, W. W., Jr.
Human experience and epidemiology
In *Biokinetics and Analysis of Uranium in Man, Proceedings of U.S. Uranium Registry Conference*, 1984, Richland, Washington, pp. H-1-H-21, USUR-05 HEHF-47, Hanford Environmental Health Foundation, Richland, Washington, 1986

Clapp, N. K., Henke, M. A., Holloway, E. C., and Tankersley, W. G.
Carcinoma of the colon in the cotton-top tamarin: A radiographic study
Original publication: *J. Am. Vet. Med. Assoc.* 183, 1328–1330, 1983
Yearb. Cancer, 1985, 461

Clapp, N. K., Lushbaugh, C. C., Humason, G. L., Gangaware, B. L., and Henke, M. A.
The cotton-top tamarin as an animal model of colorectal cancer metastasis
In *The Biology and Treatment of Colorectal Cancer Metastasis*, (A. Mastromarino, ed.), pp. 31-39, Martinus Nijhoff Publishing, 1986, Hingham, Massachusetts

Coffey, J. L., Watson, E. E., Hübner, K. F., and Stabin, M. G.
Radiopharmaceutical absorbed dose considerations
In *Clinical Aspects of Nuclear Medicine Science* (Hladik, W. B. III, Saha, G. B., and Stady, K. T., eds.), Williams and Wilkins Corp., Baltimore, Maryland (in press)

1028734

Committee on Interagency Radiation Research and Policy Coordination
Radon Protection and Health Effects
[illegible]PC Science Panel Report No.4, Oak Ridge Associated Universities, August 1986.

CIRRPC
Report on Identification of Federal Radiation Issues
Oak Ridge Associated Universities, March 21, 1986

CIRRPC
Member Agency Participation in International Radiation Activities.
Oak Ridge Associated Universities, June 1986.

CIRRPC
Second Annual CIRRPC Report
Oak Ridge Associated Universities, June 1986.

Cragle, D. L., Shy, C. M., Struba, R. J., and Siff, E. J.
A case-control study of colon cancer and water chlorination in North Carolina
In *Proceedings of the 5th Conference on Water Chlorination: Environmental Impact and Health Effects* (Jolley, R. L., Bull, R. J., Davis, W. P., Katz, S., Roberts, Jr., N. H., and Jacobs, V. A., eds.), Vol. 5, pp. 153–159, Lewis Publishers, Inc., Chelsea, Michigan, 1985

Crook, J. E.
Medical applications of short-lived radionuclides: A selective overview
In *Short-Lived Isotopes in Biology* (Minchin, P. E. H., ed.), pp. 21–24, DSIR Science Information Publishing Centre, Lower Hutt, New Zealand, 1986

[illegible], J. E., Carlton, J. E., Stabin, M., and Watson, E.
[illegible]tion dose estimates for copper-64 citrate in man
In *Fourth International Radiopharmaceutical Dosimetry Symposium* (Schlafke-Stelson, A. T., and Watson, E. E., eds.), pp. 212–216, CONF-851113, Oak Ridge Associated Universities, Oak Ridge, Tennessee, 1986

Erickson, B. H.
Effects of ionizing radiation on mammalian spermatogenesis: A model for chemical effects
In *Reproductive Toxicology* (Dixon, R. L., ed.), pp. 35–46, Raven Press, New York, 1985

Frome, E. L.
Regression methods for binomial and Poisson distributed data
In: *Proceedings of the AAPM First Midyear Topical Symposium, Multiple Regression Analysis: Applications in the Health Sciences*, 1984, Mobile, Alabama (Herbert, D., and Myers, R., eds.), pp. 84–123, American Institute of Physics, New York, 1986

Fry, R. J. M., and Fry, S. A.
Ionizing radiation: Late somatic effects
In *Encyclopedia of Materials Science and Engineering* pp. 2422–2423, Pergamon Press Ltd., Oxford, England, 1986

Groer, P. G.
Internal dosimetry for epidemiologic studies
In *Proceedings of the Department of Energy Workshop on Radiobioassay and Internal Dosimetry*, Albuquerque, New Mexico, 1[illegible] (in press)

[illegible], P. G., and Pereira, C. A.
Calibration of a radiation detector: Chromosome dosimetry for neutrons
In *Proceedings of the International Symposium on Probability and Bayesian Statistics*, Innsbruck, Austria, 1986 (in press)

Hayes, R. L.
Gallium
In *Handbook of Toxicity of Inorganic Compounds* (Seiler, H. G., Sigel, H., and Sigel, A., eds.), Marcel Dekker, Inc., New York (in press)

Hayes, R. L.
Indium
In *Handbook of Toxicity of Inorganic Compounds* (Seiler, H. G., Sigel, H., and Sigel, A., eds.), Marcel Dekker, Inc., New York (in press)

Hübner, K. F., Fry, S. A., Lushbaugh, C. C., Huff, S. H., Littlefield, L. G., Berger, C. D., Ricks, R. C., and Sipe, A. H.
Experience with chelation therapy for actinide incorporation in the United States based on chelation therapy (DTPA) registry of the Radiation Emergency Assistance Center/Training Site (REAC/TS)
In *Proceedings of a Workshop, Inkorporation von Radionukliden* February 1986, Hamburg, Germany (in press)

Hübner, K. F., Littlefield, L. G., and DuFrain, R. J.
Experience in the practical application of biological indicators
In *Biological Indicators for Radiation Dose Assessment* (Kaul, A., Dehos, A., Bogl, W., Hinz, G., Kossel, F., Schwarz, E-R., Stamm, A., and Stephan, G., eds.), pp. 17–34, MMV Medizin Verlag, München, Germany, 1986

Lee, T-c.
Enzymatic control of the cellular levels of platelet activating factor
In *Platelet Activating Factor* (Snyder, F., ed.), Plenum Publishing Corporation, New York (in press)

Lee, T-c., Blank, M. L., Fitzgerald, V., Malone, B., and Snyder, F.
Biosynthesis of intermediates in PAF metabolic pathway: Characterization of 1-alkyl-2-acetyl-*sn*-glycerol kinase in rabbit platelets
In *New Horizons in Platelet Activating Factor Research* (Lee, M. L. and Winslow, C. M., eds.), John Wiley & Sons Limited, Chichester, England (in press)

Lee, T-c., and Snyder, F.
Function, metabolism, and regulation of platelet activating factor and related ether lipids
In *Phospholipids and Cellular Regulation* (Kuo, J. F., ed.), Vol. 2, pp. 1–39, CRC Press, Inc., Boca Raton, Florida, 1985

Lushbaugh, C. C.
Radiation
In *Trauma* (Mattox, K., Moore, E., and Feliciano, D., eds.), Appleton-Century-Crofts Publishing, Houston Texas, (in press)

Lushbaugh, C. C., Brown, D. G., and Frome, E. L.
Longevity of irradiated burros
In *Proceedings, Twenty-Second Hanford Life Sciences Symposium*, 1983, Richland, Washington (in press)

Lushbaugh, C. C., Hübner, K. F., Fry, S. A., and Beck, W. L.
Ionizing radiation: Acute effects
In *Encyclopedia of Materials Science and Engineering*, pp. 2419–2421, Pergamon Press Ltd., Oxford, England, 1986

McLain, R. W., and Frome, E. L.
A system for the analysis of cohort mortality data
In *SAS Users Group International Conference Proceedings: SUGI 11*, Cary, North Carolina, pp. 874–876, SAS Institute, Inc., 1986

Ricks, R. C.
Response to a spill of radioactive material
In *Encyclopedia of Materials Science and Engineering*, pp. 4046–4048, Pergamon Press Ltd., Oxford, England, 1986

1028735

Snyder, F.
The significance of dual pathways for the biosynthesis of platelet activating factor: Alkylacetylglycerols and lyso-PAF as immediate pr rs
In . *Horizons in Platelet Activating Factor Research* (Lee, M. L., and Winslow, C. M., eds.), John Wiley & Sons Limited, Chichester, England (in press)

Snyder, F.
Enzymatic pathways for platelet activating factor, related alkylglycerolipids, and their precursors
In *Platelet Activating Factor* (Snyder, F., ed.), Plenum Publishing Corporation, New York (in press)

Snyder, F.
Biochemistry of platelet actvating factor
In *Proceedings: Platelet Activating Factor, Pulmonary Hyperreactivity and Asthma* (Holme, G., and Morley, J., eds.), L'Esterel, Quebec, Canada, 1986, Academic Press, New York (in press)

Snyder, F., Blank, M. L., Lee, T-c., Woodard, D. S., and Malone, B.
Metabolism of platelet activating factor: The role of alkylacetylglycerols in PAF biosynthesis
Adv. Inflammation Res. 10, 164–167, 1986

Snyder, F., Blank, M., Lee, T-c., Robinson, M., and Woodard, D.
Measurement of key enzyme activities involved in the metabolism of platelet activating factor (PAF)
Methods Enzymol. (in press)

Snyder, F., Lee, T-c., Blank, M., Malone, B., Woodard, D., and Robinson, M.
P activating factor (PAF): alternate pathways of biosynthesis, th hanism of activation, and reacylation of lyso-PAF with arachidonate
Adv. Prostaglandin Thromboxane Leukotriene Res. 15, 693–696, 1985

Snyder, F., Record, M., Smith, Z., Blank, M. L., and Hoffman, D. R.
Selective cytotoxic action of ether-lipid analogs of PAF: Mechanistic studies related to their metabolism, subcellular localization, and effects on cellular transport systems
In *Aktuelle Onkologie*, Zuckschwerdt Verlag, Munich, Federal Republic of Germany (in press)

Washburn, L. C., Byrd, B. L., Sun, T. T., Crook, J. E., Hübner, K. F., Coffey, J. L., and Watson, E. E.
Dosimetry of D- and L-enantiomers of ^{11}C-labeled tryptophan and valine
In *Fourth International Radiopharmaceutical Dosimetry Symposium* (Schlafke-Stelson, A. T., and Watson, E. E., eds.), pp. 239–244, CONF-851113, Oak Ridge Associated Universities, Oak Ridge, Tennessee, 1986.

Woodard, D. S.
An alternate pathway for the biosynthesis of platelet activating factor: Characterization of the dithiothreitol(DTT)-insensitive cholinephosphotransferase from rat kidney medulla
Dissertation, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge, Tennessee, 1986

Published Abstracts of Papers Presented at Meetings

Byrd, B. L., and Crook, J. E.
Uptake of C-14-labeled alicyclic α-amino acids in transplanted pheochromocytomas
J. Nucl. Med. 27, 1043, 1986

Carpenter, A. V., Flanders, W. D., Frome, E. L., Tankersley, W. G., Crawford-Brown, D. J., Sowder, C. L., Wray, M. L., Fry, S. A., and Lushbaugh, C. C.
An epidemiologic investigation of central nervous system cancers among workers at diverse nuclear facilities
Am. J. Epidemiol. 124, 530–31, 1986

Clapp, N. K., McArthur, A. H., Carson, R. L., Henke, M. A., and Peck, O. W.
Endoscopy of the colon in tamarins and marmosets
Lab. Anim. Sci. 35, 536, 1985

Clapp, N. K., McArthur, A. H. Henke, M. A., Carson, R. L., Peck, O. C., and Wood, J. D.
Is acute colitis a precursor to development of colonic cancer in cotton-top tamarins?
Gastroenterology 90, 1374, 1986

Crook, J. E., and Byrd, B. L.
Influence of nitrendipine, a calcium entry blocker, on New England Deaconess Hospital (NEDH) hypertensive rats
J. Clin. Pharmacol. 25, 466, 1985

Crook, J. E., Carlton, J. E., and Holloway, E. C.
Copper-64 citrate as a potential myocardial imaging agent
Clin. Nucl. Med. Suppl. 10, 40, 1985

Crook, J., Washburn, L., Sun, T., Byrd, B., Brill, A., Kubota, K., Bennett, G., Zanzi, I., and Yeh, S.
D- and L-enantiomers of ^{11}C-valine in the evaluation of pancreatic adenocarcinoma
Dig. Dis. Sci. 30, 968, 1985

Dobson, L., Straume, T., Carrano, A., Minkler, J., Deaven, L., Littlefield, G., and Awa, A.
Cytogenetic effectiveness of neutrons from replica of Hiroshima Bomb: Preliminary results from an interlaboratory study
In *Proceedings of the Fourth International Conference on Environmental Mutagens*, Stockholm, Sweden, 1985 (in press)

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action
Biometrics (in press)

Harrison, M. L., and Tardif, S. D.
Kin recognition in a callitrichid primate
Am. J. Phys. Anthropol. (in press)

Littlefield, G., Sayer, A., and Joiner, E. E.
"Mitotic Chiasmata" in lymphocytes of normal adults
Mamm. Chromosome Newslett. (in press)

Littlefield, G., Joiner, E., Colyer, S., and Frome, E.
Chromosome aberrations in irradiated lymphocytes cultured before or after freezing
Environ. Mutagen. 8, 46, 1986

Lowe, K. W., Holbrook, C. J., Roberts, M. G., and Linkous, S. S.
Evaluation and application of an in vivo assay for chemically induced sister chromatid exchanges and chromosome aberrations
Environ. Mutagen. 8, 127, 1986

1028736

Malone, B., Lee, T-c., and Snyder, F.
De novo biosynthesis of alkylacetylglycerols, a precursor of platelet activating factor
Fed. Proc. 45, 1529, 1986

McFee, A. F., Lowe, K. W., and Linkous, S. L.
A chemical producing unusual chromosome damage patterns in mouse bone marrow cells
Environ. Mutagen. 8, 53, 1986

McFee, A. F., and Tice, R.
Interlaboratory evaluation of a standardized protocol for determination of chemical genotoxicity in mice
Mamm. Chromosome Newslett. (in press)

Petersen, G. M., Clapp, N. K., and Tardif, S. D.
Genetic susceptibility to colon cancer in cotton-top tamarins
Clin. Res. (in press)

Petersen, G. M., Clapp, N. K., and Tardif, S. D.
Spontaneous colonic disease in cotton-top tamarins (*Saguinus oedipus oedipus*): A pan-specific genetic risk
Am. J. Phys. Anthropol. (in press)

Record, M., Hoffman, D., and Snyder, F.
Comparative subcellular distribution of platelet activating factor (PAF) and its antitumor methoxy analog in HL-60 cells
Proc. Am. Assoc. Cancer Res. 27, 14, 1986

Record, M., and Snyder, F.
Biosynthesis of platelet activating factor (PAF) via alternate pathways: Subcellular distribution of products in HL-60 cells
Fed. Proc. 45, 1529, 1986

Saall, B., Conti, P. S., and Washburn, L. C.
Accumulation of ^{11}C-labeled AIB, ACBC, and ACPC in the Dunning R3327G prostatic adenocarcinoma and Morris 5123C hepatoma tumor models
J. Nucl. Med. 26, 1049, 1986

Smith, G., Hübner, K. F., Buonocore, E., Daugherty, J., Crook, J. E., Byrd, W., and Holloway, S.
Quantitative assessment of blood-brain-barrier (BBB) in dementia with Ga-68 EDTA and positron emission tomography (PET)
J. Nucl. Med. 27, 1074, 1986

Tardif, S. D.
Status of the cotton-top tamarin (*Saguinus oedipus*) in captivity
Primate Conserv. 6, 38–39, 1985

Tardif, S. D.
Status of the cotton-top tamarin (*Saguinus oedipus*) in captivity
Primate Rep. 14, 241–241, 1986

Tardif, S. D., and Harrison, M. L.
Energetic demands of infant care in callitrichids: Species comparisons
Primate Rep. 14, 78–79, 1986

Tryka, A. F., Witschi, H., McArthur, A. H., and Clapp, N. K.
Patterns of cell proliferation during recovery from oxygen injury: Species differences
Am. Rev. Respir. Dis. 133, 1055–1059, 1986

Washburn, L. C., and Carlton, J. E.
A modified limulus lysate technique for routine pyrogen testing of gallium-68 EDTA
Clin. Nucl. Med. (in press)

Miscellaneous Papers or Talks Presented at National and International Scientific Conferences

(Published abstracts of these presentations are not available. Presented by first author.)

Cragle, D. L., and Tankersley, W. G.
What information do epidemiologists need from industrial hygienists?
Joint Conference on Occupational Health, Orlando, Florida, October 1985

Crook, J. E.
Medical applications of short-lived radionuclides: A selective overview
International Workshop on Biological Research with Short-Lived Isotopes, Lower Hutt, New Zealand, 1985

Crook, J., Byrd, B., and Elbert W.
The influence of sex hormone levels on the rate of development of implanted pheochromocytomas
III World Conference on Clinical Pharmacology and Therapeutics, Stockholm, Sweden, 1986

Dupree, E. A., Cragle, D. L., McLain, R. W., Crawford-Brown, D. J., and Teta, M. J.
Mortality among workers at a uranium processing facility: Linde Air Products Company Ceramics Plant, 1943–1949
6th Coolfont Conference on Radiation and Health, Berkeley Springs, West Virginia, 1986

Groer, P. G.
Calibration of radiation detectors
International Symposium on Probability and Bayesian Statistics, Innsbruck, Austria, 1986

Kawasaki, T., and Snyder, F.
The metabolism of lyso-PAF by a calcium-dependent lysophospholipase D in rabbit kidney medulla
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Lee, T-c., Blank, M. L., Fitzgerald, V., Malone, B., and Snyder, F.
Biosynthesis of intermediates in PAF metabolic pathway: Characterization of 1-alkyl-2-acetyl-*sn*-glycerol kinase in rabbit platelets
New Horizons in Platelet Activating Factor Research, sponsored by the Sandoz Research Institute, East Hanover, New Jersey, held at Hilton Head Island, South Carolina, 1985

Lee, T-c., Malone, B., and Snyder, F.
Kinetic studies and substrate specificities of enzymes that synthesize 1-alkyl-2-acetyl-*sn*-glycerols: A PAF precursor
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Littlefield, L. G.
Cytogenetic effectiveness of neutrons from replica of Hiroshima bomb: Preliminary results from an inter-laboratory study
International Workshop on "Reevaluation of Hiroshima and Nagasaki Cases by Chromosome Aberration Analysis for Dose Assessment and Risk Evaluation," Kyoto University, Kyoto, Japan, 1985

1028737

Littlefield, L. G.
Cytogenetic evaluations in 10 persons exposed to gamma radiation in the 1984 Mexican ^{60}Co accident
Ir tional Workshop on "Reevaluation of Hiroshima and N. ki Cases by Chromosome Aberration Analysis for Dose Assessment and Risk Evaluation," Kyoto University, Kyoto, Japan, 1985

Littlefield, L. G.
Applications and limitations of sister chromatid exchange analysis as a method for quantifying exposures to DNA-damaging agents
Annual Meeting of the Association of Cytogenetic Technologists, Atlanta, Georgia, 1986

Littlefield, L. G., Kleinerman, R. A., and Machado, S. G.
Applications and limitations of cytogenetic evaluations as an index of recent or previous radiation exposures
6th Coolfont Conference on Radiation and Health, Berkeley Springs, West Virginia, 1986

Lushbaugh, C. C.
Radiation damage to the skin
Workshop on Historical Update of Past and Recent Skin Damage from Radiation Accidents, Fundamental and Practical Aspects, Paris, France, 1985

McLain, R. W., and Frome, E. L.
Analysis of grouped cohort mortality data
146th Annual Meeting Joint Statistical Meetings of the American Statistical Association and the Biometric Society, Chicago, Illinois, 1986

Record, M., and Snyder, F.
P metabolism in HL-60 cells: Subcellular distribution of e es and metabolites
New Horizons in Platelet Activating Factor Research, sponsored by the Sandoz Research Institute, East Hanover, New Jersey, held at Hilton Head Island, South Carolina, 1985

Record, M., Wagner, M., and Snyder, F.
A kinetic study of the uptake and subcellular distribution of an anti-tumor PAF-analog ([^{3}H]alkyl-2-methoxy-GPC) in HL-60 cells
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Record, M., Wagner, M., and Snyder, F.
PAF synthesized from alkylacetylglycerol in HL-60 cells is rapidly and selectively transferred to the plasma membrane
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Siren, A.-L., Snyder, F., and Feuerstein, G.
Hemodynamic effects of platelet activating factor in the conscious rat
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Snyder, F.
Recent developments in the metabolism of PAF: Different biosynthetic routes involved in inflammatory and physiological responses
Second World Conference on Antiinflammatory Drugs, Monte , Principality of Monaco, 1986

Snyder, F.
Biochemistry of platelet activating factor
Conference on Platelet Activating Factor, Pulmonary Hyperreactivity and Asthma, L'Esterel, Quebec, Canada, 1986

Snyder, F.
The significance of dual pathways for the biosynthesis of platelet activating factor: Alkylacetylglycerols and lyso-PAF as immediate precursors
New Horizons in Platelet Activating Factor Research, sponsored by the Sandoz Research Institute, East Hanover, New Jersey, held at Hilton Head Island, South Carolina, 1985

Snyder, F., Lee, T-c., Malone, B., Blank, M., Record, M., Robinson, M., Kawasaki, T., and Wagner, M.
Enzymes responsible for the biosynthesis and catabolism of platelet activating factor and the cellular processing of their precursors and products
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Snyder, F., Record, M., Smith, Z., Blank, M. L., and Hoffman, D. R.
The selective cytotoxicity of ether-linked phospholipid analogs of platelet activating factor toward tumor cells: Their mechanism of action at the cell membrane
Symposium on The Cell Membrane as a Target of Cancer Treatment, Gottingen, Federal Republic of Germany, 1985

Surles, J. R., Morris-Natschke, S., Wykle, R. L., O'Flaherty, J. T., Snyder, F., and Piantadosi, C.
Chemical synthesis and biological evaluation of some *sn*-2 analogues of PAF
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

Woodard, D. S., Lee, T-c., Snyder, F.
The final step in the de novo biosynthesis of platelet activating factor: Properties of a unique CDP-choline:1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase in microsomes from the renal inner medulla of rats
The Second International Conference on Platelet Activating Factor and Structurally Related Alkyl Ether Lipids, Gatlinburg, Tennessee, October 26–29, 1986

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Summaries of Published Papers

(Reprints of the following published papers are available. Send requests to one of the authors or the chairman's office, Medical and Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117.)

Blank, M., Spector, A. A., Kaduce, T. L., Lee, T-c., and Snyder, F.
Metabolism of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) and 1-alkyl-2-acetyl-*sn*-glycerol by human endothelial cells
Biochim. Biophys. Acta 876, 373–378, 1986

The metabolism of platelet activating factor (1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) and 1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycerol was studied in cultures of human umbilical vein endothelial cells. Human enthothelial cells deacetylated 1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycero-3-phosphocholine to the corresponding lyso compound (1-[1,2-^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphocholine) and a portion was converted to 1-[1,2-^{3}H]alkyl-2-acyl(long chain)-*sn*-glycerol-3-phosphocholine. Lyso platelet activating factor (lyso-PAF) (1-[1,2-^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphocholine) was detected in the media very early during the incubation and the amount remained higher than the level of the lyso product observed in the cells. Cellular levels of 1-[1,2-^{3}H]alkyl-2-lyso-*sn*-glycero-3-phosphocholine were significantly higher than the acylated product (1-[1,2-^{3}H]alkyl-2-acyl(long chain)-*sn*-glycero-3-phoshocholine) at all times during the 60-min incubation period, which suggests that the ratio of acetylhydrolase to acyltransferase activities is greater in endothelial cells than in most other cells. When endothelial cells were incubated with 1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycerol, a known precursor of PAF, 1-[1,2-^{3}H]alkyl-*sn*-glycerol was the major metabolite formed (greater than 95% of the ^{3}H-labeled metabolites during 20- and 40-min incubations). At least a portion of the acetate was removed from 1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycerol by a hydrolytic factor released from the endothelial cells into the medium during the incubations. Only negligible amounts of the total cellular radioactivity (0.2%) was incorporated into platelet activating factor (1-[1,2-^{3}H]alkyl-2-acetyl-*sn*-glycero-3-phosphocholine); therefore, it is unlikely that the previously observed hypotensive activity of 1-alkyl-2-acetyl-*sn*-glycerols can be explained on the basis of the conversion to platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) by endothelial cells. Results of this investigation indicate that endothelial cells play an important role in PAF catabolism. Undoubtedly, the endothelium is important in the regulation of PAF levels in the vascular system.

P[illegible]k, M. L., Spector, A. A., Kaduce, T. L., and Snyder, F.
[illegible]osition and incorporation of [^{3}H]arachidonic acid into molecular species of phospholipid classes by cultured human endothelial cells
Biochim. Biophys. Acta 877, 211–215, 1986

Based on quantitative high performance liquid chromatographic analyses of molecular species in selected phospholipid subclasses from cultured human umbilical vein endothelial cells, the relative degree of unsaturation was ethanolamine plasmalogens > phosphatidylethanolamine > phosphatidylcholine. A total of 36 different molecular species were identified in the phosphatidylcholine fraction. Interestingly, the phosphatidylcholine contained a significant amount (11.7%) of the dipalmitoyl species, a lipid normally associated with lung surfactant. The arachidonoyl-containing molecular species of phosphatidylserine/inositol were labeled to the highest extent and the ethanolamine plasmalogens contained the lowest specific radioactivity after incubating [^{3}H]arachidonic acid with human endothelial cells for 4 h. Within each phospholipid subclass the arachidonoyl species where both acyl groups of the phospholipid are unsaturated (20:4–20:4, 18:2–20:4 + 16:1–20:4, and 18:1–20:4) had higher specific radioactivities, after labeling with [^{3}H]arachidonic acid, than those that contained saturated aliphatic chains (16:0–20:4 and 18:0–20:4). This indicates that the unsaturated species have higher turnover rates.

Brown, A. J., Glish, G. L., McBay, E. H., Snyder, F.
Alkyldihydroxyacetonephosphate synthase mechanism: ^{18}O studies of fatty acid release from acyldihydroxyacetone phosphate
Biochemistry 24, 8012–8016, 1985

Alkyldihydroxyacetonephosphate synthase (alkyl-DHAP synthase) catalyzes the exchange of the ester-linked fatty acid of 1-*O*-acyl-dihydroxyacetone phosphate (1-*O*-acyl-DHAP) for a fatty alcohol that is attached in an ether linkage to form 1-*O*-alkyl-dihydroxyacetone phosphate (1-*O*-alkyl-DHAP). In our continuing investigation of the mechanism of this enzyme, we have examined the fatty acid released during the reaction. In contrast to the reports of others using whole microsomes, we found that the cleavage of fatty acid by purified preparations of alkyl-DHAP synthase was dependent on the presence of the cosubstrate, fatty alcohol. Furthermore, the amount of fatty acid produced was equivalent to the alkyl-DHAP formed. Our previously proposed detailed mechanism for alkyl-DHAP synthase predicted that the fatty acid should retain both of the carboxyl ester oxygens upon cleavage. Reactions carried out with palmitoyl-[^{18}O]DHAP as substrate yielded [^{18}O]palmitic acid as the product in agreement with this scheme.

Conti, C. J., Clapp, N., Klein-Szanto, A. J. P., Nesnow, S., and Slaga, T. J.
Survival curves and incidence of neoplastic and non-neoplastic disease in SENCAR mice
Carcinogenesis 6, 1649–1652, 1985

The survival curves and the incidence of spontaneous diseases were studied in a population of SENCAR mice, a stock derived by a selected breeding protocol for enhanced susceptibility to chemical carcinogenesis in the skin. SENCAR mice proved to be as long-lived as other mouse strains or stocks, including one of their parental lines, Charles River CD-1. The most frequently ocurring neoplasias in SENCAR mice were lymphoma, myeloid leukemia and reticulum cell sarcoma. Other frequently occurring neoplastic diseases included lung adenomas and carcinoma and mammary gland carcinoma. However, the incidence of these tumors was not higher than the incidence in CD-1 mice or other mouse strains or stocks. A variety of non-neoplastic diseases, both inflammatory and degenerative, were also observed in old mice. The most common were liver, spleen and kidney amyloidosis, pyelonephritis and papillary necrosis. These data indicate that selective breeding for susceptibility to chemical carcinogenesis has not produced a concomitant increase in the incidence of spontaneous neoplastic and non-neoplastic disease.

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Eisele, G. R.
A perspective on gossypol ingestion in swine
Vet Human Toxicol. 28, 118–122, 1986

C seed meal has been used for many years as a protein supplement in animal rations. Due to a polyphenol pigment, gossypol, present in cottonseed meal, its use for swine has been limited. The tolerance of gossypol for monogastric animals depends upon age and species with older animals being less sensitive than younger animals. Swine are highly susceptible to gossypol poisoning, and signs commonly develop after one or more months of consumption. The use of iron salts in the ration appears to be antidotal, and lysine supplementation of gossypol containing diets appears to improve gain. Reproductive performance can also be affected, including reduced conception and litter size. New extraction methodologies are being developed to reduce the amount of gossypol in cottonseed meal.

Eisele, G. R.
Metabolism and effects of organic compounds in animals
Vet. Hum. Toxicol. 27, 417–421, 1985

In recent years, society has become increasingly aware and concerned about protection from chemicals released into the environment. The knowledge of the metabolism and effects of organic compounds in animals, specifically food-producing animals, are of paramount importance in assessing potential human health hazards. An intensive effort has been directed at detection of chemicals in the environment, determination of their physiological insult and cellular interaction; in particular their carcinogenic and mutagenic induction capability. The chemical exposure of food-producing animals can be extremely difficult to evaluate and quite devastating. The exposure of d-producing animals to polybrominated biphenyls (PBBs) and orinated biphenyls (PCBs) emphasizes the seriousness of the problem. The polycyclic aromatic hydrocarbons (PAHs), capable of inducing cancer in experimental animals, have been studied extensively in laboratory animals. Current studies deal with the mechanism of metabolism of PAHs and the ultimate carcinogenic form. Although our knowledge concerning the health hazards of organic chemicals is continually increasing, its impact on food-producing animals and man's food chain is poorly understood. Awareness of the problem by practicing veterinarians and toxicologists, environmental toxicologists and public health officials is required to evaluate the impact of organic chemicals on the human food chain.

Eisele, G. R., Tsai, S. C., Payne, J., Kyle, B., and Urso, I.
The distribution of orally administered ^{14}C-aniline HCl in tissues of dairy cattle, swine and laying pullets
J. Anim. Sci. 61, 1492–1497, 1985

Single or chronic daily (30 d) doses of ^{14}C-aniline (an aromatic amine) were administered to dairy cattle, swine and laying pullets to determine the amount of an ingested synfuel-related chemical that would remain in consumable animal products. The ^{14}C-residues were found in muscle, liver, other edible organs and fat, as well as in milk and eggs. The predominant site of deposition in acute- and chronic-exposed pullets was kidney, followed by internal yolk (immature ovum of ovary) and then liver. Egg yolk was the major site of ^{14}C-radioactivity in whole eggs. Liver, then kidney, were the major sites of deposition in acutely exposed swine; when chronically exposed, only liver showed preferential deposition with other tissues ed yielding similar concentration values. Dairy cattle yielded t distribution patterns similar to chronic swine. Within 8 h after an acute exposure, ^{14}C-residue was detected in milk which reached its maximum at 24 to 32 h. Our data demonstrate that derived concentration values of ^{14}C-residue in tissues were dependent upon the species studies as well as on the mode of exposure, acute versus chronic. Although the concentration values were variable, sufficient amounts of ^{14}C-aniline and(or) its metabolites were found in consumable products.

Fagan, T. C., Nelson, E. B., Lasseter, K. C., Crook, J., Morledge, J., and Conrad, K. A.
Once- and twice-daily nitrendipine in patients with hypertension and noninsulin-dependent diabetes
Pharmacotherapy 6, 128–136, 1986

One hundred and six patients with uncomplicated mild to moderate essential hypertension received nitrendipine 10 mg twice a day or 20 mg once a day in a double-blind, randomized design. At the end of the dosing interval, supine and standing blood pressures were lowered 6/4 and 6/3 mm Hg respectively with the former regimen, and 2/3 mm Hg with the latter. In 10 patients, blood pressure variability through the dosing interval was not increased by nitrendipine 10 mg twice daily, but in another 10, 20 mg daily increased the variability by 28% compared to placebo. Significantly more patients had adverse effects with 20 mg daily than 10 mg twice daily; and both regimens caused more side effects than placebo. In 20 patients, serum glucose levels did not change significantly during treatment with nitrendipine, but in 10 of those with noninsulin-dependent diabetes, the range of maximum plasma insulin in response to a high-carbohydrate meal was 7 times as great during treatment with nitrendipine as it was during treatment with placebo. Nitrendipine lowers blood pressure when given once or twice daily, but twice-daily administration appears to be better tolerated.

Frome, E. L., and DuFrain, R. J.
Maximum likelihood estimation for cytogenetic dose-response curves
Biometrics 42, 73–84, 1986

In vitro dose-response curves are used to describe the relation between chromosome aberrations and radiation dose for human lymphocytes. The lymphocytes are exposed to low-LET radiation, and the resulting dicentric chromosome aberrations follow the Poisson distribution. The expected yield depends on both the magnitude and the temporal distribution of the dose. A general dose-response model that describes this relation has been presented by Kellerer and Rossi (1972, *Current Topics on Radiation Research Quarterly* 8, 85–158; 1978 *Radiation Research* 75, 471–488) using the theory of dual radiation action. Two special cases of practical interest are split-dose and continuous exposure experiments, and the resulting dose-time-response models are intrinsically nonlinear in the parameters. A general-purpose maximum likelihood estimation procedure is described, and estimation for the nonlinear models is illustrated with numerical examples from both experimental designs. Poisson regression analysis is used for estimation, hypothesis testing, and regression diagnostics. Results are discussed in the context of exposure assessment procedures for both acute and chronic human radiation exposure.

Hammond, B. F., and Martin, P. G.
Calcium-cadmium effects on bone density and strength of the femur from multiparous rats
Nutr. Res., Suppl. 1, 511–514, 1985

Pregnancies, in the biologically immature teenagers, are increasing (1). The stress of multiple pregnancies, superimposed on poor dietary intake of minerals such as calcium (Ca) as well as growth of skeletal and muscle tissue in the female, depletes nutrient reserves and increases risks to both mother and child (2, 3). Animal experiments suggest that further disturbances in Ca metabolism are shown in the presence of cadmium (Cd), an environmental pollutant (4, 5). Survey studies of human populations ingesting Cd over a period of years indicate that bone loss and breakage appeared to be enhanced in the multiparous woman (5). Limited work has been done on the

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interrelationship between Ca and Cd on bone strength and density as affected by age and parity. To study the effect of chronic exposure Cd over the reproductive life of the animal, the following study undertaken.

Hubbard, J. W., Cox, R. H., Lawler, J. E., Blank, M. L., and Snyder, F.
Cardiac output and peripheral vascular resistance during intravenous infusion of 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine in conscious normotensive and spontaneously hypertensive rats
Res. Commun. Chem. Pathol. Pharmacol. 49, 459–462, 1985

Cardiovascular responses to intravenous 1-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine (56 and 112 pmol/kg/min) were examined in conscious Wistar-Kyoto (WKY) normotensive rats and spontaneously hypertensive rats (SHR) instrumented with arterial and venous catheters, and an electromagnetic flow probe around the ascending aorta. The results suggest that this compound reduces mean blood pressure via a dose related fall in peripheral vascular resistance and cardia index in conscious SHR and WKY rats.

Klein-Szanto, A. J. P., Conti, C. J., Aldaz, C. M., Clapp, N., Nesnow, S., and Slaga, T. J.
Effects of chronic topical application of 12-O-tetradecanoylphorbol-13-acetate on the skin and internal organs of SENCAR mice
Environ. Health Perspect. 68, 75–80, 1986

Repetitive topical applications of 2 μg 12-O-tetradecanoylphorbol-13-acetate (TPA) twice weekly for 37 to 52 weeks induced a sustained epidermal hyperplasia, hyperplasia of hair follicles, and increased dermal cellularity in SENCAR mice. In addition, after 52 weeks of protracted promoter treatment most animals developed generalized amyloidosis involving liver and spleen, as well as interstitial nephritis. Severe pyelonephritis and papillary necrosis were also frequently seen. Reactive lymphoid hyperplasia was also a frequent finding. Chronic administration of TPA is not an innocuous treatment affecting only the interfollicular epidermis. The general effect of the promoter on the animals was a marked decrease in their longevity, probably through impairment of the immune system.

Lee, T-c., Kirk, P., and Snyder, F.
Testosterone induction of microsomal acyl-CoA reductase and a cytosolic regulatory protein in mouse preputial glands
Biochim. Biophys. Acta 875, 129–132, 1986

Alkyl and alk-1-enyl (plasmalogens) ether-linked glycerolipids are prominent components of many mammalian cells; moreover, an acetylated form of an alkyl phospholipid was recently found to possess potent hypotensive, inflammatory and allergic properties. In our studies, preputial glands of mice were selected as a model to investigate the regulation of factors involved in the biosynthesis of ether-linked lipids, since these glands contain high concentrations of ether-linked neutral lipids that are under the influence of hormonal control. We found that a key enzyme in the ether-lipid metabolic pathway, microsomal acyl-CoA reductase that catalyzes the formation of long-chain fatty alcohols (precursor of the *O*-alkyl chain), was increased 16-fold after injecting testosterone into male, castrated mice. This induction was highly specific, since testosterone did not affect other microsomal enzyme, NADPH-cytochrome *c* reductase. Based on kinetics of enzyme activity changes, the half-life of acyl-CoA reductase was calculated to be 61-70 h. In addition, the activity of a cytosolic stimulatory protein for the acyl-CoA reductase (but not for a different cytosolic protein, lactate dehydrogenase) was also enhanced in the testosterone-treated, male, castrated mice. These findings indicate that acyl-CoA reductase is an important regulatory enzyme in the reactions that lead to the formation of the ether bond in glycerolipids and that it is modulated through hormonal control.

Lee, T-c., Malone, B., and Snyder, F.
A new *de novo* pathway for the formation of 1-alkyl-2-acetyl-*sn*-glycerols, precursors of platelet activating factor
Biochemical characterization of 1-alkyl-2-lyso-*sn*-glycero-3-P:acetyl-CoA acetyltransferase in rat spleen
J. Biol. Chem. 261, 5373–5377, 1986

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkylacetyl-GPC, platelet activating factor, i.e, PAF) can be biosynthesized either by acetylation of alkyllyso-GPC through a remodeling pathway or by the transfer of phosphocholine to alkylacetyl-*sn*-glycerol (alkylacetyl-G) via a putative de novo pathway involving a dithiothreitol-insensitive cholinephosphotransferase. However, the relevance of the de novo pathway in the biosynthesis of PAF depends on the existence of enzymes that can directly synthesize alkylacetyl-G from 1-alkyl-2-lyso-*sn*-glycero-3-P (alkyllyso-GP) or some other source. In this study, we demonstrated that microsomal preparations of rat spleen can synthesize alkylacetyl-GP by an alkyllyso-GP:acetyl-CoA acetyltransferase and that this intermediate is subsequently dephosphorylated by an alkylacetyl-GP phosphohydrolase to generate alkylacetyl-G. The properties of alkyllyso-GP:acetyl-CoA acetyltransferase were characterized under conditions where the contaminating activity of alkylacetyl-GP phosphohydrolase was minimal; this was accomplished by inhibiting the phosphohydrolase with the addition of sodium vanadate and sodium fluoride to the assay mixtures and incubating at relatively low temperatures (23°C). Alkyllyso-GP:acetyl-CoA acetyltransferase had a pH optimum of 8.4 at 23°C and was located in the microsomal fraction. The apparent K_m for acetyl-CoA under these conditions was 226 μM and the optimal concentration of alkyllyso-GP ranged between 16–25 μM. Based on pH optima, substrate inhibition studies, and sensitivities to preincubation temperatures of the microsomes, it appears that alkyllyso-GP:acetyl-CoA acetyltransferase differs from the acetyltransferase responsible for the transfer of acetate from acetyl-CoA to alkyllyso-GPC to form PAF. A variety of tissues had high activities of alkyllyso-GP:acetyl-CoA acetyltransferase, which indicates this pathway is operational in many cell types. Our results document the existence of a complete de novo biosynthetic pathway for the assembly of PAF, and this route could be responsible for maintaining physiological levels of platelet activating factor for normal cell function.

Littlefield, L. G., and Joiner, E. E.
Analysis of chromosome aberrations in lymphocytes of long-term heavy smokers
Mutat. Res. 170, 145–150, 1986

We assessed the incidence of structural chromosome aberrations in 500 diploid first-division metaphases from 48-h lymphocyte cultures from each of 6 non-smokers and from 6 persons who had smoked a minimum of 1 pack of cigarettes per day for at least 20 years. Cytogenetic analyses of coded slides revealed a single dicentric chromosome with its accompanying fragment and two symmetrical chromatid exchanges in 3000 metaphases from the non-smokers. In contrast, 9 dicentric chromosomes, 8 translocations or inversions, and 7 chromatid exchanges were observed in 3000 metaphases from lymphocyte cultures from the 6 heavy smokers. A total of 13 metaphases having chromosome-type inter- or intra-changes was noted including 9 with a single aberration, and 4 with 2 or more. Our findings provide additional evidence of the in vivo clastogenicity of cigarette smoke in long-term heavy smokers, and further demonstrate that the distribution of chromosome-type exchange aberrations is overdispersed relative to that expected based on Poisson assumptions.

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Lushbaugh, C., Humason, G., and Clapp, N.
Histology of colon cancer in *Saguinus oedipus oedipus*
Dig. Dis. Sci. 30, 119S–125S, 1985

Diagnosis of colon cancer in tamarins often requires histologic (microscopic) inspection of the entire colon before minute primary sites can be located in the flat colonic mucosa. In other cases the cancer is easily recognized grossly because infiltration produces local desmoplastic reactions. The cancer does not have a preceding benign polypoid stage. The carcinoma is most typically poorly differentiated. The PAS stain, however, demonstrates that some of the malignant cells always produce mucin. With other special stains and electron microscopy, undifferentiated stem cells, mitochondria-rich absorptive cells, and cells rich in argentaffin granules are demonstrated. Most commonly the carcinomatous cells are distributed in structureless masses, but occasionally they form tubular structures that resemble glands. Preneoplastic epithelial changes in the crypts are disguised by epithelial hyperplastic (reparative) changes without hyperchromatic nuclear changes. Nuclear pleomorphism and enlarged nuclear/cytoplasmic ratios are commonplace in nonmalignant as well as malignant cells. All colon cancers found in this species arise in association with a pre-existing, chronic ulcerating colitis.

Robinson, M., and Snyder, F.
Metabolism of platelet-activating factor by rat alveolar macrophages: Lyso-PAF as an obligatory intermediate in the formation of alkylarachidonoyl glycerophosphocholine species
Biochim. Biophys. Acta 837, 52–56, 1985

1-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (alkylacetyl-GPC; platelet-activating factor;PAF) is actively taken up and metabolized by rat alveolar macrophages maintained in culture. The major metabolic products are lyso-PAF (alkyllyso-GPC) and alkylacyl-GPC. Lyso-PAF accumulates primarily in the media, whereas alkylacyl-GPC is predominantly associated with cellular lipids. The addition of unlabeled lyso-PAF to incubations initiated with [^{3}H]PAF results in an increase in the amount of lyso-[^{3}H]PAF product formed and a decrease in the final product, [^{3}H]alkylacyl-GPC; however, the total amount of [^{3}H]PAF metabolized remains unchanged. Unlabeled lyso-PAF thus enters the metabolic pool of the cell and competes with the deacetylated product of [^{3}H]PAF, i.e., lyso-PAF, for acylation. High-performance liquid chromatography demonstrated that the reacylated product derived from lyso-PAF consisted primarily of the arachidonoyl-containing species that exists as the 16:0–20:4 molecular species. These results document that PAF is inactivated in rat alveolar macrophages via a deacetylation-reacylation reaction with lyso-PAF as an obligatory intermediate. The sequestering of arachidonic acid into the PAF precursor pool and the substantial amount of lyso-PAF secreted by macrophages into the extracellular fluid appear to be significant events in the inactivation process.

Snyder, F., Lee, T-c., Blank, M., Malone, B., Woodard, D., and Robinson, M.
Platelet-activating factor (PAF): Alternate pathways of biosynthesis, mechanism of inactivation, and reacylation of lyso-PAF with arachidonate
Adv. Prostaglandin Thromboxane Leukotriene Res. 15, 693–696, 1985

The chemical structure of the bioactive phospholipid known as platelet-activating factor (PAF) was identified in 1979 by three independent groups of workers as 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (GPC). The many diverse biological activities, in addition to the well-known effects of PAF on aggregation and degranulation of platelets or neutrophils and its potent hypotensive actions, have been reviewed elsewhere. This presentation summarizes some of our recent findings regarding (a) the biosynthesis of PAF by alternate pathways (acetyltransferase versus cholinephosphotransferase) and the possible biological significance of each route, (b) de novo synthesis of PAF precursors, (c) the mechanism of PAF inactivation, and (d) the mechanism of arachidonate specificity in reacylation of "lyso" -PAF to 1-alkyl-2-arachidonoyl-GPC.

Tardif, S. D.
Histologic evidence for age-related differences in ovarian function in tamarins (*Saguinus* sp.; primates)
Biol. Reprod. 33, 993–1000, 1985

The ovaries of 15 tamarins (Saguinus sp.) between 2 and 16 yr of age were collected at necropsy and examined for evidence of age-related differences. As expected, there was a marked decline in the number of primary follicles with advancing age. Age and the number of primary follicles available had minimal effect on the number of follicles recruited into the preantral population; however, there were significant decreases in the number of large preantral and antral follicles with increasing age. The observed decline in follicular development with age was consistent with a reproductive decline with age observed in some tamarin females. Luteal-like masses were found in all ovaries examined and their size and appearance was unrelated to age.

Tardif, S. D., Carson, R. L., and Gangaware, B. L.
Comparison of infant care in family groups of the common marmoset (*Callithrix jacchus*) and the cotton-top tamarin (*Saguinus oedipus*)
Am. J. Primatol. 11, 103–110, 1986

The involvement of parents and siblings in infant care in similarly composed groups of common marmosets (*Callithrix jacchus*) and cotton-top tamarins (*Saguinus oedipus*) was compared during the infants' first 8 weeks of life. The results indicate an earlier infant independence in *C. jacchus* than in *S. oedipus* due primarily to a more frequent rejection of carried infants in *C. jacchus*. There was no species difference in extent of maternal involvement in carrying infants. However, *S. oedipus* fathers carried infants significantly more often during weeks 5–8 than did *C. jacchus* fathers. Siblings were generally more involved in infant care at an earlier infant age in *C. jacchus* than in *S. oedipus*.

Urso, P., Gengozian, N., Rossi, R. M., and Johnson, R. A.
Suppression of humoral and cell-mediated immune responses in vitro by benzo(a)pyrene
J. Immunopharmacol. 8, 223–241, 1986

The effect of a benzo(a)pyrene (BaP) at different molar (M) concentrations on the in vitro anti-sheep red blood cell (SRBC) plaque (antibody) forming cell (PFC) response and the one-way mixed lymphocyte response (MLR) was tested. Inhibition of the PFC response and the MLR occurred when spleen cells were exposed to a wide range of BaP concentrations from 10^{-4} M to 10^{-8} M. Maximum depression of the responses occurred at 10^{-5} M for PFC production (47% of controls) and for the MLR (19% of controls) as measured by a stimulation index. No significant loss in cell viability was observed at this or lower molar concentrations of BaP. The non-carcinogenic analog of BaP, benzo(e)pyrene, did not suppress PFC responses at comparable concentrations. This in vitro system will facilitate manipulations of T and B lymphocytes and macrophages (adherent cells) in a controlled culture environment for precisely characterizing the sensitivity of these cells and their subpopulations on exposure to BaP.

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Washburn, L. C., Blair, L. D., Byrd, B. L., and Sun, T. T.
Comparison of ^{68}Ga-EDTA, [1-^{11}C]α-aminoisobutyric acid, and [^{99m}Tc]sodium pertechnetate in an experimental blood-brain barrier [illegible]
Int. J. Nucl. Med. Biol. 12, 267–269, 1985

Unilateral opening of the blood-brain barrier (BBB) in rats, induced by infusion of a hyperosmotic solution of mannitol into the left internal carotid artery, was applied to compare ^{68}Ga-EDTA, [1-^{11}C]α-aminoisobutyric acid (using the long-lived ^{14}C-labeled analog), and [^{99m}Tc]sodium pertechnetate as agents for diagnosis of BBB disruption. Of the three agents and two time intervals studied, ^{68}Ga-EDTA at 30 min. postinjection gave the highest target-to-nontarget ratio. In addition, ^{68}Ga-EDTA, unlike commonly used [^{99m}Tc]sodium pertechnetate, can be used in conjunction with positron emission tomography, which could make possible earlier and better assessment of BBB defects using ^{68}Ga-EDTA.

Washburn, L. C., Hwa Sun, T. T., Crook, J. E., Byrd, B. L., Carlton, J. E., Hung, Y.-W., and Steplewski, Z. S.
^{90}Y-Labeled monoclonal antibodies for cancer therapy
Nucl. Med. Biol. (Int. J. Radiat. Appl. Instrum., Part B) 13, 453–456, 1986

Monoclonal antibody 17-1A, which has specificity for colorectal carcinoma, was labeled with ^{90}Y (10–20% radiolabeling yield). Tissue distribution studies in tumor-bearing nude mice were carried out. ^{90}Y-labeled 17-1A showed good uptake in the SW 948 colon carcinoma cell line. However, ^{90}Y-labeled A5C3, a monoclonal antihepatitis virus antibody studied as a control, showed similar uptake in this tumor. Neither antibody was taken up well by a WM-9 melanoma. It is believed that the loss of specificity observed is due to the low specific activity of the ^{90}Y-labeled monoclonal antibody preparations used. This hypothesis is supported by radioimmunoassay data.

Major Awards, Honors, and Appointments

C. F. Allred, S. A. Fry, H. Hodges, J. S. Lambert, C. C. Lushbaugh, R. C. Ricks, and A. H. Sipe
[illegible]ented the Federal Laboratory Consortium Special Award for Excellence in Technology Transfer

D. Cragle
Member of the International Nickel Speciation Research Steering Committee

S. A. Fry
Member of the National Council on Radiation Protection and Measurements Scientific Committee's Task Force on Uranium (SC57-15)

P. Groer
Member of the National Council on Radiation Protection and Measurements Committee on Radiation Exposure and Potentially Related Injury

Member of the National Academy of Sciences' BEIR IV Alpha Committee on Health Risks of Internally Deposited Alpha Emitters

C. C. Lushbaugh
Presented the Western Regional Landauer Award by the American Association of Physicists in Medicine

L. G. Littlefield
Member of the National Council for Radiation Protection Scientific Committee Number 40 on Biological Aspects of Radiation Protection

U.S. Representative in the International Atomic Energy Agency Coordinated Research Program on Use of Chromosomal Analysis in Radiation Protection

[illegible]mber of the Editorial Board of *Environmental Mutagenesis*

[illegible]mber of the Cytogenetics Working Group on Bioassay in Response to Public Law 98-542

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Member of the Environmental Protection Agency's Gene-tox Program Committee on Mammalian Cytogenetics

F. Snyder
Member of the Editorial Board of *Archives of Biochemistry and Biophysics*

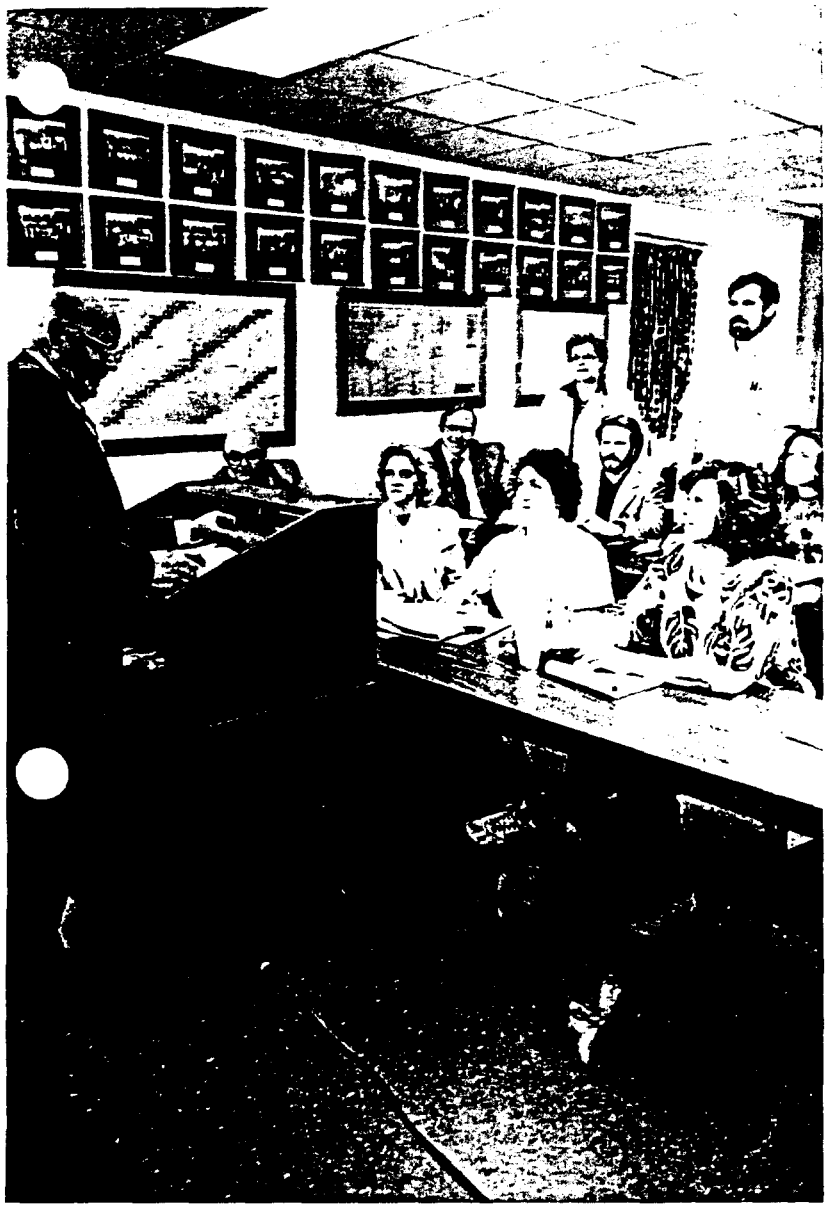
Member of the Editorial Board of *Lipids*

Member of the Editorial Board of *Journal of Biological Chemistry*

Editor of *Handbook of Lipid Research*, a continuing series, Plenum Publishing Corporation, New York

Chairman of USA Organizing Committee for the Second International Conference on Platelet Activating Factor

1028743



The REAC/TS training faculty includes staff from the Cytogenetics, Epidemiology, Nuclear Medicine, and Occupational Medicine Programs.

Training Programs

Training programs at the division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. Special short-term courses dealing with radiation health problems are available for biomedical personnel.

Graduate students participating in Ph.D. training programs have come from the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama at Birmingham, and University of Kentucky (Lexington). Students can do graduate research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals, and the advanced degrees are awarded by the parent university where the student completes the academic requirements. Stipends for competitive predoctoral fellowships have been obtained from a National Cancer Institute Training Grant administered by Oak Ridge National Laboratory, University Programs Division at ORAU (sponsored by DOE), and individual research grants.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows have been available from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health and American Cancer Society Grants, the World Health Organization (Interna-

1028744

tional Agency for Research on Cancer), ...S. DOE Postgraduate Research ...ning, the Fogarty International Center (U.S. Public Health Service), and division funds.

Summer research opportunities make it possible for faculty and advanced undergraduate students from colleges and universities throughout the United States to participate in any of the division's research programs. The DOE-sponsored summer program is administered through the University Programs Division at ORAU.

Short-term courses in the management of medical problems associated with radiation accidents are formally offered to physicians, health physicists, and paramedical personnel at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. Special courses for attorneys are planned in the future. A pilot course "Basic Concepts in Radiation for Attorneys" was successfully completed this year. A total of eleven REAC/TS courses was given during the past 12 months.

Some of the senior staff at the division conduct special courses and give lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee's Knoxville and Memphis campuses. Several staff scientists have joint appointments at ORAU universities where they teach and also serve as advisors to graduate students who pursue their research studies at ORAU.

Visiting scientists from various universities and other institutions from the United States and abroad also participate in division research programs. Many of these individuals are sponsored through the Research Participation Programs administered by ORAU's University Programs Division. In some instances, funds for the visiting scientists are also awarded through various national or international agencies, and occasionally, appointments for visiting scientists are made directly with programmatic funds from the division.

The following individuals participated in the training programs at the division during the past year:

1. **Predoctoral students**
   - Arvind Carpenter* (Epidemiology) (University of Alabama)
   - Elizabeth Dupree Ellis† (Epidemiology) (University of North Carolina)
   - Toni H. Newport (Epidemiology) (University of Alabama at Birmingham)
   - Judith Qualters (Epidemiology) (University of North Carolina)
   - Zigrida Smith, (Biochemistry) (University of Tennessee)
   - Jefferson Surles* (Medicinal Chemistry) (University of North Carolina)
   - Maria Wagner (Biochemistry) (University of Tennessee, Oak Ridge)
   - Donna Woodard† (Biochemistry) (University of Tennessee)
2. **Postdoctoral fellows**
   - Arvind Carpenter (Epidemiology)
   - Tomio Kawasaki (Biochemistry)
   - Yu-Chen C. Lee (Radiopharmaceuticals)
   - Changgeng Qian (Biochemistry)
   - Michel Record (Biochemistry)
   - David Vallari (Biochemistry)
3. **Visiting scientists**
   - John L. Hubbard (Radiopharmaceuticals) Marshall University, Huntington, West Virginia (Sponsored by University Programs Division)
   - Velma Richardson (Cytogenetics) Tuskegee University, Tuskegee, Alabama (Sponsored by University Programs Division)
   - B. DeSales Lawless (Radiopharmaceuticals) Fordham University, New York, New York (Sponsored by University Programs Division)
   - Jiang Tao (Cytogenetics) Laboratory of Industrial Hygiene, Ministry of Public Health, Beijing, China (Sponsored by the International Union Against Cancer)
   - Jixian Wang, M.D. (Epidemiology) Chinese Academy of Medical Sciences, The People's Republic of China (Sponsored by the Chinese Academy of Medical Sciences)
4. **College student research participants**
   - Andrew P. Hinck (Radiopharmaceuticals) University of Puget Sound, Tacoma, Washington
   - Jeff E. Flickinger (Biochemistry) Maryville College, Maryville, Tennessee
5. **Graduate student research participants**
   - Michael J. Keefe (Clinical Nuclear Medicine) University of North Carolina, Chapel Hill
6. **High school vocational education students**
   - Melinda Armes (Epidemiology) Harriman High School
   - Vicki Brown (Biochemistry) Anderson County High School
   - Dianne Jarnigan (Biochemistry) Anderson County High School
   - Lori McKamey (Biochemistry) Anderson County High School
   - Wendy Packan (Nuclear Medicine) Oak Ridge High School
7. **East Tennessee Human Resources students**
   - Margo Burum (Epidemiology)
   - Constance Coletta (Epidemiology)
   - Thaddeus Fortenberry (Epidemiology)
   - Kimberly Hurd (Epidemiology)

*Portion of thesis research being done at the division.
†Thesis research being completed at the division.

1028745

Non-DOE-Funded Research

W. W. Burr

Biomedical research support grant. National Institutes of Health, Division of Research Resources, $19,796* (1 April 1986 to 31 March 1987; current year of continuing award)

W. W. Burr

Evaluation of devices and techniques to determine previous radiation exposure. National Cancer Institute, $261,220* (2 September 1985 through 1 August 1987; carried over, no additional funding)

N. K. Clapp

Operation of a marmoset colony for cancer research. National Cancer Institute, $283,000*† (1 June 1986 to 31 May 1987; second yr of 3-year award)

N. K. Clapp

Support of marmoset colony. National Foundation for Ileitis and Colitis, $25,000*‡ (1 July 1986 to 30 June 1988; second year of 3-year award)

N. K. Clapp

Collaborative studies of colitis and colon cancer in tamarins. Harbor-UCLA School of Medicine, $43,600*‡ (1 September 1986 to August 1990; second of five years)

G. R. Eisele

Food chain transport of synfuels. U.S. Environmental Protection Agency-U.S. Department of Energy Interagency Agreement, $67,674* (1 October 1985 to 30 September 1986; closing year of award)

S. A. Fry

Continuous work history study. National Cancer Institute, $25,000* (19 August 1986 through 30 September 1986)

L. G. Littlefield

Studies of radiation-induced chromosome damage in humans. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $201,802* (30 September 1986 to 29 September 1987; current year of continuing award)

A. F. McFee

Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $362,770* (30 September 1986 to 29 September 1987; second year of 2-year award)

R. C. Ricks

Development and implementation of radiological training courses for health physics and medical personnel and to conduct a national teleconference entitled "Handling the Radiation Accident Victim in the Hospital Emergency Departmentment," December 17, 1986. Federal Emergency Management Agency, $178,370* (30 September 1986 through 30 September 1987)

R. C. Ricks

Reactor status considerations in emergency decision making. Federal Emergency Management Agency, $98,000* (1 June 1985 through 30 September 30 1987; carried over to FY87; no additional funding)

F. Snyder

An antihypertensive class of neutral lipids in kidney. National Heart, Lung, and Blood Institute, $80,839§ (1 December 1985 to 30 November 1986; first year of 3-year award)

F. Snyder

A novel class of biologically active phospholipids. National Heart, Lung, and Blood Institute, $97,950§ (1 April 1986 to 31 March 1987; first year of 5-year award)

F. Snyder

Metabolism, regulation, and function of ether-linked glycerolipids and other lipid analogs in cancer cells. American Cancer Society, $97,200* (1 January 1986 to 31 December 1986; first year of a 2-year award)

F. Snyder

Ether lipids in cancer—enzyme mechanisms. National Cancer Institute, $70,147§ (30 September 1986 to 30 June 1987; first 9 months of a 5-year award)

S. D. Tardif

Determinants of reproductive competence in tamarins. National Institutes of Health, Division of Research Resources, $45,350§ (5 September 1986 to 4 September 1987; third year of 3-year award)

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer therapy. National Cancer Institute, $89,032§ (1 September 1986 to 31 August 1987; second year of 3-year award)

*Total costs, i.e., direct plus overhead (current year).
†Not under DOE prime contract.
‡These collaborative awards are reimbursable to the NCI contract.
§Direct costs (current year).

1028746

Collaborative Research

N. K. Clapp

Genetic epidemiology and immunologic parameters in tamarin colitis and colon cancer. Harbor-UCLA School of Medicine (Los Angeles), Department of Medicine, G. Petersen; Division of Gastroenterology, W. Snape, S. Targan

In vitro tetraploidy in skin fibroblasts of colon cancer tamarins. Cornell Medical College (New York), Laboratory of Cell Biology, B. S. Danes

Coronaviruses in colitis and colon cancer. University of Tennessee (Knoxville), College of Veterinary Medicine, D. Brian

Ontogenetic skeletal changes in *Saguinus fuscicollis* and *Saguinus oedipus*. University of Tennessee (Knoxville), Department of Anthropology, W. Bass

Lectin binding in colitis and colon cancer in cotton-top tamarins. VA Medical Center (Ann Arbor), Gastroenterology Section, C. R. [illegible]d

[illegible]al toxins in tamarin colon disease. University of Tennessee Medical Center (Knoxville), J. Fuhr

Idiopathic ulcerative colitis in marmosets. University of Nevada, School of Medicine (Reno), O. Peck; The Ohio State University (Columbus), Physiology Department, J. Wood

CEA and LDH_x in colon-cancer bearing tamarins. Roswell Park Memorial Institute (Buffalo), Surgical Developmental Oncology Department, N. Petrelli

Heavy metal toxicity in tamarins. University of Tennessee (Knoxville), School of Veterinary Medicine, W. Farkas

Marmoset colonic antigen reactivity with human ulcerative colitis antibody. Albert Einstein College of Medicine (Bronx), K. Das

Electrophoretic survey of genetic diversity of tamarin colony. Laboratory Viral Carcinogenesis (Frederick, Maryland), Section of Genetics, L. Forman

Evaluation of immunosuppression in marmosets. University of Tennessee Memorial Medical Center (Knoxville), J. Fuhr

Oncogenicity of Epstein-Barr Virus. The Ohio State University (Columbus), Department of Medical Microbiology and Immunology, R. Glaser

Nutritional requirements of marmosets. Laboratory Animal Nutritionist, National Institutes of Health (Bethesda), J. Knapka

Expression of oncogenes in inflammatory bowel diseases in cotton-top tamarins. Harbor-UCLA Medical Center (Torrance), Department of Medicine, S. Meltzer

[illegible]ication of fecal mutagens and fecal microflora in tamarins and [illegible]osets. National Cancer Institute (Frederick, Maryland), Laboratory of Comparative Carcinogenesis, J. Rice, P. Donovan

Monoclonal antibodies to detect tamarin colon cancer. The University of Texas (Galveston), Department of Surgery, C. Townsend

J. E. Crook

Development of software for quantitative positron tomography. The University of Tennessee Medical Center (Knoxville), Department of Medicine, G. Smith; Vanderbilt University (Nashville), Department of Biomedical Engineering, P. H. King

^{11}C-Amino acids/positron ECT for pancreatic studies. Brookhaven National Laboratory (Upton, New York), Medical Department, A. B. Brill

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp

Clinical use of radiopharmaceuticals in human disease. The University of Tennessee Medical Center (Knoxville), Department of Radiology, K. F. Hübner

Use of radiopharmaceuticals in animal models of human disease. The University of Tennessee (Knoxville), College of Veterinary Medicine, R. E. Gompf

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. Fordham University (New York), Department of Science, B. D. Lawless

The study of prejunctional alpha-adrenoceptors in New England Deaconess Hospital rats. The University of Houston, Department of Pharmacology, D. C. Eikenburg

S. A. Fry

Morbidity among workers exposed to mercury at the Oak Ridge Y-12 plant. University of Michigan (Ann Arbor), Department of Environmental and Industrial Health, L. Fine

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Radiation Epidemiology Branch (Bethesda), R. Kleinerman, J. Boice

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons. Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straume, A. Carrano, J. Minkler; Los Alamos National Laboratory (Los Alamos), L. Devan; Radiation Effects Research Foundation (Hiroshima), A. Awa

Coordinated research program on Routine Use of Chromosomal Aberration Analysis in Occupationally Exposed Radiation Workers. International Atomic Energy Agency (Vienna), A. Bianco

Cytogenetic follow-up studies of a survivor exposed to ^{241}Am. Hanford Environmental Health Foundation (Washington), B. D. Breitenstein

Cytogenetic follow-up studies in Mexican ^{60}Co accident patients. Ciudad Universitaria (Mexico), Christina de Nava, P. Ostrosky

Evaluations of HGPRT mutants in lymphocytes of previously irradiated patients. University of Vermont, Patrick O'Neill.

Human sperm cytogenetics (assessment of radiation-induced chromosomal aberrations after accidental exposure). Lawrence Livermore National Laboratory, B. Brandriff

The effect of methyl acetimidate on cellular proliferation and chromosome structure. Tuskegee University (Alabama), Department of Biology, V. Richardson

C. C. Lushbaugh

United States Transuranium Registry. Hanford Environmental Health Foundation, B. D. Breitenstein

1028747

C. C. Lushbaugh and N. K. Clapp

Marmoset T and B cellular immunology. Meharry Medical College (' 'ille), Department of Microbiology, P. Patel

C. C. Lushbaugh and S. A. Fry

Epidemiology of human carcinogenesis. University of Alabama at Birmingham, Department of Epidemiology, P. Cole

Comprehensive epidemiology of DOE atomic workers. University of North Carolina (Chapel Hill), Department of Epidemiology, C. M. Shy

C. C. Lushbaugh and L. C. Washburn

Diethylenetriaminepentaacetate (DTPA) project. Department of Energy contractors, 45 coinvestigators

A. F. McFee

In vivo mouse assay for chemically induced SCE and chromosomal aberrations. Brookhaven National Laboratory, R. Tice

F. Snyder

Biological effects of unique phospholipid analogs. California State University (Northridge), Department of Chemistry, J. Hajdu

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Pi---adosi

(vascular and sympathetic effects of 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein

Synthesis and biochemical studies of analogs of platelet-activating factor. Queens College of the City University of New York, Flushing, Department of Chemistry, R. Bittman

Platelet activating factor levels and acetylhydrolase activities in plasma from cancer patients treated with interleukin II. Loyola Medical Center (Maywood, Illinois), J. Godwin

Platelet activating factor metabolism in insects. Louisiana State University (Baton Rouge), E. N. Lambremont

S. D. Tardif

Reproductive aging in tamarin females. University of Wisconsin (Madison), Department of Psychology, C. Snowdon and T. Ziegler

Demographic analyses and computerized colony management. Southwest Foundation for Biomedical Research (San Antonio), Department of Genetics, B. Dyke, T. Gage

Kin recognition and sexual interactions in marmosets and tamarins. Kent State University (Ohio), Departments of Anthropology and Biology, M. L. Harrison

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. The Wistar Institute (Philadelphia), Z. Steplewski

Production of ^{11}C- and ^{18}F-labeled radiopharmaceuticals. Brookhaven National Laboratory, Chemistry Department, J. S. Fowler

Short-lived alpha-emitting radionuclides for labeling monoclonal antibodies. Oak Ridge National Laboratory, Chemical Technology Division, J. E. Bigelow

Synthesis of labeled compounds using organoborane techniques. University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka

Synthesis of ^{18}F-labeled amino acids for tumor imaging. Marshall University (Huntington, West Virginia), Department of Chemistry, J. L. Hubbard

1028748

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1028749

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*Personnel no longer here
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[3]Educational Leave
[4]Consultant
[5]Student Research Participant (University Programs)
[6]Part-time Temporary
[7]Part-time Continuing
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1028750

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1028751

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ORAU 88/B-14



Oak Ridge Associated Universities
Medical and Health Sciences Division

Research Report 1986–1987

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This report is based on work performed primarily under contract number DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities.

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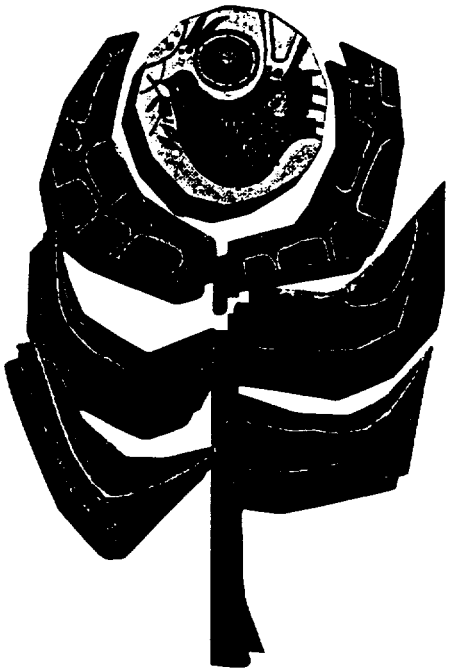
Printed in the United States of America
Available from
National Technical Information Service
U.S. Department of Commerce
5285 Port Royal Road
Springfield, VA 22161

NTIS price codes
Printed copy: A03
Microfiche copy: A01

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Research Report 1986–1987

"Hymn to Life," a mural by Charles Counts, was originally commissioned in honor of patients with cancer who were treated at ORAU's Medical and Health Sciences Division, a pioneer in the use of radionuclides to diagnose and treat cancer. Although the Division no longer operates a hospital, this mural still symbolizes the diversity of the research programs that contribute to the goals and objectives of the Division. According to the artist, the mural's small clay tile pieces, or "tessarae," come together in this work to represent the natural growth and symbiosis of the Medical and Health Sciences Division's activities.



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Preface

Our Research Report for 1986–87 reflects continued growth and a bright outlook for research programs at the Medical and Health Sciences Division. This report provides a brief overview of the current status of our research, training, and service programs in light of the progress and changes of the past year. For more comprehensive coverage of our research activities, the reader is referred to staff publications in the literature and to other special reports cited elsewhere in the report.

The following highlights reflect some of the more significant accomplishments and illustrate the diversity of our research efforts.

A significant achievement in the biochemistry program was the completion of an in-depth study of the properties of two key biosynthetic enzymes (alkyllyso-glycero-P acetyltransferase and alkylacetylglycero-P phospho-hydrolase) and the identification of the regulatory steps in the de novo pathway of biosynthesis that control physiological levels of PAF, a potent bioactive phospholipid. These findings extend major earlier contributions that originally described the two enzymatic pathways involved in PAF biosynthesis.

A new ^{90}Y radiolabeled monoclonal antibody that was developed and tested by the Preclinical Nuclear Medicine Program in animal trials showed promise as a potential clinical agent in detecting colorectal and pancreatic cancers.

Cytogenetic researchers continued to investigate the mechanisms involved in radiation damage in human cells. This year, they observed that the relative effectiveness of radiation in causing chromosome damage can be reduced up to 50 percent when human or other mammalian cells are exposed to X-rays in the presence of dimethyl sulfoxide; the latter is an excellent in vitro radioprotective agent since it selectively scavenges OH radicals that are responsible for inducing DNA lesions.

New collaborative arrangements in the Marmoset Research Program were established with several university groups and the Frederick Cancer Research Facility to investigate the role of colitis, mutagenicity, and depressed immune functions in the development of colonic carcinoma in the cotton-top tamarin. Also, grant support from the National Institutes of Health was awarded for studies of behavioral and endocrine factors as determinants of reproductive competence in the marmoset.

Radiation Emergency Assistance Center/Training Site personnel provided help and advice to Brazilian physicians, health physicists, dosimetry experts, and environmentalists following an accident in Goiania, Brazil, in which 244 persons were exposed to radioactive cesium-137.

1028757

Results of two published studies by the Center for Epidemiologic Research revealed there was no association between occupational exposure to radiation or uranium and increased cancer death rates at the occupational exposures encountered by workers at nuclear plants operated for DOE and its predecessor agencies.

The Committee on Interagency Radiation Research and Policy Coordination, now in its fourth year of operation, issued a report of national importance on the guidance for U.S. policy on use of the international system of units for radiation quantities and measurements.

A Center of Excellence for DOE's Human Reliability Program, designated as the Center for Human Reliability Studies, was established as a unit of this division. The Center provides DOE with technical support, education and training, operational review, research, and analysis for all aspects of the DOE Human Reliability Program.

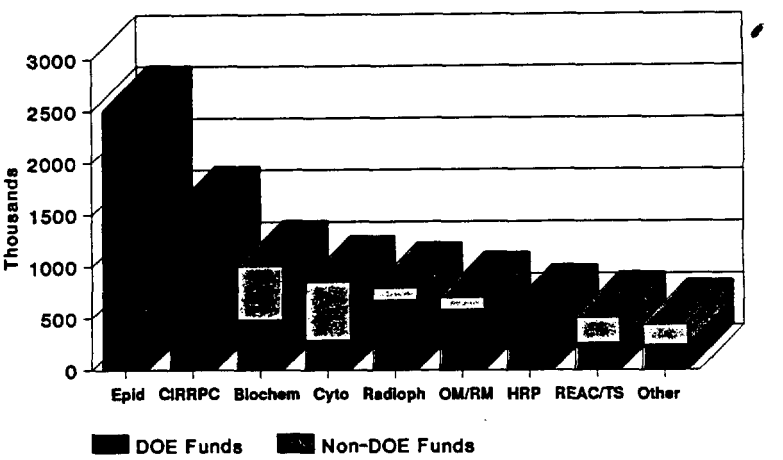
Descriptions of all ongoing research programs can be found in the scope section of this report; also summarized is a special project conducted for the National Cancer Institute of the National Institutes of Health. An update of other activities covers publications and presentations at national and international meetings; collaborative research efforts; training programs; current staffing and funding; and major awards, honors, and appointments of division staff.

Additional information about the Medical and Health Sciences Division can be obtained from the Chairman's Office.

W. W. Burr, Jr.
Chairman

Fred Snyder
Associate Chairman

FY 1987 Funding Sources
Medical and Health Sciences Division



1028758

Table of Contents

1028759

Scope of Programs

Biochemistry 1
Preclinical and Clinical Nuclear Medicine 2
Cytogenetics 5
Marmoset Research 8
Radiation Emergency Assistance Center/ Training Site 10
Center for Epidemiologic Research 12
Committee on Interagency Radiation Research and Policy Coordination 14
Occupational Medicine 15
Evaluations and Techniques to Determine Previous Radiation Exposure 16
Center for Human Reliability Studies 17

Publications, Abstracts, Summaries, and Invited Lectures

Full-Length Journal Articles 18
Chapters, Review Articles, Government Reports, and Other Publications 19
Abstracts (Published) of Papers Presented at Meetings 22
Summaries 23
Invited Lectures Presented at National and International Scientific Conferences 28

Collaborative Research 30

Training Programs 32

Major Awards, Honors, and Appointments 34

Patents Pending 34

Non-DOE-Funded Research 35

Medical and Health Sciences Division Staff 36

Administration and Committees 39

1028760

Scope of Programs

1028761

Biochemistry

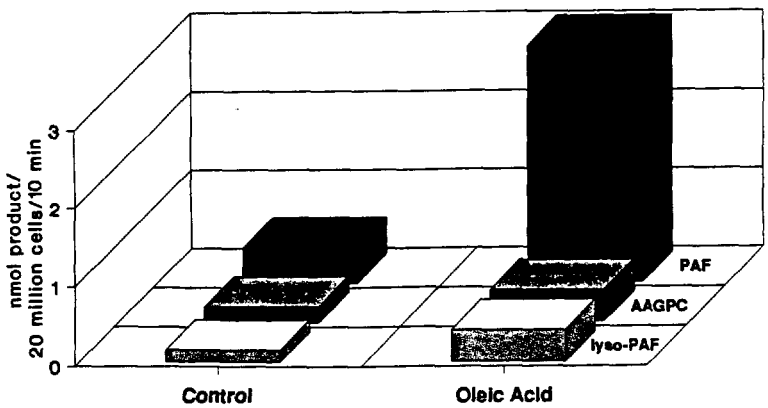
Program Director:
Fred Snyder

Research efforts in the Biochemistry Program continue to center on three major areas of research: phospholipids as cell mediators that include platelet activating factor (PAF), selective antitumor ether-linked phospholipid analogs, and the role of phospholipids in cell membranes. This program focuses on enzymatic pathways, cellular metabolism and regulation, mechanisms of action of biologically active lipids, and the function of ether-linked and diacyl types of glycerolipids in membranes of mammalian cells. Experimental models range from molecular and cell culture systems to intact animals. Objectives are to establish the precise role of phospholipids and other glycerolipids in fundamental biochemical processes required for health and how such processes are altered in pathological conditions such as cancer, hypertension, inflammation, and respiratory disorders. Recent significant achievements in the Biochemistry Program are described in the following highlights.

Detailed properties of two important enzymes (a phosphohydrolase and a cholinephosphotransferase) in the de novo biosynthesis of platelet activating factor (PAF) were characterized. These enzymes catalyze essential steps required in the de novo route for the biosynthesis of PAF, a potent cellular phospholipid mediator involved in blood pressure, inflammatory, and allergic responses.

Kinetic features of lysophospholipase D (an enzyme known to selectively degrade lyso forms of ether-linked lipids) were recently

Stimulation of the de Novo Pathway for the Biosynthesis of Platelet Activating Factor (PAF) in Cells with Minimal Endogenous Production of PAF



Erlich ascites cells were incubated in buffer for 10 minutes in the absence or presence of oleic acid; phenylmethylsulfonylfluoride, an acetylhydrolase inhibitor, was present in all incubations to minimize the catalytic hydrolysis of the acetate from PAF. These data combined with other results (J. Biol. Chem., in press) indicate that the de novo pathway for PAF biosynthesis can be influenced by physiological factors and that in this instance, the cytidylyltransferase activity (which catalyzes a rate-limiting step) is enhanced by the addition of oleic acid to the cell media.

1028762

delineated in rabbit kidney membranes. The results revealed distinctly different cation requirements for this enzyme among animal species and emphasize that lysophospholipase D can exert a decisive catabolic role in the intermediary metabolism of PAF.

A novel acyltransferase (distinctly different from other acyltransferases) was discovered that forms an acetylated neutral lipid, a new class of lipids thought to function as an inactive reservoir of an immediate precursor available for direct conversion to biologically active PAF molecules. This enzymatic evidence for a neutral lipid storage form of acetylated lipids in these experiments further stresses the potential importance and ubiquity of PAF-related lipids as physiological mediators.

A new direction in the PAF project focuses on the regulatory steps that control PAF levels in both cellular and extracellular compartments. A breakthrough in understanding the regulation of the de novo biosynthesis of PAF resulted from experiments that identified the two enzymes catalyzing the rate-limiting steps in this pathway. The first, cytidylyltransferase, is required for the production of the water-soluble precursor of the phosphocholine moiety of PAF. The second, alkyllysoglycerophosphate acetyltransferase, is responsible for the formation of the lipid (alkylacetylglycerol) portion of PAF.

Mechanistic kinetic studies on the acylation of alkyl lysophospholipids by acyltransferases in tumor cell membranes have demonstrated that transacylation by a CoA-independent transacylase generates a much greater degree of polyunsaturation in membrane phospholipids than occurs via either the acyl-CoA-dependent acyltransferase or the CoA-dependent transacylase. Results of this study also emphasize the complexity of factors and/or conditions that affect the turnover of fatty acids at the *sn*-2 position of membrane phospholipids.

A new high performance liquid chromatographic method was developed for the separation of the three different subclasses (diacyl, alkylacyl, and alk-1enylacyl) of glycerol-containing phospholipids. The approach used—phospholipase C and benzoate derivatization—offers major advantages over any other procedure by providing rapid automatic direct on-line quantitation during chromatography and increased sensitivity of detection.

Collaborative work with Dr. Robert Bittman at Queens College of the City of New York led to the synthesis and subsequent biochemical studies of two structurally related analogs of PAF (2-methyl-2-methoxy- and 2-methyl-2-lyso-PAF). Studies of PAF analogs such as these in biochemical systems provide useful information about structural-functional relationships with respect to both enzymatic and tumor cytotoxic responses. For example, the present investigation revealed that a lyso-grouping at the *sn*-2 position (2 methyl-2-lyso-PAF) greatly diminishes the tumor cytotoxic activity of such compounds. Also, neither PAF analog was found to inhibit the primary enzymes in the PAF remodeling pathway; however, the lyso grouping of the 2-methyl-2-lyso analog appears to be acetylated by the same acetyltransferase that acetylates lyso-PAF.

Preclinical and Clinical Nuclear Medicine

Program Director:
James E. Crook

The major objectives of the Preclinical and Clinical Nuclear Medicine programs are to develop new radiopharmaceutical agents and to carry out phase I and phase II testing of such materials in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of potential agents for radiodiagnosis and radiotherapy, tests of these substances in animals for effectiveness and toxicity, and clinical efficacy trials in humans according to U.S. Food and Drug Administration rules and guidelines.

Monoclonal Antibodies

Preclinical development of radiolabeled monoclonal antibodies for diagnosis and therapy of cancer continues to be a major research interest. The National Cancer Institute grant, "Radiolabeled Monoclonal Antibodies for Cancer Therapy," is in its third year of support. This study investigates the ability of radiolabeled monoclonal antibodies to tumor-

1028163

associated antigens to specifically deliver tumoricidal dosages of therapeutic radionuclides to malignant sites. Monoclonal antibodies labeled with diagnostic radionuclides can similarly be used for tumor localization and staging.

Monoclonal antibodies have been labeled, both at ORAU and elsewhere, with radionuclides of iodine and various gamma-, beta-, and alpha-emitting metal ion radionuclides. Although still often used, monoclonal antibodies labeled with radioiodine have an unfortunate tendency to deiodinate in vivo, thus limiting their utility.

Monoclonal antibodies are labeled with metal ion radionuclides via bifunctional chelate techniques in which a chelating agent is covalently coupled, under mild conditions, to the monoclonal antibody molecule. Although a number of bifunctional chelate techniques have been developed, the bulk of our work to date has used the cyclic anhydride of diethylenetriaminepentaacetic acid (DTPA, a commonly used chelating agent) to couple DTPA to monoclonal antibodies. Using this method, we have successfully radiolabeled DTPA-conjugated monoclonal antibodies in high yield with beta-emitting yttrium-90, alpha-emitting einsteinium-253 and fermium-255, and gamma-emitting indium-111.

CO17-1A, a monoclonal antibody with specificity for colorectal and pancreatic carcinomas, was developed and supplied by Dr. Zenon Steplewski, a collaborator at the Wistar Institute in Philadelphia, Pennsylvania. ^{90}Y-Labeled CO17-1A was shown to be taken up selectively by the tumors of nude mice bearing xenografts of human colorectal carcinoma cell line SW 948. We found that the specificity of tumor uptake was improved by both coadministration of 100 µg of the unlabeled monoclonal antibody per mouse and by purification of the ^{90}Y-labeled CO17-1A by high-performance liquid chromatography rather than column gel filtration chromatography. A study of the time course of the tissue distribution of ^{90}Y-labeled CO17-1A permitted calculation of absorbed radiation dose estimates. In an experimental radiotherapy study, a definite relationship was observed between the dosage of ^{90}Y-labeled CO17-1A administered to nude mice bearing SW 948 human colorectal carcinoma xenografts and the tumor growth response. Tumors in animals of the higher dosage groups grew much more slowly than those in the control and lower dosage groups.

Our results with ^{90}Y-labeled CO17-1A indicate that the Y-90 dissociates in vivo to some extent from the chelate-conjugated monoclonal antibody, leading to higher than desirable uptakes in both bone and organs of the reticuloendothelial system (liver, spleen, and bone marrow). As a result, dose-limiting bone marrow toxicity has been observed for ^{90}Y-labeled monoclonal antibodies produced using the currently available bifunctional chelate techniques. Future work in this area will emphasize developing new bifunctional chelate techniques which will produce radiolabeled monoclonal antibodies with greater in vivo stability and will also focus on reducing bone marrow toxicity through using radioprotective agents and chelation therapy.

Immunological Studies of the Cotton-Top Tamarin

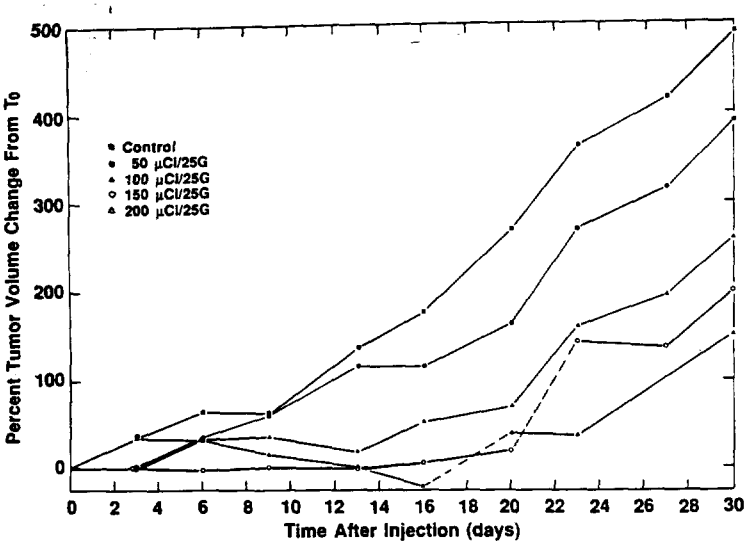
We also conducted a number of experiments involving the integrity of the immunological surveillance systems of cotton-top tamarins, *Saguinus oedipus*, with acute colitis and colon adenocarcinoma. The assessment of helper/inducer (OKT4+) to suppressor/cytotoxic (OKT8+) T-lymphocyte ratios is an increasingly useful method of monitoring immune competence. The OKT4+/OKT8+ ratios were significantly lower in these two groups when compared to negative control animals. Participating in this set of experiments was Dr. B. D. Lawless, a DOE Faculty Research Participant from Fordham University in New York.

We have found autoantibodies in the sera of tamarins with colon cancer that are not found in control animals. Serum immunoglobulin fractions of control and test animals were isolated and biotinylated. The human adenocarcinoma cell line, SW 948, and the tamarin adenocarcinoma cell line, CTT, were incubated with the biotinylated immunoglobulins, washed thoroughly, and then incubated with FITC-avidin. Fluorescent cells were those complexed with autoantibodies. The presence and specificity of the autoantibodies were also established by ELISA, using antigens extracted from the colon cancer cells. Autoantibodies in the sera may indicate the presence of colon cancer.

The mouse anti-human monoclonal antibody BR55.2, with specificity for human colon adenocarcinoma, was biotinylated and fluorescently stained with peripheral blood mononuclear cells from cancerous tamarins. These experiments were performed in collaboration with Dr. Joseph Fuhr of the University of Tennessee Medical Center at Knoxville. The results showed cross-reactivity of mouse anti-human colon cancer monoclonal antibody to peripheral blood mononuclear cells of cancerous tamarins. Antibodies from either cancerous or chronic colitis tamarins were also biotinylated. The fluorescently labeled cells were detected when peripheral blood mononuclear cells from cancerous tamarins were incubated with biotinylated autoantibodies from cancerous tamarins. This result was confirmed using cytofluorographic analysis which showed a significant difference ($P = 0.0043$) of the percent of fluorescently labeled peripheral blood mononuclear cells between cancerous tamarins and chronic colitis tamarins when stained with biotinylated autoantibodies from

1028764

Dose-Response Curve for ^{90}Y-Labeled CO17-1A in Nude Mice Bearing SW 948 Colorectal Carcinomas



ORAU researchers observed a definite relationship between the dosage of ^{90}Y-labeled CO17-1A and the tumor growth response in nude mice bearing SW 948 human colorectal carcinomas. Tumors in animals receiving higher dosages grew much more slowly than those in the control and lower dosage groups. Specificity of tumor uptake was found to be improved by coadministration of 100 µg of unlabeled monoclonal antibody and by purification using high-performance liquid chromatography rather than column gel filtration chromatography.

cancerous tamarins.

NEDH Rat—An Animal Model of Hypertension

Pheochromocytomas are hypertension-producing tumors of the adrenal medulla. Since surgical removal of the tumor is possible, this represents a potentially curable form of hypertension. Thus, detection and early surgical or, where appropriate, medical treatment is desirable. Additional experiments with the New England Deaconess Hospital (NEDH) rat bearing subcutaneously implanted pheochromocytomas were carried out. Christine Drivdahl, a DOE Undergraduate Student Research Participant, participated in one series of experiments exploring changes in OKT4+/OKT8+ ratios in hypertensive NEDH rats. Changes in the helper and suppressor subsets of peripheral blood lymphocytes before and after transplantation of the pheochromocytomas were assessed using fluorescence-activated cell sorting. Quantitation of catecholamines were performed concurrently. There appeared to be a correlation of OKT8+ cells with the catecholamine concentrations.

In collaboration with the Cytogenetics group, which has continued to study and maintain the pheochromocytoma cells in culture, a series of experiments was undertaken by William Grever, a DOE Undergraduate Student Research Participant, to evaluate the in vitro uptake of several ^{14}C-labeled unnatural alicyclic amino acids, e.g., 1-aminocyclobutanecarboxylic acid (ACBC), 1-aminocyclopentanecarboxylic acid (ACPC), and 1-aminocyclohexanecarboxylic acid (ACHC), by pheochromocytoma cells. Preliminary results showed an apparent preferential uptake of ACHC by the tumor cells.

Positron Emission Tomography

Emphasis continues to be placed on the use of positron emission tomography (PET) to produce high-resolution, three-dimensional images as well as to act as a noninvasive probe of biochemical function. Thus, it can be used specifically to detect metabolic alterations associated with disease processes. The Clinical Nuclear Medicine Program uses PET to evaluate positron-emitting radiopharmaceuticals for diagnostic and research applications in cardiovascular and neoplastic diseases, with emphasis on a more quantitative evaluation of the in vivo distribution of such radiopharmaceuticals.

Through the work of consultants Dr. Paul King of Vanderbilt University and Dr. Ron Keyser of EG&G ORTEC, an Oak Ridge company, along with Mr. Mitch Russell of ORAU's Computer Services group, the facilities of the Clinical Nuclear Medicine Program have been improved through the installation of new interactive software for ORAU's ECAT II™ positron tomographic scanner. It is anticipated that these programs will lead to improved quantitative imaging of regions of interest located in the thorax.

In-house availability of short-lived, positron-emitting radionuclides is anticipated in the near future through the division's interaction with a small Oak Ridge corporation, ORBIT, Inc., headed by Dr. John Neiler. ORBIT, Inc. has obtained phase II support from DOE's Small Business Innovation Research Program to construct a small, proton-only, low-energy (8 MeV) cyclotron and to test its utility as a source of clinically important radionuclides for PET (carbon-11, nitrogen-13, oxygen-15, and fluorine-18). DOE has approved installation of this cyclotron in MHSD's Vance Road building in a well-shielded room which formerly housed the medium-energy total-body irradiation (METBI) facility. Modification of the facility as

1028765

required for installation of the cyclotron is under way and the cyclotron is expected to be in place by February 1988. We envision that the 8 MeV cyclotron and ECAT II™ positron tomographic scanner will be used for preclinical development and clinical feasibility studies of new positron-emitting radiopharmaceuticals. Additional clinical evaluation of selected agents conceivably could then be carried out in collaboration with the University of Tennessee Medical Center at Knoxville using state-of-the-art PET facilities there.

Our clinical PET studies with ^{11}C-labeled D- and L-tryptophan and D- and L-valine for pancreatic imaging and with ^{11}C-labeled 1-aminocyclobutane-carboxylic acid (ACBC) for tumor imaging will resume using the radionuclide production capability of the ORBIT, Inc., 8 MeV cyclotron. In addition, ^{18}F-labeled DL-α-monofluoro-methylalanine, an unnatural fluorine-containing amino acid which we have shown in studies using the ^{14}C-labeled compound to have a tumor specificity similar to that of ACBC, will be synthesized and tested as a potential tumor-imaging agent for use with PET.

Local availability of F-18 will also aid in the development of ^{18}F-labeled ω-substituted primary aliphatic amines as agents for PET studies of pulmonary function. One of the major metabolic activities of the lungs involves regulation of various biogenic amines. Lung endothelial cells sequester ^{11}C-labeled straight-chain primary aliphatic amines very selectively. However, the rapid metabolic loss of the label makes it difficult to accurately quantitate the pulmonary uptake. We have developed a synthetic route to ω-fluorine-substituted primary aliphatic amines which is conducive to radiolabeling these compounds with F-18. Compared to our previous work with amines labeled in the α-position with C-11, the presence of a radiofluorine substituent in the ω-position should delay the loss of radiolabel, permitting more accurate quantitation of uptakes.

A patent for copper-64 citrate, a positron-emitting radiopharmaceutical, as a new myocardial-imaging agent based on experimental work performed in dogs is pending at this time. A cooperative study with Computer Technology and Imaging, Inc. (CTI) of Knoxville has afforded the opportunity to compare canine myocardial images obtained using ORAU's ECAT II™ and CTI's state-of-the-art ECAT Model 931 instrument. Collaboration has continued with Dr. R. E. Gompf of the University of Tennessee's College of Veterinary Medicine to study the role of copper-64 citrate in the diagnosis of mitral valve disease, a type of acquired heart disease in which the size of the left ventricle is increased. Plans to extend these observations to clinical studies are under consideration.

Chelation Therapy

Chelation therapy with DTPA drugs is the method of choice for decorporation of internally deposited actinide contaminants. As part of MHSD's project to coordinate national use of DTPA (see REAC/TS section), the Preclinical Nuclear Medicine and Biochemistry Programs collaborate in research on new, highly lipophilic chelating agents. Current research involves a study in rats of the decorporation efficacy and pharmacological tolerance of calcium-DTPA encapsulated in liposomes prepared as large unilamellar vesicles (LUV). Preliminary results have indicated that the LUV can effectively deliver the chelator to tissue sites for removal of actinides. These agents also do not produce the large spleen weight increases previously seen with calcium-DTPA encapsulated in a different type of liposome (multilamellar vesicles).

Cytogenetics

Program Director:
L. Gayle Littlefield

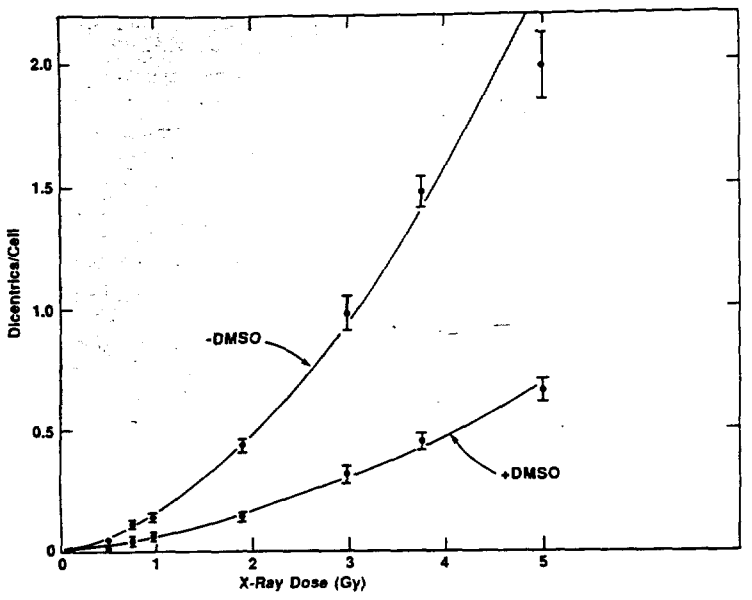
The Cytogenetics Program has three major research objectives: to further delineate the physical and biological mechanisms involved in the induction, repair, and persistence of chromosome damage induced by radiation and DNA-damaging chemicals; to develop and apply cytogenetic methods as biological indicators of dose in persons having real or suspected radiation over-exposures; and to define and quantify the basic sequelae of radiation or chemical damage at the cellular and chromosomal level through the use of experimental animals as surrogates for humans.

Studies of Mechanisms Involved in Aberration Induction

It is well known that ionizing radiation induces several types of

1028766

Effect of DMSO on X-Ray Induced Chromosome Aberrations



Studies conducted by cytogeneticists at ORAU demonstrated that the proportion of radiation-induced chromosome aberrations is reduced by about 70% when lymphocytes are exposed to X-radiation in culture medium containing 1M DMSO.

lesions in DNA, including single-strand breaks, double-strand breaks, and base damage. Such lesions may be induced "directly," by prompt ionizations of DNA, or "indirectly," via mechanisms involving interactions of target molecules with highly reactive free radicals. We have continued studies employing dimethyl sulfoxide (DMSO) as a model chemical to further investigate the role of OH radicals in producing DNA lesions involved in chromosome aberration induction. DMSO, which has low acute toxicity and high permeability, is an excellent selective scavenger of OH radicals. Several previous studies have demonstrated that DMSO is an effective radio-protector in both cellular and chemical systems.

In experiments reported previously in this report, we exposed human lymphocytes to X-radiation in culture medium containing DMSO. These studies demonstrated that DMSO affects a concentration-dependent modification of radiation-induced chromosome aberrations in human lymphocytes with maximum protectable fractions of approximately 70% at DMSO concentrations ≥ 1M. Based on calculations of the concentration of DMSO required to produce one-half maximum protection, we were able to estimate that the rate constant for reaction of OH radicals with DNA targets involved in the formation of chromosome aberrations is approximately $6 \times 10^8 sec^{-1}$.

Further studies demonstrated that similar reductions in the proportion of radiation-induced chromosome aberrations are observed in human lymphocytes exposed to X-ray doses ranging from 51 to 500 rad in medium containing 1M DMSO. Based on mathematical fittings of the dicentric-per-cell data to various dose response curves, we conclude the OH-radical-mediated lesions in DNA account for 50% of the dose dependency for dicentrics resulting from either one-track or two-track events, following exposures of noncycling cells to moderate-to-high doses of low LET radiation. Additional studies involving the in vitro irradiation of human lymphocytes demonstrated that DMSO exerts a similar radioprotective effect against the induction of chromosome aberrations in proliferating cell populations.

Last year additional studies were conducted to determine whether DMSO was equally effective in protecting against radiation-induced aberrations in lymphocytes of other mammalian species. In these studies we exposed human, pig, and rabbit lymphocytes to varying doses of X-radiation in medium with or without 1M DMSO. Evaluations of radiation-induced aberrations demonstrated differences in relative radiation sensitivity between the three species as expected. Furthermore, at all doses, pronounced reductions in X-ray-induced dicentrics were observed in lymphocytes irradiated in the presence of DMSO. Over the radiation dose and DMSO concentration employed, these preliminary findings suggest that DMSO is effective in modifying the induction of aberrations in mammalian lymphocytes from species having differing chromosomal radiation sensitivity. Studies are under way to determine whether the concentration of DMSO required to achieve maximum protection differs among these three species and whether response differs among individual animals within each species.

Cytogenetic Dosimetry Follow-Up Evaluations

During the past year, we provided cytogenetic dosimetry estimates for three persons referred to REAC/TS because of suspected radiation over-exposures. Under the support of an interagency agreement between the National Cancer Institute and DOE, we also continued detailed cytogenetic evaluations in control

1028167

and irradiated persons selected by NCI from various cohorts having medical exposures 20 to 40 years ago.

We completed ongoing cytogenetic evaluations in cultured lymphocytes from a population of 200 adults who received radiation therapy for enlarged thymus during infancy and from 25 women who received high-dose radiotherapy for cancer of the cervix 2 to 15 years ago. In collaboration with scientists at the NCI Radiation Epidemiology Branch, we will complete statistical evaluations to determine whether evidence of radiation exposure can still be observed in circulating lymphocytes of these adults who received neonatal radiation exposures. We will also determine the rate of loss of T-lymphocytes bearing asymmetrical chromosome-type aberrations as a function of time after radiation exposure in the cervical cancer patients.

In Vitro Dose-Response Studies

The Cytogenetics Program continued to participate in an International Cooperative Program on the Routine Use of Chromosome Aberration Analysis in Occupationally Exposed Radiation Workers, sponsored by the International Atomic Energy Agency (IAEA). Detailed results of a collaborative study of cytogenetic dosimetry for simulated whole-body and partial-body accidental radiation were published in 1987.

As part of our continuing participation in this program, we conducted studies aimed at developing improved techniques for use in biological dosimetry. Several laboratories have proposed that the analysis of radiation-induced micronuclei in cultured lymphocytes may provide a simpler and less expensive method for quantifying radiation exposure levels than the more time-consuming technique of scoring chromosome aberrations in metaphase preparations.

The primary disadvantage of this system has been that micronuclei can only be observed in interphase daughter nuclei of cells that have completed one cell division after radiation exposure, and simple and reliable methods have not previously been available for distinguishing between first and second division interphase nuclei. Recently, a method which allows selective analysis of micronuclei frequencies in such second-division interphase nuclei was published. Last year we initiated collaborative studies with Dr. Soha Kholeif, an IAEA Fellow from the University of Alexandria, Egypt, to define variables that may affect the induction or expression of micronuclei using this new methodology. We completed statistical analyses of data derived from the in vitro exposure of blood samples from three normal adults to varying X-ray doses. These studies revealed no significant differences in the levels of response between three individuals. In addition, the studies showed that increases in micronuclei were readily observable at doses as low as 25 rad and that their frequency increased as a curvilinear function of dose over the range of 25 to 400 rad.

Last year we also continued to collaborate with Dr. Velma B. Richardson of Tuskegee University. In earlier published studies, we investigated the cytogenetic effects of methyl acetimidate, a lysine-specific protein crosslinking agent, using human peripheral lymphocytes in culture. Recently, we initiated studies to evaluate the efficacy of folic acid deprivation as a method for obtaining mitotic synchrony of mammalian cells in tissue culture. Evaluations of radiation-induced chromosome aberrations in human lymphocytes exposed to X-radiation during methotrexate-induced block suggest that mitotic arrest occurred during the period of DNA synthesis rather than during the G1 phase, as previously reported by other investigators.

Studies in Animal Models

We continue to use experimental animal models in studies designed to provide information on the basic sequelae of damage at the cellular and chromosomal levels. Mammalian cytogenetics also has useful applications for the study of chemical effects. Under the terms of a contract with the National Toxicology Program, we have been examining variables and evaluating the effectiveness of an in vivo cytogenetic system for assessing the genotoxicity of chemicals. A considerable battery of in vitro assays is available and has been used to measure the carcinogen/mutagen potency of chemicals. However, none of these have proven very predictive of a chemical's in vivo action. Thus, while other systems may be useful for preliminary screening purposes, the final definitive evaluation of a chemical should be conducted in an in vivo assay system, and cytogenetic measurements in mouse bone marrow cells provide convenient endpoints for such assays.

In a cooperative study conducted in conjunction with Brookhaven National Laboratory, we have identified a series of potential variables in the mouse in vivo cytogenetic assay procedure and have systematically evaluated their possible contribution to the variability inherent in the system. Based on these results, we have developed a standard protocol for evaluating the genotoxic effects of chemicals in the intact animal based on the induction of chromosome aberrations and sister chromatid exchanges, as well as on interference with cell division kinetics. The qualitative similarity of the results obtained in the two laboratories has demonstrated an ability to minimize interlaboratory variability, which is a major contributor to the uncertainty surrounding many quantitative tests.

While this program emphasizes testing chemicals, we are also continuing to investigate other areas dealing with basic factors influencing in vivo cytogenetic

1028768

testing. One new area of investigation involves development of the capability for in vitro culture of mouse lymphocytes. For years, cultured peripheral blood lymphocytes have been the cell system of preference for studying cytogenetic alterations in humans as well as other species; however, rats and mice, the species in which most genetic and carcinogenic data have been gathered, have consistently defied successful in vitro culture of their lymphocytes. Recent reports from other investigators have indicated success in the culture of mouse lymphocytes, and there are a number of in vivo/in vitro comparisons of cytogenetic endpoints that could be made if this methodology can be used to support our in vivo testing. During the past year we developed basic capabilities for culturing mouse lymphocytes. These procedures should lead to the ability to make comparisons between specific cell systems as well as between in vivo and in vitro treatment regimes.

Another area of related investigation examines the potential influence which the solvent used for administration of a chemical may have on the animal's response to the compound. DMSO is frequently used for the in vivo administration of chemicals which are insoluble in water. This presents the possibility that compounds may crystalize from DMSO solution upon contact with an aqueous environment. To evaluate possible differences in chemical effects due to the characteristics of the solvent in which they are administered, we quantitated sister chromatid exchange induction in bone marrow cells of mice following administration of chemicals in three different carriers. Mice were injected intraperitoneally with equal doses of compounds dissolved or mechanically suspended in saline, corn oil, or DMSO, and SCEs were quantitated in bone marrow cells recovered 24 hours later. Of the four chemicals tested thus far, three have shown no difference due to the solvent in which they were administered; however, one chemical demonstrated a significant mutagenic potency when administered in corn oil or DMSO, but not when delivered as a suspension in saline. These preliminary results indicate that the choice of vehicle in which the chemical is administered to test animals can significantly influence the outcome of mutagenic evaluations.

Marmoset Research

Program Director:
Neal K. Clapp

The Medical and Health Sciences Division continues to operate a National Cancer Institute contract to breed and raise tamarins and marmosets for cancer research. The contract's objectives are (1) to maintain a breeding colony of *Saguinus oedipus* and to produce a maximum number of healthy animals for research purposes at minimum cost; (2) to provide facilities for experimental studies of oncogenic substances in tamarins; and (3) to provide professional and technical support for others to safely and accurately perform experimental protocols approved and requested by the National Cancer Institute. Reproductive efficiency of the cotton-top tamarin colony remains excellent; colony-born animals are now used almost exclusively as breeding pairs. Additional marmoset colony support, which began in September 1985, continues from the National Foundation for Ileitis and Colitis.

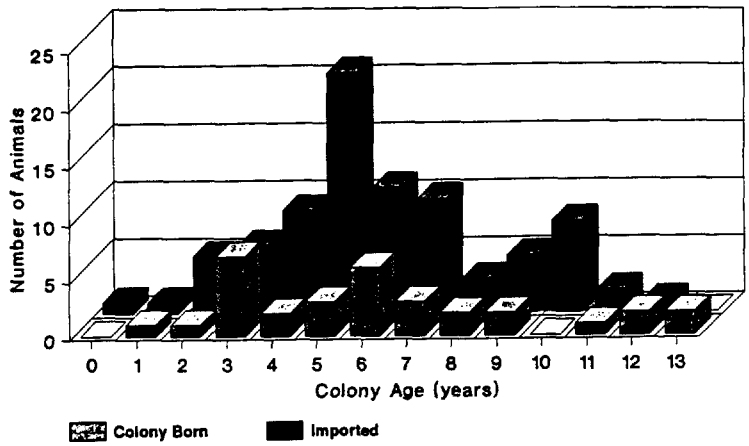
ORAU marmoset research personnel are systematically visualizing and biopsying tamarin and marmoset colonic tissues to determine the pathogenesis (both the time course and pathology of the disease process) of colitis and its contribution to and association with the development of colon cancer in cotton-top tamarins. This study is conducted in collaboration with gastroenterologists at the University of Nevada School of Medicine and the Ohio State University College of Medicine. The identification of a strong correlation in necropsied animals between colon cancer and acute colitis suggests a possible causality relationship. Current prospective longitudinal studies of colitis have shown varied patterns for the course of the inflammatory disease; however, animals with acute colitis are at very high risk for developing colonic carcinoma. Epithelial changes that are recognized as criteria for dysplasia in humans have been identified in tamarin colons but, to date, have only been found in the presence of

1028769

acute colitis. Information from these studies augments the paucity of information that is now available regarding the pathogenesis of the similar human diseases. Future plans include determining the efficacy of chemotherapeutic agents in controlling exacerbations of acute colitis that may in turn ultimately reduce the development of colonic carcinoma.

Research concerned with the behavioral and endocrine determinants of reproductive competence in the cotton-top tamarin was conducted through support from the Division of Research Resources of the NIH. The initial objective was to define the importance of the social aspects of rearing infant siblings on the subsequent maternal behavior of females. Combined studies of behavior and the relation of demographic parameters to reproductive performance have enhanced our ability to select competent breeders. Reproductive aging in female tamarins is being examined to determine whether these species display endocrine, ovarian, or fertility changes similar to menopausal women. Preliminary results indicate that a significant decline in ovarian follicular development and reduced fertility occurs with age in tamarin females, but an absolute cessation of follicular development has not been seen in aging animals. Collaborative studies with the University of Wisconsin are providing data on endocrine function in aging tamarin females. Results indicate the hormone profiles of the aged tamarin are different from those observed in breeding females. It appears that the aged tamarin has greater variation in ovarian hormone levels and a greater chance of being acyclic. In collaboration with investigators at the University of Tennessee, morphological ovarian changes are being visualized by laparoscopy. In these studies, correlations will be attempted between hormonal status, ovulation rate, and luteal function.

S. oedipus Colon Cancer Deaths
1981–1987



This figure shows the frequency of colonic carcinoma by age in recent S. oedipus *deaths, both imported and colony-born animals. Prior to 1981, all tamarins dying with colon carcinoma were less than 7 years of age. Despite a general increase in average age of the ORAU colony and the appearance of cancer deaths in animals equal to or greater than 7 years of age, colon cancer deaths still follow a normal distribution with a median colony age of 5 years. The association with onset and activity of colitis is being critically evaluated by ORAU staff and collaborators as a possible mechanism for colon cancer expression.*

The cooperative efforts initiated in 1984 to establish an international studbook for the endangered cotton-top tamarin resulted in the publication of the first edition in July, 1987. The studbook is crucial for the sound conservation of tamarins and the planning and use of this species for research purposes. Collaborative research projects with extramural investigators are designed to characterize the biology, biochemistry, physiology, nutritional aspects, and behavioral characteristics of the marmoset as an experimental animal and to relate these findings to human disease processes. These investigations involve the following studies: T and B cell immunology, cell culture of tamarin colon cancers, presence of oncogenes, genetic diversity of species, genetic epidemiology and familial relationships in tamarin colon cancer, and idiopathic ulcerative colitis in colon cancer susceptibility and expression. In other projects we are investigating the effects of such diverse entities as serum and fecal enzymes, fecal microflora and fecal mutagens, coronaviruses, lectin binding, toxins, and bile acids on marmoset colonic diseases.

Extensive collaborations are under way with researchers in the Laboratory of Comparative Carcinogenesis at Frederick Cancer Research Facility, National Cancer Institute. These concerted efforts will correlate changes in the fecal contents (both fecal mutagens and microbial flora) with their mutagenicity and carcinogenesis efficacy. Preliminary results indicate that mutagenicity is extremely high in anaerobically incubated fecal samples from tamarins that have acute colitis but not in aerobic samples nor in samples from tamarins with chronic colitis. Identification of the mutagenic agents may offer answers to the significance of compounds produced by these microbes. Studies will compare these parameters in the cancer-

1028770

prone cotton-top tamarin and the other nonsusceptible species housed in the ORAU tamarin and marmoset colony. Other studies are evaluating adducts in DNA that may identify the molecular changes that precede and/or lead to the development of colonic carcinoma.

An exciting collaborative research relationship has developed with the Harbor-UCLA School of Medicine. An NIH-funded center for the study of Inflammatory Bowel Disease has been established at UCLA. The marmoset model of inflammatory bowel disease is being used to investigate such diverse parameters as genetic epidemiology, the role of mucosal leukotrienes in colitis, and the presence of oncogenes in colonic disease processes that are observed and followed clinically by ORAU personnel. Comparisons will be made to follow the onset of colon carcinoma in colony-born cotton-top tamarins that have colon cancer in their family history and animals that have no cancer in their history; the unique species susceptibility for this disease appears to be panspecific rather than familial in nature. These results contrast with observations in humans where a significant percentage of colonic neoplasms are familial.

The strongly supported view that depressed immune function may be related to the development of cancer is the basis for our experiments designed to investigate the possibility that the colon-cancer-susceptible cotton-top tamarin may have immune deficiencies. In collaboration with ORAU nuclear medicine program personnel and a visiting investigator from Rockefeller University, we developed methodology for determining the T4+/T8+ lymphocyte ratios. Animals with acute colitis have a dramatically depressed ratio, and further reduction occurs when the tamarin has colon cancer. These results are even more interesting because a similar reduced OKT4+/OKT8+ ratio has been identified in human AIDS patients.

Radiation Emergency Assistance Center/ Training Site

Program Director:
Robert Ricks

The Radiation Emergency Assistance Center/Training Site (REAC/TS) continued to provide support for medical management of radiation accidents. REAC/TS support includes on-site emergency medical assistance and consultation services. REAC/TS also continued to work closely with the International Atomic Energy Agency (IAEA) and with the World Health Organization (WHO) in the role of the Western Hemisphere Collaboration Center for radiation accident management.

Medical Assistance

Last year, REAC/TS provided assistance in matters related to the Chernobyl reactor accident and its follow-up through participation in the International Conference on Nonmilitary Radiation Emergencies sponsored by the American Medical Association in Washington, D.C. Through other international meetings, REAC/TS continued to maintain direct and indirect consultation with the Soviets regarding medical effects of the Chernobyl accident and to otherwise assist the U.S. and world communities in analyzing Chernobyl data.

At the request of the government of Brazil and the IAEA, two members of the division, Drs. C. C. Lushbaugh and Robert C. Ricks, were part of a five member international response team that travelled to Rio de Janeiro and Goiania, Brazil, to consult with Brazilian physicians, health physicists, dosimetrists, and environmentalists following the cesium-137 accident in Goiania in September. Twenty persons were severely injured; four of the radiation victims subsequently died from their radiation insult. In addition to consultation services, cytogenetic analysis was performed on blood samples from nine persons involved in the Goiania accident.

In the Brazilian cesium-137 accident, the clinical use of Prussian Blue was evaluated as a therapeutic agent for the first time in persons who had received high levels of internal contamination with radioactive cesium. Preliminary analysis of these results conducted by IRD-CNEN indicates that Prussian Blue was effective in removing cesium in highly contaminated patients. A more complete evaluation of the effectiveness of Prussian Blue will take place in FY 1988 when the Brazilian data are published.

Clinical use of DTPA continued

1028711

to be coordinated by REAC/TS for the evaluation of individuals undergoing DTPA chelation therapy for known or suspected internal contamination with plutonium-239 or americium-241. Results of such evaluations (through whole-body counting) are used to determine the efficacy of therapy and to determine a need for continued use of DTPA in persons contaminated internally. As part of the REAC/TS Registry System, the DTPA Registry continues to be expanded and updated as more information becomes available.

Training

During the past year, REAC/TS continued to offer courses of instruction in medical management of radiation accidents to medical, paramedical, and health physics personnel. Participants included 92 physicians, 64 nurses, 72 health physicists, and 46 others, for a total of 274. Since 1976, a total of 103 training courses have been offered at REAC/TS with 1,948 health professionals trained.

Graduates of REAC/TS courses continue to comprise a broad base of occupational backgrounds and locales within the United States and foreign countries. The courses offer various degrees of formal training in radiation medicine that are sponsored by government, private industry, medical/health physics societies, hospitals, and various other professional groups.

Last year, REAC/TS again offered a training course entitled "Basic Concepts of Radiation for Attorneys." The course was judged to be useful by the 23 participants, and plans are to offer the course on a regular basis in the future.

REAC/TS also participated in Busy Force One, a full-scale nuclear weapons accident exercise conducted near Salina, Kansas. Three REAC/TS staff members participated on-site and were part of DOE's accident response group. The REAC/TS on-site team also worked with both military and private practice medical personnel at the drill. On-the-scene activities were closely coordinated with other REAC/TS staff members who remained behind at the REAC/TS facility.

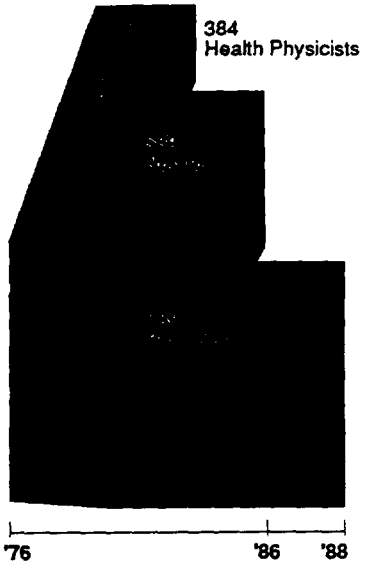
REAC/TS continued to be closely associated with the Federal Emergency Management Agency (FEMA). FEMA provides funding for conducting REAC/TS training courses in the management of health problems related to radiation accidents. Last year, REAC/TS conducted for FEMA a national teleconference entitled "Handling the Radiation Accident Victim in the Hospital Emergency Department." This teleconference was broadcast through the Emergency Education Network (EENET). The teleconference, broadcast live from studios in Washington, D.C., was well received not only in the United States, but in Canada and Mexico as well. In the coming year, REAC/TS will conduct for FEMA another national teleconference dealing with mass casualty and evacuation during radiological emergencies.

Registries

Through the REAC/TS Registry System, long-term follow-up of persons involved in earlier radiation accidents continues. The REAC/TS Registry at present contains information on 902 accidents or incidents involving more than 141,000 persons. The registry has identified 292 radiation accidents worldwide that include more than 136,000 persons and 62 acute fatalities. This number includes the persons involved in the Chernobyl reactor accident.

The DTPA Registry has been expanded to include 56 facilities, with 47 coinvestigator physicians reporting annually on 592 persons who have received at least one dose of DTPA chelate for actual or suspected internal contamination. Information on foreign radiation accidents continues to be added to the registry system through contact with physicians and health physicists in various foreign countries and through close liaison with the WHO Collaboration Center in Paris. Finally, through the DOE >5 Rem Study Registry, follow-up of persons exceeding the 5 rem occupational exposure limit continues for the purpose of establishing trends in morbidity and mortality.

REAC/TS Course Participants



Since 1976, REAC/TS has provided training in the medical management of radiation accidents for medical, paramedical, and health physics personnel. Two years ago, REAC/TS broadened its training efforts by offering a special course in radiation concepts for attorneys. Response was favorable, and this year 23 participants attended the second such course at ORAU.

1028772

Center for Epidemiologic Research

Program Director:
Shirley A. Fry

The Epidemiology Program carries out interdisciplinary research to examine the relationships between occupational exposure to low levels of ionizing radiation and the subsequent health and mortality of nuclear industry workers. The health effects of chemical toxicants, particularly uranium and other toxic metals, also are being studied as primary and potentially confounding or modifying factors with respect to radiation. The basis of our research is ongoing epidemiologic studies of 11 populations, totaling approximately 200,000 persons, of DOE and DOE contractor (and their predecessors) employees. The goal is to identify the untoward effects, if any, of their employment at some time since 1942 at designated facilities nationwide. Our responsibilities were expanded last year by an assignment to develop and conduct epidemiologic studies of an estimated additional 70,000 persons ever employed at the Lawrence Radiation Laboratories at Livermore and Berkeley, California.

Supporting research and technical activities at the Center for Epidemiologic Research include: (1) developing, testing, and applying new and improved statistical methods, including probabilistic modeling, for analyzing epidemiologic data; (2) critically evaluating methods and results of studies among other populations exposed to ionizing radiation and other industrial toxicants; (3) advancing methodologies in the areas of data collection, records management, assessment of occupational hazards, and development of measures of exposure for use in epidemiologic analyses; and (4) developing and implementing improved computer systems to ensure the high quality and validity of the data bases and to facilitate the processing, manipulation, and analysis of large volumes of data.

Our studies are a part of the overall Health and Mortality Study of DOE workers that was initiated in 1964 by the Atomic Energy Commission and that has an estimated total study population of 600,000 present and former workers at more than 70 sites nationwide. In our studies we have the collaboration of the School of Public Health of the University of North Carolina, Chapel Hill, other DOE-sponsored epidemiology groups at Los Alamos and Hanford, and of researchers at the Lawrence Livermore National Laboratory.

Completed Studies

Major accomplishments last year included reporting the results of studies concerning mortality among workers formerly employed at Linde Corporation's MED uranium processing site and the results of an evaluation of occupational exposure to radiation and selected chemicals, and of several nonoccupational factors, as risk factors in the development of primary brain cancer among workers at the Oak Ridge Y-12 plant and Oak Ridge National Laboratory. No association was found between the Linde employees' exposure to uranium dust and mortality from diseases of interest identified a priori. Also, no association was found between the Oak Ridge workers' occupational exposure to radiation from external or internal sources and their subsequent development of brain cancer. Descriptions of these studies and the results are summarized in the abstracts of published papers in this report. In addition to publication in the scientific literature, the results of these studies were reported directly to plant management and labor representatives and via the local media to the public in the areas around the plants involved.

We also provided collaborative scientific and technical input and support to a study reported last year by the University of Michigan on the subject of morbidity among workers exposed to elemental mercury while employed at the Oak Ridge Y-12 plant between 1953 and 1958. This study indicated that the current health status of the majority of the workers evaluated had not been adversely affected by their mercury exposure. The results of completed studies of mortality among cohorts of white males employed at the Oak Ridge Y-12 and Savannah River (SRP) plants are scheduled to be published soon. Evaluation is continuing of the mortality experience of SRP workers with respect to their radiation exposure and among a subgroup of the workers among whom an

1028773

unexplained specific disease excess was identified in the preliminary analysis.

Ongoing Studies

Analysis was completed of mortality among all workers employed between 1943 and 1947 at any of DOE's Oak Ridge facilities; the results of this analysis are in the interpretative phase. In this study, the Mortality Analysis System (MAS) developed in-house was implemented using external and internal comparison populations. This system expedited evaluation of the effects on mortality of several levels of risk factors of interest. Further development of the MAS is in progress to enable more detailed analysis involving multiple risk factors.

Other studies currently in progress include mortality among workers at DOE's Gaseous Diffusion Plant in Oak Ridge and at the former Manhattan Engineer District uranium processing site at Mallinckrodt Chemical Works. Mortality among workers at the Fernald Feed Materials Production Center is being updated to include deaths through 1982 in preparation for analysis of the mortality experience of this cohort with respect to occupational exposure to uranium dust.

Progress also was made in the follow-up by telephone interview of the cohort of DOE workers (approximately 3,000) who received ≥5 rem per year and of their matched comparison subjects. The objective of this study is to determine the morbidity experience of the ≥5 rem cohort and whether or not it differs significantly from that of workers who never received ≥5 rem in a year. Mortality among this population was updated through 1983 in preparation for analysis with respect to radiation exposure.

Other Activities

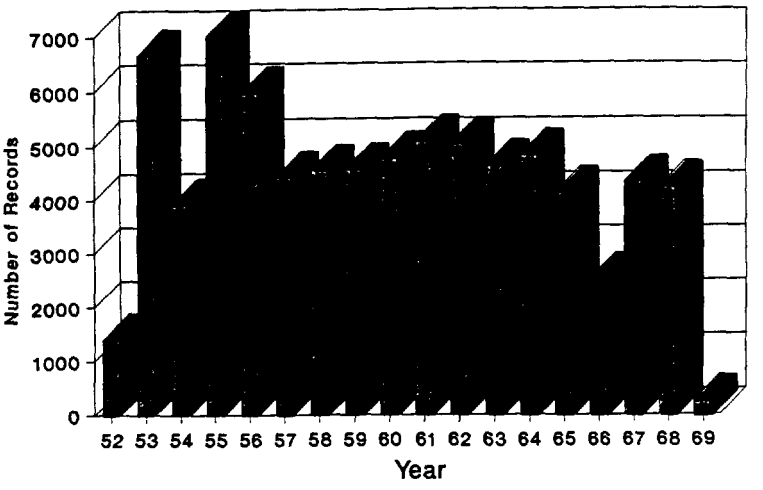
A program-dedicated mainframe computer system, including data base management and statistical analysis software systems, was purchased last year. Plans were developed for redesigning in-house developed software and corresponding data base files. Migration to the new system is in progress. When in full operation we expect this system to significantly increase our computer capabilities and overall productivity.

A major effort in hazards assessment last year was directed toward developing uranium exposure measures that are comparable across facilities and over time at the same facility. Such measures are necessary to enable planned studies of populations of uranium processing workers at four geographically separated sites.

In pursuit of the long-term goals of the program we anticipate becoming increasingly involved with improving and developing methodologies and technologies for use in parallel and combined analyses of data for the several worker populations currently being studied as part of the nationwide DOE study. Plans are being made for such analyses involving four populations for whom uranium dust is the major exposure and lung cancer the health effect of primary interest. We also will be collaborating with the other DOE-sponsored epidemiology groups on a parallel analysis of data from worker populations at Oak Ridge National Laboratory, Hanford, and Rocky Flats exposed to external radiation. These studies will be aimed at comparing effects of these exposures on mortality across and between populations.

We continue to operate the Death Certificate Retrieval Office (DCRO) in support of the ORAU and other DOE epidemiology groups, primarily those at Los Alamos and Hanford, who are conducting studies of other DOE worker populations as part of the Health and Mortality Study. Data on vital status and deaths among DOE employees are retrieved primarily through the Social Security Administration and the

Health and Mortality Study
Air Monitoring Records Analyzed



Last year, CER staff members computerized more than 77,000 hard copy air monitoring records from a uranium processing plant that is part of the Health and Mortality Study of DOE Workers. These computerized data, when combined with work history data, will enable researchers to develop measures of uranium exposure for individual workers. Air monitoring data from 1952–1969 at this plant is complemented by other types of data for later years. In the future, CER will computerize other records from the plant represented by this figure, along with records from three other sites as the basis for a case-control study.

1028774

vital statistics departments of the states. Other sources of these data include the National Death Index, state death indexes, state motor vehicle records, the Civil Service Commission, the Veterans' Administration, and other large retirement systems. With the permission of the individual states we have expanded our activities in this area and now are searching for information about deaths in selected epidemiologic studies being conducted by the National Cancer Institute.

Committee on Interagency Radiation Research and Policy Coordination

Program Director:
Anthony H. Ewing

The White House Office of Science and Technology Policy created the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) in 1984, chartering it under the Federal Coordinating Council for Science, Engineering, and Technology. CIRRPC coordinates radiation matters among federal agencies, evaluates radiation research, and provides advice on the formulation of radiation policy. Seventeen federal agencies have designated policy representatives to CIRRPC, and fourteen of these agencies have designated senior scientists as members of CIRRPC's Science Panel.

ORAU provides the necessary personnel and facilities to serve as a technical assistance office to handle technical, administrative, and logistical functions for CIRRPC. ORAU/CIRRPC staff assist CIRRPC by analyzing and drafting technical documents, providing guidance on radiation-related issues, managing technical assistance projects contracted to other organizations, determining consultant needs and arranging for such services, convening meetings and seminars to address radiation issues of concern to the federal agencies, participating in subpanel deliberations, and assisting with CIRRPC reports from their inception to publication.

In the next several months, subpanels will submit draft reports on major radiation issues either to the Science Panel or the CIRRPC Policy Panel for review and approval. Subjects of the subpanel reports are:

Policy recommendations on indoor radon.

Application to veterans' compensation of the National Institutes of Health Report on the Radioepidemiologic Tables.

Review of the agricultural and ecological effects of nuclear war as postulated in the SCOPE 28 Report.

The neutron quality factor.

Several projects under the direction of the ORAU technical support staff will also result in reports early next year. These are:

A report by the National Council on Radiation Protection and Measurements of the radiation exposure to the U.S. population.

An overview of the federal

CIRRPC Member Agencies

Department of Agriculture	Department of State
Department of Commerce	Department of Transportation
Department of Defense	Environmental Protection Agency
Department of Energy	Federal Emergency Management Agency
Department of Health & Human Services	National Aeronautics & Space Administration
Department of Housing and Urban Development	Office of Management and Budget
Department of Interior	Veterans Administration
Department of Justice	National Security Council
Department of Labor	Nuclear Regulatory Commission

CIRRPC coordinates radiation matters among 18 federal agencies, evaluates radiation research, and provides advice on the formulation of radiation policy.

1028775

government's radiation research agenda.

A compendium of legal and technical facts contained in the major U.S. radiation standards and guides for protection of workers and the public.

Future plans call for other studies including: examining the biological effects of low levels of ionizing radiation on populations, researching a more precise way to determine the biological effectiveness of neutron radiation, addressing questions concerning advance planning of health effects research following a major nuclear accident, and assessing the research needs in the health effects of nonionizing radiation.

Our *Third Annual Report*, published this year, discusses two recent reports: *Radon Protection and Health Effects* and *Report of the CIRRPC Policy Subpanel on SI Metric Radiation Units*. For more information on any of our programs, or to receive a copy of our *Third Annual Report*, contact Anthony H. Ewing, ORAU/CIRRPC Program Director, 1019 Nineteenth Street N.W., Washington, D.C., 20036.

Occupational Medicine

Program Director:
T. A. Lincoln, M.D.

The Occupational Medicine Support Program (OMSP) provides assistance to the Medical Director, Office of the Deputy Assistant Secretary, Safety, Health and Quality Assurance (SHQA) of the Department of Energy. We conduct biennial reviews of DOE contractor occupational medical departments and prepare a detailed report that becomes the basis of the official DOE appraisal report. Selected data from these reviews are stored in a management data base that is used to enable prompt retrieval of information on the content, staffing, and scope of these programs and to prepare a brief annual report. Assistance is also provided to SHQA and DOE contractors on a variety of other occupational medical problems such as substance abuse, beryllium disease, and occupational medicine policy development.

OMSP plans, organizes, and implements an annual Contractor Medical Directors' Workshop, for which continuing medical education credits are provided to participants. The first workshop was held in San Francisco in October 1987 and included lectures by faculty from the University of California-San Francisco School of Medicine and Lawrence Livermore National Laboratory. Topics included chemical dependency, AIDS, neurotoxicity of solvents, melanoma, mapping human chromosome 19, and many other subjects.

ORAU occupational medical personnel also participate in the REAC/TS program, providing lectures and consultation in REAC/TS training and service activities.

In addition, OMSP contributes to the Medical Division's ability to assist the DOE General Counsel and other legal agencies of the federal government with medical case reviews, medicolegal depositions, and expert testimony.

1028776

Evaluations and Techniques to Determine Previous Radiation Exposure

Principal Investigator:
W. W. Burr, Jr.

Program Coordinator:
G. R. Eisele

One special project completed last year illustrates the Medical and Health Sciences Division's ability to respond to requests for assistance outside the realm of existing division projects. Working with expert collaborators around the world, we completed a series of four reports for the National

Whole-Body Counting Working Group

Richard Toohey, Ph.D., Chairman
Argonne National Laboratory

Larry Anderson, A.A.
Lawrence Livermore National Laboratory

Carol Berger, M.S.
International Technology Corporation

Norman Cohen, Ph.D.
New York University Medical Center

Earl Palmer, M.S.
Battelle Pacific Northwest Laboratory

Bioassay Working Group

Bruce Boecker, Ph.D., Chairman
Lovelace Inhalation Toxicology Research Institute

Roscoe Hall, Jr., M.S.
E. I. du Pont de Nemour and Company
Savannah River Plant

Kenneth G. W. Inn, Ph.D.
National Bureau of Standards
Center for Radiation Research

James Lawrence, Ph.D.
Los Alamos National Laboratory

Paul Ziemer, Ph.D.
School of Health Sciences
Purdue University

Cytogenetics Working Group

Michael Bender, Ph.D., Chairman
Brookhaven National Laboratory

Akio Awa, Ph.D.
Radiation Effects Research Foundation
Japan

Antone Brooks, Ph.D.
Lovelace Inhalation Toxicology Research Institute

John Evans, Ph.D.
Clinical and Population Cytogenetic Unit
Medical Research Council
Western General Hospital
Scotland, United Kingdom

Peter Groer, Ph.D.
Oak Ridge Associated Universities

Carlos Pereira, Ph.D.
Operations Research Center
University of California

Gayle Littlefield, Ph.D.
Oak Ridge Associated Universities

Julian Preston, Ph.D.
Oak Ridge National Laboratory

Biological Indicators Working Group

C. C. Lushbaugh, Ph.D., M.D., Chairman
Oak Ridge Associated Universities

William Burr, Jr., Ph.D., M.D.
Oak Ridge Associated Universities

G. R. Eisele, Ph.D.
Oak Ridge Associated Universities

Karl Hübner, M.D.
University of Tennessee Memorial Research Center and Hospital

Experts from the U.S. and several foreign countries were members of the four working groups assembled to respond to a request from the National Cancer Institute to review devices and techniques that may be ueful in determining previous radiation exposure.

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Cancer Institute.

The project arose as a result of a 1984 Senate Veterans' Affairs Committee hearing that raised the question of whether it was possible to determine or estimate radiation doses that might have been received in the early period following World War II by military personnel who were present in Hiroshima or Nagasaki as members of the occupation forces and radiation doses that may have been received by military personnel as a result of exposure during atmospheric testing of nuclear weapons (24–42 years ago). The resulting Public Law 98-542 Section 7(a)(2) instructs the Secretary of Health and Human Services, through the director of the National Institutes of Health, to "(A) conduct a review of the reliability and accuracy of the scientific and technical devices and techniques (such as 'whole-body counters') which may be useful in determining previous radiation exposure; (B) submit to the Administrator of Veterans' Affairs and the Committees on Veterans' Affairs of the House of Representatives and the Senate . . . the results of such review, including information concerning the availability of such devices and techniques, the categories of exposed individuals as to whom use of such devices and techniques may be appropriate, and the reliability and accuracy of dose estimates which may be derived from such devices and techniques. . . ." The National Institutes of Health requested the National Cancer Institute to accept responsibility for this review.

At the request and under the guidance of the National Cancer Institute, the Medical and Health Sciences Division undertook a review of devices and techniques that may be useful in determining previous radiation exposure. To accomplish this task, four expert working groups were formed to review the applicability of biological indicators, bioassay, whole-body counting, and cytogenetic techniques in radiation dose assessment. These expert working groups reviewed the status of current technology and methods in these fields as well as new experimental techniques being investigated. The four reports prepared and submitted to the National Cancer Institute are:

Current Status of Cytogenetic Procedures to Detect and Quantify Previous Exposures to Radiation

Current Status of Bioassay Procedures to Detect and Quantify Previous Exposures to Radioactive Materials

Current Status of Biological Indicators to Detect and Quantify Previous Exposures to Radiation

Current Status of Whole-Body Counting to Detect and Quantify Previous Exposures to Radioactive Materials

A listing of the working groups and their members appears in the table to the left.

Center for Human Reliability Studies

Center Director:
W. W. Burr, Jr.

Program Director:
G. R. Eisele

Last year, the Office of Safeguards and Securities of the Department of Energy established the Center for Human Reliability Studies in MHSD to support DOE's new Human Reliability Program (HRP). The HRP has been established to assure that individuals occupying positions affording access to special nuclear materials meet the highest standard of reliability. The Center will assist DOE in achieving consistency in the HRP from facility to facility by providing technical assistance and operational guidance for the program. In addition, the Center will conduct research and provide analyses in areas related to personnel security.

Specifically, the Center will support DOE by providing: (1) Policy Analysis—Preparing position papers dealing with specific HRP components, policy options, technical guidance, and program implementation; (2) Experts and Advisors—Identifying experts in each of the HRP component areas and establishing technical advisory working groups; (3) Technical Guidance—Assisting in preparing the HRP implementation guidelines which will contain standards, criteria, and training material; (4) Legal Analyses—Identifying legal issues, research statutes, legal regulations, case histories, and solution options relevant to HRP objectives; (5) Education and Training—Conducting training courses, workshops, and case study seminars for managers and employees.

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Publications, Abstracts, Summaries, and Invited Lectures

Full-length Journal Articles

Bittman, R., Witzke, N. M., Lee, T-c., Blank, M. L., and Snyder, F.
Synthesis and biochemical studies of analogs of platelet-activating factor bearing a methyl group at C2 of the glycerol backbone
J. Lipid Res. 28, 733–738, 1987

Blank, M. L., Cress, E. A., and Snyder, F.
Separation and quantitation of phospholipid subclasses as their diradylglycerobenzoate derivatives by normal-phase high performance liquid chromatography
J. Chromatogr. 392, 421–425, 1987

Blank, M. L., Lee, T-c., Cress, E. A., Fitzgerald, V., and Snyder, F. Plasmalogen biosynthesis in Madin-Darby canine kidney cells: Selectivity in the acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphoethanolamine and the subsequent desaturation step
Arch. Biochem. Biophys. 251, 55–60, 1986

Boland, C. R. and Clapp, N. K.
Oncodevelopmental mucins in the colons of New World monkeys with ulcerative colitis: The association between inflammation and neoplasia
Gastroenterology 92, 625–634, 1987

Carpenter, A. V., Flanders, W. D., Frome, E. L., Cole, P., and Fry, S. A.
Brain cancer and nonoccupational risk factors: A case-control study among workers at two nuclear facilities
Am. J. Public Health 77, 1180–1182, 1987

Carpenter, A. V., Flanders, W. D., Frome, E. L., Crawford-Brown, D. J., and Fry, S. A.
CNS cancers and radiation exposure: A case-control study among workers at two nuclear facilities
J. Occup. Med. 29, 601–604, 1987

Checkoway, H., Pearce, N. E., Crawford-Brown, D. J., and Cragle, D. L.
Radiation doses and cause-specific mortality among workers at a nuclear materials fabrication plant
Am. J. Epidemiol. (in press)

Clapp, N. K., McArthur, A. H., Carson, R. L., Henke, M. A., Peck, O. C., and Wood, J. D.
Visualization and biopsy of the colon in tamarins and marmosets by endoscopy
Lab. Anim. Sci. 37, 217–219, 1987

Clapp, N. K. and Tardif, S. D.
Current conservation and biomedical research: Status of cotton-top tamarins (*Saguinus oedipus*)
Lab. Primate Newslett. 25, 1–3, 1986

Dupree, E. A., Cragle, D. L., McLain, R. W., Crawford-Brown, D. J., and Teta, M. J.
Mortality among workers at a uranium processing facility: Linde Air Products Company Ceramics Plant, 1943–1949
Scand. J. Work Environ. Health 13, 100–107, 1987

Eisele, G. R., Bernard, S. R., and Nestor, C. W., Jr.
Gastrointestinal absorption of americium-241 by orally exposed swine: Comparison of experimental results with predictions of metabolic models
Radiat. Res. (in press)

Ezra, D., Laurindo, F. R. M., Czaja, J. F., Snyder, F., Goldstein, R. E., and Feuerstein, G.
Cardiac and coronary consequences of intracoronary platelet activating factor infusion in the domestic pig
Prostaglandins 34, 41–57, 1987

Gilbert, R. O., Smith, D. D., Engel, D. W., Shinn, J. H., Anspaugh, L. R., and Eisele, G. R.
Transfer of plutonium to tissues of cattle grazing a desert ecosystem
Health Phys. (in press)

Hoffman, D. R., Hoffman, L. H., and Snyder, F.
Cytotoxicity and metabolism of alkyl phospholipid analogues in neoplastic cells
Cancer Res. 46, 5803–5809, 1986

Kawasaki, T. and Snyder, F.
The metabolism of lyso-platelet-activating factor (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) by a calcium-dependent lysophospholipase D in rabbit kidney medulla
Biochim. Biophys. Acta 920, 85–93, 1987

1028779

Littlefield, L. G., Joiner, E. E., Colyer, S. P., and Frome, E. L.
Effects of cryopreservation on the recovery of radiation-induced chromosome aberrations in human lymphocytes
Radiat. Prot. Dosim. 18, 25–29, 1987

Lloyd, D. C., Edwards, A. A., Prosser, J. S., Barjaktarovic, N., Brown, J. K., Horvat, D., Ismail, S. R., Koteles, G. J., Almassy, Z., Krepinsky, A., Kucerova, M., Littlefield, L. G., Mukherjee, U., Natarajan, A. T., and Sasaki, M. S.
A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation
Mutat. Res. 179, 197–208, 1987

Lushbaugh, C. C., Fry, S. A., and Ricks, R. C.
Medical and radiobiological basis of radiation accident management
Br. J. Radiol. (in press)

Lushbaugh, C. C., Fry, S. A., Ricks, R. C., Hübner, K. F., and Burr, W. W.
Historical update of past and recent skin damage radiation accidents
Brit. J. Radiol. Suppl. 19, 7–12, 1986

Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. F., and Shelby, M.
Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells
Mutat. Res. (in press)

Preston, R. J., San Sebastian, J. R., and McFee, A. F.
The in vitro human lymphocyte assay for assessing the clastogenicity of chemical agents
Mutat. Res. (in press)

Richardson, V. B., Littlefield, L. G., and Colyer, S. P.
Cytogenetic evaluations in human lymphocytes exposed to methyl acetimidate, a lysine-specific protein crosslinking agent
Mutat. Res. 180, 121–129, 1987

Robinson, M., Blank, M. L., and Snyder, F.
Highly unsaturated phospholipid molecular species of rat erythrocyte membranes: Selective incorporation of arachidonic acid into phosphoglycerides containing polyunsaturation in both acyl chains
Arch. Biochim. Biophys. 250, 271–279, 1986

Snyder, F., Blank, M. L., Johnson, D., Lee, T-c., Malone, B., Robinson, M., and Woodard, D.
Alkylacetylglycerols versus lyso-PAF as precursors in PAF biosynthesis and the role of arachidonic acid in PAF metabolism
Pharmacol. Res. Commun. 18 (Suppl.), 33–41, 1986

Tardif, S. D., Carson, R. L., and Clapp, N. K.
Breeding performance of captive-born cotton-top tamarin (*Saguinus oedipus*) females: Proposed explanations for colony differences
Am. J. Primatol. 11, 271–275, 1986

Tice, R. R., Ivett, J. L., and McFee, A. F.
The effect of agent treatment time on the induction of sister-chromatid exchanges in mouse bone marrow cells in vivo
Mutat. Res. 182, 15–29, 1987

Woodard, D. S., Lee, T-c., and Snyder, F.
The final step in the de novo biosynthesis of platelet activating factor: Properties of a unique CDP-choline:1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase in microsomes from the renal inner medulla of rats
J. Biol. Chem. 262, 2520–2527, 1987

Zeiger, E., Shelby, M. D., Ivett, J., and McFee, A. F.
Mutagenicity testing of 5-(4-nitrophenyl)-2,4-pentadien-1-al ("spy dust") and its metabolites in vitro and in vivo
Environ. Mutagen. 9, 269–280, 1987

Chapters, Review Articles, Government Reports, and Other Publications

Blank, M. L., Byrd, B. L., Cress, E. A., Washburn, L. C., and Snyder, F. S.
Use of liposomal DTPA in heavy metal detoxification
In *Liposomes as Drug Carriers: Recent Trends and Progress*, (Gregoriadis, G., ed.), John Wiley & Sons Ltd., Chichester, England (in press)

1028780

Blank, M. L., Robinson, M., and Snyder, F.
Chromatographic analyses of ether-linked lipids involved in PAF metabolism
In *Platelet Activating Factor and Related Lipid Mediators*, (Snyder, F., ed.), pp. 33–52, Plenum Publishing Corporation, New York, 1987

Carpenter, A. V.
Radiation exposure and central nervous system cancers: A case-control study among workers at two nuclear facilities
Oak Ridge Associated Universities, Oak Ridge, Tennessee, ORAU-282, 1987

Clapp, N. K. (ed.)
Uniscience Monograph "The Cotton-Top Tamarin: A Primate Model for the Study of Colon Carcinoma."
CRC Press, Baco Raton, Florida (in press)

Committee on Interagency Radiation Research and Policy Coordination
Report of the CIRRPC Policy Subpanel on SI Metric Radiation Units
Oak Ridge Associated Universities, December 1986

CIRRPC
Third Annual CIRRPC Report
Oak Ridge Associated Universities, June 1987

CIRRPC
Update of the International Activities Report
Oak Ridge Associated Universities, May 1987

Coffey, J. L., Watson, E. E., Hübner, K. F., and Stabin, M. G.
Radiopharmaceutical absorbed dose considerations
In *Essentials of Nuclear Medicine Science*, (Hladik, W. B. III, Saha, G. B., and Study, K. T., eds.), pp. 51–74, Williams and Wilkins Corp., Baltimore, Maryland, 1987

Dobson, L., Straume, T., Carrano, A., Minkler, J., Deaven, L., Littlefield, G., and Awa, A.
Cytogenetic effectiveness of neutrons from replica of Hiroshima Bomb: Preliminary results from an interlaboratory study
In *Proceedings of the Fourth International Conference on Environmental Mutagens*, 1985, Stockholm, Sweden (in press)

DuMouchel, W. and Groer, P. G.
A Bayesian methodology for combining radiation studies
In *The Effects on Populations of Internally Deposited Alpha-Emitting Radionuclides: 1987*, Committee on the Biological Effects of Internal Radiations, National Academy Press, Washington, D.C. (in press)

Frome, E. L.
Statistical analysis of cytogenetic dose-response curves
In *Statistical Methods in Toxicological Research*, (Krewski, D. and Franklin, C., eds.), Gordon & Breach Science Publications, New York, New York (in press)

Groer, P. G.
Internal Dosimetry for Epidemiologic Studies
In *Proceedings of the Department of Energy Workshop on Radiobioassay and Internal Dosimetry*, January 20–22, 1987, Albuquerque, New Mexico, PNL-SA-14043, CONF-860146, UC-41, 1987

Groer, P. G. and De B. Pereira, C. A.
Calibration of a radiation detector: Chromosome dosimetry for neutrons
In *Probability and Bayesian Statistics*, (Viertl, R., ed.), Plenum Publishing Corporation, New York, New York (in press)

Hayes, R. L.
Gallium
In *Handbook of Toxicity of Inorganic Compounds*, (Seiler, H. G., Sigel, H., and Sigel, A., eds.), pp. 297–300, Marcel Dekker, Inc., New York, 1987

Hayes, R. L.
Indium
In *Handbook of Toxicity of Inorganic Compounds*, (Seiler, H. G., Sigel, H., and Sigel, A., eds.), pp. 323–326, Marcel Dekker, Inc., New York, 1987

Hübner, K. F., Fry, S. A., Lushbaugh, C. C., Huff, S. H., Littlefield, L. G., Berger, C. D., Ricks, R. C., and Sipe, A. H.
Experience with chelation therapy for actinide incorporaton in the United States based on chelaton therapy (DTPA) registry of the Radiation Emergency Assistance Center/Training Site (REAC/TS)
In [Proceedings] *Workshop, Inkorporation von Radionukliden, 1986*, (Maintz, E., ed.), pp. 168–208, Hamburgische Electricitats-Werke AG, Hamburg, Federal Republic of Germany, 1987

Lee, T-c.
Enzymatic control of the cellular levels of platelet activating factor
In *Platelet Activating Factor and Related Mediators*, (Snyder, F., ed.), pp. 115–133, Plenum Publishing Corporation, New York, 1987

Lee, T-c., Blank, M. L., Fitzgerald, V., Malone, B., and Snyder, F.
Biosynthesis of intermediates in PAF metabolic pathway: Characterization of 1-alkyl-2-acetyl-*sn*-glycerol kinase in rabbit platelets
In *New Horizons in Platelet Activating Factor Research*, (Winslow, C. M. and Lee, M. L., eds.), pp. 37–44, John Wiley & Sons Ltd., Chichester, England, 1987

1028781

Lincoln, T. A.
Educational opportunities in occupational medicine
In *Handbook of Occupational Medicine*, (McCunney, R. J., ed.), Little Brown & Co., Boston, Massachusetts (in press)

Littlefield, L. G.
Applications and limitations of cytogenetic evaluations as an index of recent or previous radiation exposures
In *Proceedings of ASA Conference on Radiation and Health, Coolfont VI, 1986*, Berkeley Springs, West Virginia (in press)

Littlefield, L. G., Joiner, E. E., Frome, E. L., Colyer, S. P., and Sayer, A. M.
Modification of X-ray induced chromosome aberrations: radioprotection by DMSO
In *Radiation Research, Proceedings of the 8th International Congress of Radiation Research, Edinburgh, Scotland, UK*, Vol. 1, (Fielden, E. M., Fowler, J. F., Hendry, J. H., and Scott, D., eds.), p. 146, Taylor and Francis, New York, 1987

Littlefield, L. G., Frome, E. L., Joiner, E. E., and Colyer, S. P.
Modulation of radiation-induced chromosome aberrations by DMSO: preliminary comparisons in three mammalian species
In *Proceedings of DOE Contractor's Workshop, Cellular and Molecular Aspects of Radiation Induced DNA Damage and Repair* (in press)

Lushbaugh, C. C.
Radiation
In *Trauma*, (Mattox, K., Moore, E., and Feliciano, D., eds.), Appleton-Century-Crofts Publishing, Houston, Texas (in press)

Lushbaugh, C. C.
Some examples of failure in X-ray safety
Adv. X-Ray Anal. (in press)

Lushbaugh, C. C., Ricks, R. C., and Fry, S. A.
A U.S. perspective on Chernobyl
In [Proceedings] *International Conference on Non-Military Radiation Emergencies, 1986*, Pan American Health Organization, Washington, D.C. (in press)

Lushbaugh, C. C., Brown, D. G., and Frome, E. L.
Longevity of irradiated burros
In *Life-Span Radiation Effects Studies in Animals: What Can They Tell Us?* (Thompson, R. C. and Mahaffey, J. A., eds.), Proceedings of the Twenty-Second Hanford Life Sciences Symposium, Richland, Washington, 1983, pp. 107–115, CONF-830951, Office of Scientific and Technical Information, Oak Ridge, Tennessee, 1986

Snyder, F., ed.
Platelet Activating Factor and Related Lipid Mediators, 471 p., Plenum Publishing Corporation, New York, 1987

Snyder, F.
The significance of dual pathways for the biosynthesis of platelet activating factor: Alkylacetylglycerols and lyso-PAF as immediate precursors
In *New Horizons in Platelet Activating Factor Research*, (Winslow, C. M. and Lee, M. L., eds.), pp. 13–25, John Wiley & Sons Ltd., Chichester, England, 1987

Snyder, F.
Historical aspects of alkyl lipids and their biologically active forms (ether lipids, platelet activating factor, and antihypertensive renal lipids)
In *Platelet Activating Factor and Related Lipid Mediators*, (Snyder, F., ed.), pp. 1–8, Plenum Publishing Corporation, New York, 1987

Snyder, F.
Enzymatic pathways for platelet activating factor, related alkyl glycerolipids, and their precursors
In *Platelet Activating Factor and Related Lipid Mediators*, pp. 89–114, (Snyder, F., ed.), Plenum Publishing Corporation, New York, 1987

Snyder, F.
Biochemistry of platelet actvating factor
In *Proceedings: Platelet Activating Factor, Pulmonary Hyperreactivity and Asthma*, (Holme, G. and Morley, J., eds.), L'Esterel, Quebec, Canada, 1986, Academic Press, New York (in press)

Snyder, F., Blank, M., Lee, T-c., Robinson, M., and Woodard, D.
Measurement of key enzyme activities involved in the metabolism of platelet activating factor (PAF)
Methods Enzymol. 141, 379–396, 1987

Snyder, F., Record, M., Smith, Z., Blank, M. L., and Hoffman, D. R.
Selective cytotoxic action of ether-lipid analogs of PAF: Mechanistic studies related to their metabolism, subcellular localization, and effects on cellular transport systems
Aktuel. Onkol. (in press)

Surles, J. R.
A structure-activity relationship study of platelet-activating factor
Dissertation, University of North Carolina, Chapel Hill, 1987

1028782

Abstracts (Published) of Papers Presented at Meetings

Clapp, N. K., Henke, M. A., McArthur, A. H., Gangaware, B. L., and Lushbaugh, C. C.
Colonic epithelial changes in tamarins: Are these premalignant changes leading to colon carcinoma?
Proc. Am. Assoc. Cancer Res. 28, 109, 1987

Crook, J., Byrd, B., and Elbert, W.
The influence of sex hormone levels on the rate of development of implanted pheochromocytomas
Acta Pharmacol. Toxicol. 59, 63, 1986

Crook, J. E., Mroczkowski, P. J., Cress, E. E., Blank, M. L., and Snyder, F.
Serum platelet activating factor acetylhydrolase activity in white and black essential hypertensive patients
Circulation 74, II–329, 1986

Das, K. M., Vecchi, M., Sakamaki, S., and Clapp, N. K.
Ulcerative colitis in humans and spontaneous colitis in tamarins: A common link through a crossreactive colonic antigen
Gastroenterology 92, 1363, 1987

Frome, E. L. and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action
Biometrics (in press)

Harrison, M. L. and Tardif, S. D.
Kin recognition in a callitrichid primate
Am. J. Phys. Anthropol. 72, 208, 1987

Harrison, M. L. and Tardif, S. D.
Species differences in responses to conspecific intruders in cotton-top tamarins and common marmosets
Am. J. Primatol. (in press)

Kawasaki, T. and Snyder, F.
Enzymatic synthesis of a novel acetylated neutral lipid (related to platelet activating factor, PAF) by acyl-CoA:1-alkyl-2-acetyl-*sn*-glycerol acyltransferase
Fed. Proc. 46, 2033, 1987

Lee, T-c., Blank, M. L., Fitzgerald, V., and Snyder, F.
Mechanisms of acylation for alkyllysophospholipids
Fed. Proc. 46, 2126, 1987

Lee, Y. C., Lawless, B. D., Crook, J. E., and Clapp, N. K.
Altered T4+/T8+ ratios in tamarins with colitis and colon cancers
Clin. Res. 35, 802A, 1987

Littlefield, L. G., Joiner, E. E., Frome, E. L., and Colyer, S. P.
Modulation of radiation-induced chromosome aberrations by DMSO—An OH radical scavenger
Environ. Mutagen. 9, 61, 1987

Littlefield, G., Sayer, A., and Joiner, E. E.
"Mitotic Chiasmata" in lymphocytes of normal adults
Mamm. Chromosomes Newslett. 27, 9, 1986

Littlefield, L. G., Sayer, A. M., Kleinerman, R. A., and Machado, S. G.
Radiation-induced chromosome aberrations in lymphocytes of women who received radiotherapy for cancer of the cervix > 20 years ago
Mamm. Chromosomes Newslett. (in press)

Lowe, K. W., Holbrook, C. J., Linkous, S. L., and Roberts, M. R.
Preliminary comparison of three cytogenetic assays for genotoxicity in mouse bone marrow cells
Environ. Mutagen. 9, 63, 1987

Malone, B., Lee, T-c., and Snyder, F.
PAF biosynthesis: characterization of alkylacetyl-GP phosphohydrolase in rat spleens
Fed. Proc. 46, 2033, 1987

McFee, A. F., Lowe, K. W., and Linkous, S. L.
A chemical producing unusual chromosome damage patterns in mouse bone marrow cells
Environ. Mutagen. 8, 53, 1986

McFee, A. F. and Tice, R.
Interlaboratory evaluation of a standardized protocol for determination of chemical genotoxicity in mice
Mamm. Chromosomes Newslett. (in press)

Petersen, G. M., Clapp, N. K., and Tardif, S. D.
Spontaneous colonic disease in cotton-top tamarins (*Saguinus oedipus oedipus*): a pan-specific genetic risk
Am. J. Phys. Anthropol. 72, 241–242, 1987

Petersen, G. M., Clapp, N. K., and Tardif, S. D.
Genetic susceptibility to colon cancer in cotton-top tamarins
Clin. Res. 35, 185A, 1987

Petersen, G. M., Clapp, N. K., and Tardif, S. D.
Cotton-top tamarins are genetically susceptible to colon cancer
Gastroenterology 92, 1575, 1987

Tardif, S. D. and Ziegler, T. E.
Age-related effects on ovarian function in tamarins (*Saguinus*)
Primate Rep. 14, 146, 1986

1028783

Washburn, L. C. and Carlton, J. E.
A modified limulus lysate technique for routine pyrogen testing of gallium-68 EDTA
J. Nucl. Med. 27, 1798, 1986

Washburn, L. C., Sun, T. T., Lee, Y. C., Byrd, B. L., Crook, J. E., and Steplewski, Z.
Y-90-Labeled monoclonal antitumor antibodies: Effect of purification method and coinjection of unlabeled antibody
J. Nucl. Med. 28, 721, 1987

Summaries

Bittman, R., Witzke, N. M., Lee, T-c., Blank, M. L., and Snyder, F.
Synthesis and biochemical studies of analogs of platelet activating factor bearing a methyl group at C2 of the glycerol backbone
J. Lipid Res. 28, 733–738, 1987

Two platelet activating factor (PAF) analogs containing a methyl group at C2 of the glycerol moiety were synthesized, and some of their biochemical properties were investigated. 1-*O*-Hexadecyl-2-*C*,*O*-dimethyl-*rac*-glycero-3-phosphocholine (2-methyl-2-methoxy PAF) was prepared in a synthetic scheme beginning with the etherification of 2-methylpropen-1-ol. A reaction sequence involving hydroxylation, tritylation, alkylation, and detritylation afforded 1-*O*-hexadecyl-2-*C*,*O*-dimethyl-*rac*-glycerol, which was converted into the phosphocholine. A 2-lyso derivative of this PAF analog (2-methyl-lyso PAF) was synthesized from 1-*O*-hexadecyl-2-*C*-methyl-3-*O*-trityl-*rac*-glycerol. Benzylation followed by detritylation gave 1-*O*-hexadecyl-2-*C*-methyl-2-*O*-benzyl-*rac*-glycerol, which was converted into the phosphocholine compound. Hydrogenolysis afforded 1-*O*-hexadecyl-2-*C*-methyl-*rac*-glycero-3-phosphocholine (2-methyl-lyso PAF). The 2-methyl-lyso PAF analog served as a substrate for the acetyl-CoA-dependent acetyltransferase that acetylates 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine. However, 2-methyl-lyso PAF did not have a significant effect on the activities of a CoA-independent transacylase or of the acetylhydrolase that inactivates PAF, and thus does not appear to be a substrate or an inhibitor, respectively, for these enzymes. In addition, this analog exhibited only one-half of the antitumor activity of *rac*-1-*O*-alkyl-2-methoxy-*rac*-glycero-3-phosphocholine in human leukemic (HL-60) cells, and elicited no hypotensive response in rats and no platelet-activating activity. In contrast, the 2-methyl-2-methoxy analog of PAF had the same cytotoxic activity against HL-60 cells as *rac*-1-*O*-alkyl-2-methoxyglycero-3-phosphocholine; however, it had no activation effect on rabbit platelets and no hypotensive effect on rats, and it did not inhibit plasma acetylhydrolase activity.

Blank, M. L., Lee, T-c., Cress, E. A., Fitzgerald, V., and Snyder, F.
Plasmalogen biosynthesis in Madin-Darby canine kidney cells: Selectivity in the acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphoethanolamine and the subsequent desaturation step
Arch. Biochem. Biophys. 251, 55–60, 1986

Acyl group specificity in the acylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphoethanolamine (1-alkyl-2-lyso-GroPEtn) to form 1-alkyl-2-acyl-*sn*-glycero-3-phosphoethanolamine (1-alkyl-2-acyl-GroPEtn) and the subsequent desaturation of 1-alkyl-2-acyl-GroPEtn to form plasmalogens (1-alk-1'-enyl-2-acyl-*sn*-glycero-3-phosphoethanolamine, i.e., 1-alk-1'-enyl-2-acyl-GroPEtn) was investigated in intact Madin-Darby canine kidney (MDCK) cells and cell-free membrane preparations. We found 1-[^{3}H]alkyl-2-lyso-GroPEtn was selectively acylated with polyunsaturated fatty acids in the order 20:4>20:5>20:3(n-9)>22:6 by cell-free membrane preparations of MDCK cells. The same pattern of acyl specificity was seen in intact MDCK cells, although the intact cells produced significantly larger amounts of 1-[^{3}H]alkyl-2-acyl-GroPEtn containing oleic acid. There was an increased desaturation of the 1-[^{3}H]alkyl-2-acyl-GroPEtn species containing docosahexaneoic acid to plasmalogens (1-[^{3}H]alk-1'-enyl-2-acyl-GroPEtn) by both intact MDCK cells and the cell-free membrane preparations. The relatively rapid disappearance of the 1-[^{3}H]alk-1'-enyl-2-docosahexaenoyl-GroPEtn species during a 20-h incubation of prelabeled intact MDCK cells suggests a more rapid turnover of this molecular species. Our results indicate there is a high selectivity in the final acylation and desaturation steps of the biosynthetic pathway for plasmalogens.

Boland, C. R. and Clapp, N. K.
Oncodevelopmental mucins in the colons of New World monkeys with ulcerative colitis: The association between inflammation and neoplasia
Gastroenology 92, 625–634, 1987

A spontaneously occurring experimental model of chronic colitis has been described in three closely related species of New World monkeys. One species, *Saguinus oedipus oedipus*, has the additional feature of developing odenocarcinoma of the colon in this setting. Pathological and lectin histochemistry features were associated with the concomitant development of cancer. Chronic inflammation was found in all of the colons examined, and 14% had features of acute inflammation, which was significantly associated with those animals that developed cancer. An oncofetal glycoconjugate structure has been defined in human colons by the ability to bind peanut lectin. A similar glycoconjugate, probably a mucin, was expressed in the colons of 65% of these animals. A marked degree of expression of the mucin was significantly associated with those animals that developed cancer. A highly significant association

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was found between the presence of acute inflammation and the expression of the mucin. These studies demonstrate similarities in glycoconjugate expression between the human colon and the New World monkey model of spontaneous colitis. A form of mucin is expressed in these animals that is highly associated with both the presence of acute inflammation and the development of cancer elsewhere in the colon. In addition, an association between acute inflammatory activity and neoplasia was found, suggesting a potential etiologic linkage between the two.

Carpenter, A. V., Flanders, W. D., Frome, E. L., Cole, P., and Fry, S. A.
Brain cancer and nonoccupational risk factors: A case-control study among workers at two nuclear facilities
Am. J. Public Health 77, 1180–1182, 1987

In a nested case-control study of nuclear workers, 82 brain cancer cases were compared with 328 matched controls to investigate the possible association with nonoccupational risk factors such as histories of epilepsy or head injury. We observed a moderately strong association between brain cancer occurrence and history of epilepsy (OR = 5.7, 95 per cent CI: 1.0, 32.1), but did not find a positive association with previous head injury (OR = 0.9, 95 per cent CI: 0.2, 4.2).

Carpenter, A. V., Flanders, W. D., Frome, E. L., Crawford-Brown, D. J., and Fry, S. A.
CNS cancers and radiation exposure: A case-control study among workers at two nuclear facilities
J. Occup. Med. 29, 601–604, 1987

A nested case-control study was conducted among workers employed in two nuclear facilities to investigate the possible association of primary CNS cancers with occupational exposure to radiation from external and internal sources. External radiation monitoring data from film badges were available for 27 cases and 90 matched controls. The radiation dose to the lung from internally deposited uranium was estimated for 47 cases and 120 matched controls from area and personnel monitoring data and was used in analyses in lieu of the brain dose. No association was observed between deaths from CNS cancers and exposure to ionizing radiation from external or internal sources. However, due to the small number of monitored subjects and low doses, a modest association could not be ruled out.

Clapp, N. K., McArthur, A. H., Carson, R. L., Henke, M. A., Peck, O. C., and Wood, J. D.
Visualization and biopsy of the colon in tamarins and marmosets by endoscopy
Lab. Anim. Sci. 37, 217–219, 1987

Endoscopic visualization and biopsy have been performed under anesthesia in more than 65 tamarins and marmosets to study the pathogenesis of colitis and cancer of the colon. This procedure allows examination of the large bowel from the anus to the cecum and has been repeated at 2–6 month intervals with few complications. However, care must be exercised not to perforate the colon. Successful use of this technique will permit study of the pathogenesis of colonic diseases throughout the life of the animal and should provide cause-effect information about colitis and colon cancer in tamarins that may apply to the human diseases.

Dupree, E. A., Cragle, D. L., McLain, R. W., Crawford-Brown, D. J., and Teta, M. J.
Mortality among workers at a uranium processing facility: Linde Air Products Company Ceramics Plant, 1943–1949
Scand. J. Work Environ. Health 13, 100–107, 1987

A retrospective cohort mortality study of 995 white males employed more than 30 d at a uranium processing facility in upstate New York between 1943 and 1949 investigated the association between excess observed deaths and long-term occupational exposure via inhalation to uranium compounds. Two comparison groups were used, the white male population of the United States and the white male population in the New York counties of Erie and Niagara. The vital status determination was 94.3% complete through 31 December 1979. With the use of the national comparison group, statistically significantly increased standardized mortality ratio (SMR) values were observed for all causes (SMR 118), laryngeal cancer (SMR 447), all circulatory diseases (SMR 118), arteriosclerotic heart disease (SMR 119), all respiratory diseases (SMR 152), and pneumonia (SMR 217). Site-specific outcomes of special interest with a statistically increased number of deaths above expected were laryngeal cancer (observed 5) and pneumonia (observed 17). No association was found with length of employment or work in the most hazardous areas of the plant. The comparison with regional rates gave similar results.

Ezra, D., Laurindo, F. R. M., Czaja, J. F., Snyder, F., Goldstein, R. E., and Feuerstein, G.
Cardiac and coronary consequences of intracoronary platelet activating factor infusion in the domestic pig
Prostaglandins 34, 41-57, 1987

In previous studies we have shown that platelet activating factor (PAF) is a potent vasoactive substance with deleterious effects on coronary blood flow (CBF) and myocardial performance. The present study further investigates the effects of PAF during its sustained intracoronary infusion in the blood-perfused domestic pig (n=16). PAF infusion (1-9 nmol/min) produced triphasic changes in CBF (n=7): an initial brief phase of coronary dilation (14 ± 2% above baseline), followed by severe reduction in CBF due to increase in coronary vascular resistance and a third

1028785

phase of escape that was characterized by return of CBF towards baseline in spite of continuing PAF infusion. In 9 remaining pigs PAF infusion had a biphasic response: the first phase of coronary dilation rapidly turned into severe coronary constriction accompanied by severe systemic hypotension and death within a few min. PAF infusion caused a profound rise in systemic arterial and coronary venous thromboxane B_2 levels, while 6-keto-$PGF_{1\alpha}$ and leukotriene C_4-immunoreactivity levels were not changed. Indomethacin completely blocked the rise in thromboxane level during PAF infusion and abolished the constrictor effect of PAF on the coronary vessels. These data suggest that PAF might play a detrimental role on the coronary circulation and cardiac function, primarily through thromboxane A_2 mediated mechanism.

Hoffman, D. R., Hoffman, L. H., and Snyder, F.
Cytotoxicity and metabolism of alkyl phospholipid analogues in neoplastic cells
Cancer Res. 46, 5803–5809, 1986

The cytotoxic response of several types of neoplastic cells to analogs of unnatural alkyl phospholipids (e.g., *rac*-1-hexadecyl-2-methoxy-glycero-3-phosphocholine) has been partially attributed to their accumulation as a result of the low activity of the alkyl cleavage enzyme (a tetrahydropteridine-dependent monooxygenase) in tumor cells. We tested this possibility by comparing the alkyl cleavage enzyme activity in cells that exhibit differences in sensitivity toward the cytotoxic effects of the *rac*-1-hexadecyl-2-methoxy-glycero-3-phosphocholine. Human promyelocytic leukemia cells (HL-60), a cell line highly sensitive to the cytotoxic alkyl phospholipid analog, possessed an alkyl cleavage enzyme activity (0.25 pmol/min/μg protein) similar to that found in three cell types known to be relatively resistant to the cytotoxic activity of the analog: immature human promyeloblastic leukemia cells (K562) (0.22 pmol/min/μg protein), human polymorphonuclear neutrophils (PMN) (0.34 pmol/min/μgprotein), and Madin-Darby canine kidney cells (0.37 pmol/min/μg protein). Moreover, our results indicate that the cytotoxic *rac*-1-octadecyl-2-methoxy-glycero-3-phosphocholine analog is not a substrate for the alkyl cleavage enzyme with an active microsomal preparation of the enzyme from rat liver; cleavage of this analog was 200-fold less than the rate obtained with 1-octadecylglycerol as substrate. In cultures of either sensitive or resistant type cells, approximately 90% of the added *rac*-1-[9',10'-^{3}H] octadecyl-2-methoxy-glycero-3-phosphocholine was not metabolized during a 24-hr incubation. The amount of radiolabel in fatty acids, a major product of alkyl cleavage activity, was small and essentially identical amounts were produced in all four cell types (3.1 ± 0.2%). These data indicate that differences in the cellular activities of the alkyl cleavage enzyme are not responsible for the differential cytotoxic responses between normal and specific types of neoplastic cells toward *rac*-1-octadecyl-2-methoxy-glycero-3-phosphocholine. On the other hand, the cellular uptake of the alkyl phospholipids could be a factor in explaining the cytotoxic response of certain tumor cells, since more radiolabeled 1-octadecyl-2-methoxy-glycero-3-phosphocholine was associated with the susceptible HL-60 cells than the resistant cell types.

Autoradiography revealed that the radiolabeled 2-methoxy analog accumulates at the periphery of HL-60 leukemia cells, whereas the label was more uniformly distributed in PMN and K562 cells. In contrast the relatively nontoxic naturally occurring 1-[1',2'-^{3}H] alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (platelet-activating factor), an important cellular mediator involved in hypersensitivity and cardiovascular responses, is rapidly metabolized by HL-60 cells and appears evenly distributed throughout the leukemia cells as judged by autoradiography. Data from these experiments indicate that the unnatural alkyl phospholipids accumulate at the surface membrane of "sensitive" cancer cells, the site where their cytotoxic action appears to be elicited.

Kawasaki, T. and Snyder, F.
The metabolism of lyso-platelet-activating factor (1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine) by a calcium-dependent lysophospholipase D in rabbit kidney medulla
Biochim. Biophys. Acta 920, 85–93, 1987

A Ca^{2+}-dependent lysophospholipase D activity in microsomal preparations from the rabbit kidney medulla hydrolyzes the choline moiety from 1-*O*-[9,10-^{3}H]hexadecyl-2-lyso-*sn*-glycero-3-phosphocholine (lyso-PAF) to form 1-*O*-[9,10-^{3}H] hexadecyl-2-lyso-*sn*-glycero-3-P; the latter is subsequently dephosphorylated by a phosphohydrolase to 1-*O*-[9,10-^{3}H]hexadecyl-*sn*-glycerol. Sodium vanadate, which is known to inhibit phosphohydrolases, reduces the proportion of hexadecylglycerol and increases the formation of hexadecyllysoglycero-phosphocholine. Essentially no hydrolysis occurs when the *sn*-2 position of the hexadecyllyso-GPC substrate contains an acyl moiety. The lysophospholipase D in rabbit kidney is of microsomal origin and has a broad pH optimum between 8.0 and 8.8, with the activity decreasing sharply from pH 7.6 to 7.2. Wykle et al. (*Biochim. Biophys. Acta* 619 (1980) 58–67) have previously demonstrated the existence of a microsomal lysophospholipase D (specific for ether lipid substrates) in rat tissues that requires Mg^{2+} and exhibits a pH optimum of 7.2; high activities of the Mg^{2+}-dependent lysophospholipase D were found in liver and brain, but not in kidney. In contrast to the Mg^{2+}-dependent lysophospholipase D in rat tissues, the renal enzyme from rabbits requires Ca^{2+} (5 mM),

1028786

whereas Mg^{2+} (5 mM) exhibits little stimulatory action. Under optimal assay conditions (0.1 M Tris-HCl (pH 8.4)/5 mM $CaCl_2$), lysophospholipase D in the rabbit kidney medulla has an activity of 2.7 nmol/min per mg protein compared to 0.9 nmol/min per mg protein for the lysophospholipase D in the rat kidney medulla (0.1 M Tris-HCl (pH 7.2)/5 mM $MgCl_2$). The Ca^{2+}-dependent lysophospholipase D is highest in the liver and kidney medulla from rabbits, but is very low in rat tissues; similar activities were found in male and female rabbits. Our data indicate that the divalent metal ion requirements for expression of maximum lysophospholipase D activities can differ markedly among animal species and also suggest the microsomal Ca^{2+}-dependent lysophospholipase D is an important catabolic route for lyso-PAF metabolism in rabbit renomedullary tissue.

Lloyd, D. C., Edwards, A. A., Prosser, J. S., Barjaktarovic, N., Brown, J. K., Horvat, D., Ismail, S. R., Koteles, G. J., Almassy, Z., Krepinsky, A., Kucerova, M., Littlefield, L. G., Mukherjee, U., Natarajan, A. T., and Sasaki, M. S.
A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation
Mutat. Res. 179, 197–208, 1987

An experiment sponsored by the International Atomic Energy Agency was undertaken to compare dose estimation by cytogenetic analysis on aliquots of samples of irradiated blood sent by air to participating laboratories. Accidental acute whole-body irradiations to 0.7 and 2.34Gy and half-body irradiations to 3.5 Gy were simulated with X rays and gamma rays. For the partial irradiations, the size of the irradiated fraction and its dose were estimated by the Qdr and contaminated Poisson techniques. Each laboratory's in vitro dose-response data were fitted to the quadratic model by the iteratively reweighted least squares method.

Interlaboratory variations in dose-response curves and in the aberration yields and dose estimates for the simulated accidents were noted. However, in general, most participants consistently obtained results acceptably close to the true values.

Littlefield, L. G., Joiner, E. E., Colyer, S. P., and Frome, E. L.
Effects of cryopreservation on the recovery of radiation-induced chromosome aberrations in human lymphocytes
Radiat. Prot. Dosim. 18, 25–29, 1987

Experiments were conducted to determine whether irradiated blood samples may be preserved by freezing without compromising the accurate assessment of radiation-induced chromosome aberrations in lymphocyte cultures initiated at later dates. Human whole blood at 37°C was exposed *in vitro* to 0,1,2, or 4 Gy cobalt-60 gamma radiation, and lymphocytes were cultured immediately after exposure or after one week's storage at -70°C. A slight depression in cellular proliferation and a significant increase in chromatid breakages were observed in cultures initiated from the previously frozen lymphocytes. In preparations from both fresh and frozen lymphocytes the dose response relationships for radiation-induced dicentrics and acentrics were adequately described by the linear-quadratic dose response model ($Y=\alpha D+\beta D^2$) with no significant differences in values of the alpha or beta coefficients between the two sets of cultures. This finding provides evidence that lymphocytes bearing radiation-induced chromosome aberrations are not at selective risk for cell death as a result of cryopreservation.

Lushbaugh, C. C., Fry, S. A., Ricks, R. C., Hübner, K. F., and Burr, W. W.
Historical update of past and recent skin damage radiation accidents
Brit. J. Radiol. Suppl. 19, 7–12, 1986

Records of radiation accidents worldwide since 1944 are maintained at the Radiation Accident Registry of the Radiation Emergency Assistance Center/Training Site (REAC/TS) in Oak Ridge. These records show that in the 263 major radiation accidents which have occurred, there have been 150 severe local radiation injuries, of which 117 have been from exposure to sealed radioactive sources. Most of these lesions have resulted from the unsafe handling of ^{192}Ir radiography sources. Recent redesign of these devices, used for testing the integrity of welds, promises to eliminate these accidents. However, many other kinds of irradiators used in industry and scientific research still remain in the public domain, capable of causing irreparable dermal damage. The Registry records reveal that there are many unsolved physical and medical problems whose solution is urgently needed to improve the prognosis and therapy of such lesions. Pathologically, radiation-induced skin lesions are well described and an approximate dose-response relationship is universally accepted even though the actual "dose" is rarely known when the patient is first seen. Radiation dose is estimated biologically after the lesion has run its pathological course or after a medical physicist has prepared a "mock-up" of the accident from a retrospective scenario.

Most of the dermal injuries reported here are described by anecdotal histories, which underscore the urgent need for better, quantitative, non-destructive methods for defining the extent of the clinically significant radiation exposure as early as possible after its occurrence.

1028787

Richardson, V. B., Littlefield, L. G., and Colyer, S. P.
Cytogenetic evaluations in human lymphocytes exposed to methyl acetimidate, a lysine-specific protein crosslinking agent
Mutat. Res. 180, 121–129, 1987

The cytogenetic effects of methyl acetimidate (MAI), a lysine-specific protein crosslinking reagent, were investigated using human peripheral lymphocytes in culture. Lymphocytes were treated with the chemical either prior to PHA exposure or 2–3 days following mitogenic stimulation and assessed for perturbations in cellular proliferation and induction of SCEs. Severe reductions in the mitotic index (MI) and pronounced decreases in the proportion of metaphases proceeding beyond M(1) were observed following G_0 exposure to MAI concentrations of as low as 2 mM; with complete suppression of mitotic activity in all cultures exposed to levels of 3 mM MAI or greater. Concentrations resulting in severe depression in MI caused only moderate increases in SCEs. Cells exposed to less than 10 mM MAI during the late S-G_2 stages of the cell cycle and harvested at the first metaphase following treatment exhibited profound mitotic delay, impaired prophase to metaphase transitions and abnormal mitotic configurations. These findings demonstrate that protein-specific crosslinking agents may induce a wide spectrum of adverse cytogenetic outcomes in both cycling and noncycling lymphocytes.

Robinson, M., Blank, M. L., and Snyder F.
Highly unsaturated phospholipid molecular species of rat erythrocyte membranes: Selective incorporation of arachidonic acid into phosphoglycerides containing polyunsaturation in both acyl chains.
Arch. Biochem. Biophys. 250, 271–279, 1986

This study describes for the first time the complete molecular species composition and turnover of [^{3}H]arachidonic acid in various glycerophospholipid classes of rat erythrocytes, a model system that has been extensively used to investigate numerous membrane phenomena. Quantitative analysis of the individual molecular species of the choline, ethanolamine, serine, and inositol glycerophospholipid classes was made possible by preparing their diradylglycerobenzoate derivatives that could be quantitated by on-line UV detection in conjunction with high performance liquid chromatography; turnover of the molecular species containing arachidonate was evaluated in erythrocytes labeled with [^{3}H]arachidonic acid. A unique observation was the significant amounts of 22:6-20:4, 20:4-20:4, and 18:2-20:4 species observed in the diacyl fractions of phosphatidylethanolamine and phosphatidylserine. Moreover, the analysis of the specific radioactivities of individual phospholipid species from erythrocytes incubated with [^{3}H]arachidonic acid demonstrated a selective incorporation of arachidonic acid into the most highly unsaturated molecular species in all of the phospho-lipid classes examined. Although the 22:6-20:4, 20:4-20:4, and 18:2-20:4 species represented only 4.5% of the total mass of the diacyl phosphoglycerides, these species accounted for a major portion (37%) of the arachidonic acid incorporated into the phospholipids. These results demonstrate the existence of unique populations of phospholipid molecules in rat erythrocytes with a high degree of unsaturation that exhibit a very rapid metabolic turnover rate.

Tardif, S. D., Carson, R. L., and Clapp, N. K.
Breeding performance of captive-born cotton-top tamarin (*Saguinus oedipus*) females: Proposed explanations for colony differences
Am. J. Primatol. 11, 271–275, 1986

Successful breeding by captive-born *Saguinus oedipus* females has now been reported from several colonies, but a marked parity effect (ie, differences in infant survival with the number of litters produced) is usually observed; survival of infants of primiparous females is extremely low but increases with subsequent litters. This parity effect was not observed in the captive-born females in the Oak Ridge Associated Universities colony, with survival of offspring of primiparous females at 58.6%. Two explanations are proposed for this difference: (1) pairing with a male having previous experience in siring and rearing offspring may improve infant survival for primiparous females; and (2) postponing mating to a later age may increase infant survival for primiparous mothers.

Tice, R. R., Ivett, J. L., and McFee, A. F.
The effect of agent treatment time on the induction of sister-chromatid exchanges in mouse bone marrow cells in vivo
Mutat. Res. 182, 15–29, 1987

The effect of time of agent administration, via intraperitoneal injection, on the yield of SECs in bone marrow acells of male B6C3F1 mice was determined for cyclophosphamide (CP), 7,12-dimethylbenz[a] anthracene (DMBA) and mitomycin C (MMC). Animals were treated with several doses of each carcinogen/mutagen at 3 different treatment times: -1, +1 and +8 h in relation to the onset of BrdUrd administration. The results of these studies indicate that the optimal treatment time for inducing a maximal SCE response is agent-specific. For CP, the slope of the SCE response was greatest at the +8 h treatment time, while the maximal response for DMBA occurred at the -1 h treatment time. For MMC, the slope of the SCE response was independent of treatment time and of the method of bromodeoxyuridine administration (intravenous infusion vs. tablet implantation) but dependent on the laboratory conducting the study (Brookhaven National Laboratory vs. Oak Ridge Associated Universities). Based on the results of these

1028788

studies, the 1 h acute treatment time is considered optimal for the in vivo cytogenetic evaluation of suspect chemicals for genotoxic activity when bone marrow is used as the target cell population.

Woodard, D. S., Lee, T-c., and Snyder, F.
The final step in the de novo biosynthesis of platelet activating factor: Properties of a unique CDP-choline:1-alkyl-2-acetyl-*sn*-glycerol cholinephosphotransferase in microsomes from the renal inner medulla of rats
J. Biol. Chem. 262, 2520–2527, 1987

Final steps in the synthesis of platelet activating factor (PAF) occur via two enzymatic reactions: a) the acetylation of 1-alkyl-2-lyso-*sn*-glycero-3-phosphocholine by a specific acetyltransferase or b) the transfer of the phosphocholine base group from CDP-choline to 1-alkyl-2-acetyl-*sn*-glycerol by a DTT-insensitive cholinephosphotransferase. Our studies demonstrate that rat kidney inner medulla microsomes synthesize PAF primarily via the DTT-insensitive cholinephosphotransferase since the specific activity of this enzyme is >100-fold higher than the acetyltransferase. The two cholinephosphotransferases that catalyze the biosynthesis of phosphatidylcholine and PAF have similar Mg^{2+} or Mn^{2+} requirements and are inhibited by Ca^{2+}. Also topographic experiments indicated both activities are located on the cytoplasmic face of microsomal vesicles.

PAF synthesis was slightly stimulated by 10 mM DTT, whereas the enzymatic synthesis of phosphatidylcholine was inhibited >95% under the same conditions. The concept of two separate enzymes for PAF and phosphatidylcholine synthesis is further substantiated by the differences in the two microsomal cholinephosphotransferase activities with respect to pH optima, substrate specificities, and their sensitivities to temperature, deoxycholate, or ethanol.

Study of the substrate specificities of the DTT-insensitive cholinephosphotransferase showed that the enzyme prefers a lipid substrate with 16:0 or 18:1 *sn*-1 alkyl chains. Short chain esters at the *sn*-2 position (acetate or propionate) are utilized by the DTT-insensitive cholinephosphotransferase but analogs with acetamide or methoxy substituents at the *sn*-2 position are not substrates. Also, CDP-choline is the preferred water-soluble substrate when compared to CDP-ethanolamine. Utilization of endogenous neutral lipids as a substrate by the DTT-insensitive cholinephosphotransferase demonstrated that sufficient levels of alkylacetylglycerols are normally present in rat kidney microsomes to permit the synthesis of physiological quantities of PAF. These data suggest the renal DTT-insensitive cholinephosphotransferase could be a potentially important enzyme in the regulation of systemic blood pressure.

Zeiger, E., Shelby, M. D., Ivett, J., and McFee, A. F.
Mutagenicity testing of 5-(4-nitrophenyl)-2,4-pentadien-1-al ("spy dust") and its metabolites in vitro and in vivo
Environ. Mutagen. 9, 269–280, 1987

5-(4-Nitrophenyl)-2,4-pentadien-1-al (NPPD; spy dust) was tested for mutagenicity in Salmonella and for its ability to induce chromosome aberrations and sister chromatid exchanges (SCE) in cultured Chinese hamster ovary (CHO) cells and mouse bone marrow. Two metabolites of NPPD produced by the rat, 4-nitrobenzoic acid (NBA) and 4-nitrohippuric acid (NHA), were also tested in Salmonella and CHO cells. NPPD was mutagenic in Salmonella, and induced low-level increases in chromosome aberrations and SCE in bone marrow. It did not induce aberrations in CHO cells. NBA was positive in Salmonella and CHO cells, while NHA was negative. The mutagenicity of NPPD in Salmonella was partially, but not completely, eliminated in a strain lacking one of the bacterial nitroreductases.

Invited Lectures Presented at National and International Scientific Conferences

(Published abstracts of these presentations are not available.)

Fry, S. A., Carpenter, A. V., Checkoway, H., Cragle, D. L., Crawford-Brown, D. J., Dupree, E. A., Frome, E. L., Groer, P. G., Lushbaugh, C. C., Shy, C. M., and Watson, J. E.
Studies of mortality among populations of U.S. nuclear industry workers
Presented at the British Nuclear Energy Society Conference on Health Effects of Low Dose Ionising Radiation—Recent Advances and Their Implications, London, England, 1987

Lee, T-c.
Regulation and metabolism of PAF
Gordon Research Conference, Medicinal Chemistry, New London, NH, 1987

Lushbaugh, C. C.
Chernobyl, early radiation effects
Physicians Seminar on Radiation Effects on Man, White Haven, PA, 1986

Lushbaugh, C. C.
Medical response and responses of health physicists and physicians to radiation emergencies
Special Session at the 32nd Annual Meeting of the Health Physics Society, Salt Lake City, UT, 1987

1028789

Snyder, F.
Structure, metabolism, and function of ether lipids in mammalian cells
Presented at the First International Symposium on Ether Lipids in Oncology, Gottingen, Federal Republic of Germany, 1986

Snyder, F.
Metabolism and intracellular processing of PAF and selective antitumor PAF analogues: Enzymatic and subcellular sites for potential pharmacological intervention
Presented at The Promise of PAF, An International Seminar on the Therapeutic and Commercial Potential of Drugs Affecting Platelet Activating Factor, London, England, 1986

Snyder, F.
Relation of polyenoic transacylation to platelet activating factor and plasmalogen metabolism
Gordon Research Conference, Lipid Metabolism, Meriden, NH, 1987

Snyder, F.
Biosynthesis and membrane processing of platelet activating factor (PAF)
Twelfth International Conference on Biological Membranes, Crans-sur-Sierre, Switzerland, 1987

Snyder, F., Lee, T-c., Blank, M. L., and Malone, B.
Enzyme specificities and regulatory controls in the biosynthesis of platelet-activating factor (PAF)
Tenth International Congress of Pharmacology, Sydney, Australia, 1987

Miscellaneous Presentations at Meetings With No Published Abstracts

Crook, J. E., Mroczkowski, P. J., Cress, E. A., Blank, M. L., and Snyder, F.
Serum platelet activating factor acetylhydrolase activity in white and black essential hypertensive patients
Presented at the American Heart Association 59th Scientific Sessions, Dallas, TX, 1986

Crook, J. E., Som, P., Kubota, K., Brill, A. B., and Knapp, F. F., Jr.
Myocardial substrate utilization in catecholamine excess hypertension as assessed by quantitative autoradiography
Presented at the V International Symposium on Radiopharmacology, Buenos Aires, Argentina, 1986

1028790

Collaborative Research

N. K. Clapp

Genetic epidemiology and immunocologic parameters in tamarin colitis and colon cancer. Harbor-UCLA School of Medicine (Los Angeles), Department of Medicine, G. Petersen; Division of Gastroenterology, W. Snape, S. Targan

Coronaviruses in colitis and colon cancer. University of Tennessee (Knoxville), College of Veterinary Medicine, D. Brian

Ontogentic skeletal changes in *Saguinus fuscicollis* and *Saguinus oedipus*. University of Tennessee (Knoxville), Department of Anthropology, W. Bass

Lectin binding in colitis and colon cancer in cotton-top tamarins. VA Medical Center (Ann Arbor), Gastroenterology Section, C. R. Boland

Fecal toxins in tamarin colon disease. University of Tennessee Medical Center (Knoxville), J. Fuhr

Idiopathic ulcerative colitis in marmosets. University of Nevada (Reno), School of Medicine, O. Peck; The Ohio State University (Columbus), Physiology Department, J. Wood

CEA and LDH_x in colon-cancer-bearing tamarins. Roswell Park Memorial Institute (Buffalo), Surgical Developmental Oncology Department, N. Petrelli

Heavy metal toxicity in tamarins. University of Tennessee (Knoxville), School of Veterinary Medicine, W. Farkas

Marmoset colonic antigen reactivity with human ulcerative colitis antibody. Albert Einstein College of Medicine (Bronx), K. Das

Electrophoretic survey of genetic diversity of tamarin colony. Laboratory of Viral Carcinogenesis (Frederick, Maryland), Section of Genetics, L. Forman

Evaluation of immunosuppression in marmosets. University of Tennessee Memorial Medical Center (Knoxville), J. Fuhr

Oncogenicity of Epstein-Barr Virus. The Ohio State University (Columbus), Department of Medical Microbiology and Immunology, R. Glaser

Nutritional requirements of marmosets. Laboratory Animal Nutritionist, National Institutes of Health (Bethesda), J. Knapka

Expression of oncogenes in inflammatory bowel diseases in cotton-top tamarins. Harbor-UCLA Medical Center (Torrance), Department of Medicine, S. Meltzer

Identification of fecal mutagens and fecal microflora in tamarins and marmosets. National Cancer Institute (Frederick, Maryland), Laboratory of Comparative Carcinogenesis, J. Rice and P. Donovan

Monoclonal antibodies to detect tamarin colon cancer. The University of Texas (Galveston), Department of Surgery, C. Townsend

J. E. Crook

Development of software for quantitative positron tomography. Vanderbilt University (Nashville), Department of Biomedical Engineering, P. H. King; EG&G ORTEC (Oak Ridge), R. M. Keyser

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp, Jr.

Clinical use of radiopharmaceuticals in human disease. The University of Tennessee Medical Center (Knoxville), Department of Radiology, K. F. Hübner

Flow cytometry and OKT4+/OKT8+ measurements. The University of Tennessee Medical Center (Knoxville), Department of Biology, J. Fuhr

Use of radiopharmaceuticals in animal models of human disease. The University of Tennessee (Knoxville), College of Veterinary Medicine, R.E. Gompf

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. Fordham University (New York), Department of Science, B. D. Lawless

Development of imaging techniques for positron emission tomography. Computer Technology and Imaging, Inc. (Knoxville), M. Andreaco

Evaluation of an 8 MeV proton cyclotron as a source of radionuclides for positron emission tomography. ORBIT, Inc. (Oak Ridge), J. H. Neiler

S. A. Fry

Morbidity among workers exposed to mercury at the Oak Ridge Y-12 plant. University of Michigan (Ann Arbor), Department of Environmental and Industrial Health, L. Fine

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Radiation Epidemiology Branch (Bethesda), R. Kleinerman and J. Boice

1028791

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons. Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straume, A. Carrano, and ⁻. Minkler; Los Alamos National Laboratory (Los Alamos), L. Devan; Radiation Effects Research Foundation (Hiroshima), A. Awa

Coordinated research program on Routine Use of Chromosomal Aberration Analysis in Occupationally Exposed Radiation Workers. International Atomic Energy Agency (Vienna), A. Bianco

Chromosome aberrations in human lymphocytes exposed to monoenergetic neutrons in the presence or absence of DMSO. Radiological Research Accelerator Facility, Columbia University, Paul Goldhagen, Stephen Marino

Evaluations of HGPRT mutants in lymphocytes of previously irradiated patients. University of Vermont, Patrick O'Neill

The effect of methyl acetimidate on cellular proliferation and chromosome structure. Tuskegee University (Alabama), Department of Biology, V. Richardson

C. C. Lushbaugh

United States Transuranium Registry. Hanford Environmental Health Foundation, B. D. Breitenstein

C. C. Lushbaugh and N. K. Clapp

Marmoset T and B cellular immunology. Meharry Medical College (Nashville), Department of Microbiology, P. Patel

C. C. Lushbaugh and S. A. Fry

Epidemiology of human carcinogenesis. University of Alabama (Birmingham), Department of Epidemiology, P. Cole

Comprehensive epidemiology of DOE atomic workers. University of North Carolina (Chapel Hill), Department of Epidemiology, C. M. Shy

C. C. Lushbaugh and L. C. Washburn

Diethylenetriaminepentaacetate (DTPA) project: Department of Energy contractors, 45 coinvestigators

A. F. McFee

In vivo mouse assay for chemically induced SCE and chromosomal aberrations. Brookhaven National Laboratory, R. Tice

F. Snyder

Biological effects of unique phospholipid analogs. California State University (Northridge), Department of Chemistry, J. Hajdu

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Piantadosi

Cardiovascular and sympathetic effects of 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein

Synthesis and biochemical studies of analogs of platelet-activating factor. Queens College of the City University of New York (Flushing), Department of Chemistry, R. Bittman

Platelet activating factor levels and acetylhydrolase activities in plasma from cancer patients treated with interleukin II. Loyola Medical Center (Maywood, Illinois), J. Godwin

Platelet activating factor metabolism in insects. Louisiana State University (Baton Rouge), E. N. Lambremont

S. D. Tardif

Reproductive aging in tamarin females. University of Wisconsin (Madison), Department of Psychology, C. Snowdon and T. Ziegler

Demographic analyses and computerized colony management. Southwest Foundation for Biomedical Research (San Antonio), Department of Genetics, B. Dyke and T. Gage

Kin recognition and sexual interactions in marmosets and tamarins. Kent State University (Kent), Departments of Anthropology and Biology, M. L. Harrison

Morphology of tamarin ovaries. University of Tennessee (Knoxville), College of Veterinary Medicine, S. Orosz, P. Morris, and R. Funk

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. The Wistar Institute (Philadelphia), Z. Steplewski

Short-lived alpha-emitting radionuclides for labeling monoclonal antibodies. Oak Ridge National Laboratory, Chemical Technology Division, J. E. Bigelow

Synthesis of labeled compounds using organoborane techniques. University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka

1028792

Training Programs

Training programs at the division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. In addition, special short-term courses, conducted by REAC/TS, provide training for biomedical personnel in matters related to nuclear accidents and associated radiation health problems.

Graduate students doing Ph.D. dissertation research in our division have been affiliated with the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, University of Tennessee (Knoxville and Memphis campuses), University of North Carolina (Chapel Hill), University of Alabama (Birmingham), and University of Kentucky (Lexington). These students have done research in biochemistry, epidemiology, cytogenetics, and radiopharmaceuticals. The Ph.D. is awarded by the parent university at which the student must meet all of the academic requirements for the degree. Financial support for these graduate studies comes from the National Cancer Institute Training Grant administered by the Oak Ridge National Laboratory, the University Programs Division at ORAU (sponsored by DOE), and individual reseach grants.

Postdoctoral training continues to be an important part of the division's research efforts. Funds for support of postdoctoral fellows have been available from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health, American Cancer Society, the World Health Organization (International Agency for Research on Cancer), the Fogarty International Center (U.S. Public Health Service), and division funds.

Summer research opportunities enable faculty and undergraduate students from colleges and universities throughout the United States to participate in our division's research programs. The DOE-sponsored summer program is administered through the University Programs Division at ORAU.

Visiting scientists from various universities and other U.S. and foreign institutions also participate in division research programs. Some of these scientists are sponsored through the Research Participation Program administered by ORAU's University Programs Division. In some instances, funds for visiting scientists are awarded through various national or international agencies, and occasionally, such appointments are made directly using programmatic funds from the Medical Division.

Physicians, health physicists, and paramedical personnel attend short-term courses in the management of medical problems associated with radiation accidents offered at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A special course, offered for the second year, also provides basic concepts in radiation for attorneys. A total of 13 REAC/TS courses were given during the past year.

Several members of the senior staff conduct special courses and provide lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee (Knoxville). Several staff scientists have joint appointments at ORAU member universities. There, they teach and also advise graduate students who pursue their research studies at ORAU.

The individuals listed here participated in the training programs at the division during the past year:

1028793

Predoctoral Students

Elizabeth Dupree Ellis* (Epidemiology) (University of North Carolina,Chapel Hill)
Judith Qualters (Epidemiology) (University of North Carolina, Chapel Hill)
Zigrida Smith (Biochemistry) (University of Tennessee, Knoxville)
Jefferson Surles** (Medicinal Chemistry) (University of North Carolina, Chapel Hill)

Postdoctoral Fellows

Arvind Carpenter (Epidemiology)
Tomio Kawasaki (Biochemistry)
Joseph Y. Lee (Biochemistry)
Yu-Chen C. Lee (Radiopharmaceuticals)
Changgeng Qian (Biochemistry)
David S. Vallari (Biochemistry)

Visiting Scientists

Velma Richardson (Cytogenetics) (Tuskegee University, Tuskegee, Alabama)
Sponsored by University Programs Division
B. DeSales Lawless (Radiopharmaceuticals) (Fordham University, New York, New York)
Sponsored by University Programs Division
Soha Kholeif, (Cytogenetics) Alexandria, Egypt *International Atomic Energy Agency Fellowship*

College Student Research Participants

William Grever (Radiopharmaceuticals) (University of Wisconsin, Madison)
Christine Drivdahl (Radiopharmaceuticals) (Carroll College, Helena, Montana)
Ray J. Lee (Radiopharmaceuticals) (Washington University, St. Louis, Missouri)
Elizabeth Odell (Biochemistry) (Belmont Abbey College, Belmont, North Carolina)
Barbara Perkins (Marmoset Research) (The College of St. Rose, Albany, New York)

High School Vocational Office Education Students

Melinda Armes (Epidemiology) (Harriman High School)
Tisha L. Duncan (Biochemistry) (Clinton High School)
Karen Kennedy (Epidemiology) (Oak Ridge High School)
Lori McKamey (Biochemistry) (Clinton High School)
Angela Roberts (Epidemiology) (Roane County High School)

*Thesis research being completed at the division.
**Portion of thesis research being done at the division.

1028794

Major Awards, Honors, and Appointments

D. Cragle

Member of the International Nickel Speciation Research Project Steering Committee

S. A. Fry

Member of the National Council on Radiation Protection and Measurements Scientific Committee's Task Force on Uranium (SC57-15)

P. G. Groer

Member of the Cytogenetics Working Group for NCI in response to Public Law 98-542

Consultant to the National Council on Radiation Protection and Measurement (NCRP), Committee on Radiation Exposure and Potentially Related Injury

Consultant to CIRRPC Science Subpanel on Radioepidemiological Tables

Member of the National Academy of Sciences' BEIR IV Alpha Committee on Health Risks of Internally Deposited Alpha Emitters

Consultant to the Mining, Safety, and Health Administration on setting of new WL-standard for underground mining

P. G. Groer and S. H. Huff

Awarded the Federal Laboratory Consortium Special Award for Excellence in Technology Transfer

L. G. Littlefield

Member of the National Council for Radiation Protection, Scientific Committee Number 40 on Biological Aspects of Radiation Protection

U.S. Representative in the IAEA Coordinated Research Program on Use of Chromosomal Analysis in Radiation Protection

Member of the Editorial Board of *Environmental Mutagenesis*

A. McFee

Member of the American Society for Testing and Materials Committee E-47.09 on Genetic Toxicology

Member of the Environmental Protection Agency's Gene-tox Program Committee on Mammalian Cytogenetics

F. Snyder

Executive Editor and Member of the Editorial Committee, *Archives of Biochemistry and Biophysics*

Member of the Editorial Board, *Lipids*

Member of the Editorial Board, *Journal of Biological Chemistry*

Editor, *Handbook of Lipid Research*, A continuing series, Plenum Publishing Corporation, New York

Patents Pending

Fred Snyder and Merle Blank
Alkyl phospholipid antihypertensive agent

Fred Snyder and Merle Blank
1-Alkyl-2-acetyl-*sn*-glycerol and its use as an antihypertensive agent

Gayle Littlefield and Shirley Colyer
Continuous cell-line tamarin adenocarcinoma of the colon (ORAU TAC-1)

James Crook
Copper-64 citrate as a myocardial imaging agent

1028795

W. W. Burr, Jr.

Radon regulation in uranium in mines. Department of Labor, $104,850[a] (1 June 1987 to 30 September 1988)

W. W. Burr, Jr.

Biomedical research support grant. National Institutes of Health, Division of Research Resources, $23,727[b] (1 April 1987 to 31 March 1988; current year of continuing award)

W. W. Burr, Jr.

Evaluation of devices and techniques to determine previous radiation exposure. National Cancer Institute, $261,220[a] (2 September 1985 through 1 August 1987; carried over, no additional funding)

N. K. Clapp

Operation of a marmoset colony for cancer research. National Cancer Institute, $294,000[a,c] (1 June 1987 to 31 May 1988; third year of 3-year award)

N. K. Clapp

Support of marmoset colony. National Foundation for Ileitis and Colitis, $25,000[a,d] (1 July 1987 to 30 June 1988; third year of 3-year award)

N. K. Clapp

Collaborative studies of colitis and colon cancer in tamarins. Harbor-UCLA School of Medicine, $15,000[a,d] (1 September 1987 to August 1990; third year of 5-year award)

S. A. Fry

Epidemiological studies—death data retrieval. National Cancer Institute, $80,000[a] (1 October 1987 through 30 September 1988; first year of 2-year award)

L. G. Littlefield

Studies of radiation-induced chromosome damage in humans. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $201,802[a] (30 September 1986 to 29 September 1987; current year of continuing award)

A. F. McFee

Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations. National Cancer Institute-U.S. Department of Energy Interagency Agreement, $362,770[a] (30 September 1986 to 29 September 1987; second year of 2-year award)

R. C. Ricks

Radiological training courses for health physics and medical personnel. Federal Emergency Management Agency, $178,727[a] (30 September 1986 to 30 September 1987)

R. C. Ricks

Reactor status considerations in emergency decision-making. Federal Emergency management Agency, $56,787[a] (1 June 1986 to 30 September 1987)

F. Snyder

An antihypertensive class of neutral lipids in kidney. National Heart, Lung, and Blood Institute, $85,629[b] (1 December 1986 to 30 November 1987; second year of 3-year award)

F. Snyder

A novel class of biologically active phospholipids. National Heart, Lung, and Blood Institute, $100,353[b] (1 April 1987 to 31 March 1988; second year of 5-year award)

F. Snyder

Plasmalogens and related ether lipids in cancer cells. National Cancer Institute, $98,297[b] (1 July 1987 to 30 June 1988; second year of 5-year award)

F. Snyder

Metabolism, regulation, and function of ether-linked mediators and related phospholipids in cancer cells and their antitumor analogs. American Cancer Society, $97,200[a] (1 January 1987 to 31 December 1987; second year of 2-year award)

S. D. Tardif

Determinants of reproductive competence in tamarins. National Institutes of Health, Division of Research Resources, $45,350[b] (5 September 1986 to 30 November 1987; third year of 3-year award)

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer therapy. National Cancer Institute, $94,002[b] (1 September 1987 to 31 August 1988; third year of 3-year award)

a Total costs, i.e., direct plus overhead (current year)
b Direct costs (current year)
c Not under DOE prime contract
d These collaborative awards are reimbursable to the NCI contract

1028796

Medical and Health Sciences Division Staff

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1028797

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James C. White, Ph.D. (HRP)
Jill D. Wright, Ph.D. (HRP)
*Paul L. Ziemer, Ph.D. (ADM)

---

*Personnel no longer here
[1]Educational Leave
[2]DOE Fellow
[3]Postdoctoral Research Associate
[4]Part-time Temporary
[5]Part-time Continuing
[6]Full-time Temporary

1028798

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1028799

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1028800

Oak Ridge Associated Universities is a private, not-for-profit association of 49 colleges and universities and a management and operating contractor of the U.S. Department of Energy. ORAU's mission is to foster, encourage, and engage in the identification and development of solutions to scientific, engineering, technical, medical, and human resource problems through the resources available to ORAU and its member universities. In support of this mission, ORAU provides diverse services (principally academic outreach, research, training and education, technical assistance, and technology transfer) for DOE, ORAU's member institutions, other colleges and universities, and other private and governmental organizations. Established in 1946, ORAU was one of the first university-based, science-related corporate management groups.

The **Medical and Health Sciences Division** (MHSD) conducts research on major biomedical problems, develops and offers training in conjunction with current MHSD research programs and in the management of radiation accidents, and provides technical assistance.

MHSD *research* on biomedical problems principally involves the respiratory, vascular, pancreatic, and gastrointestinal systems, with emphasis on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Research programs in the division are focused on the following areas: (1) preclinical and clinical development of new radiopharmaceutical agents for in vivo diagnosis of diseases in humans, (2) biochemical aspects of cellular lipid mediators and membrane lipids in disease processes, (3) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (4) cytogenetic investigations designed to assess possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), and (5) interdisciplinary investigations of the marmoset as a primate model for spontaneous colon cancer and other human diseases.

Training is offered in areas of current MHSD research. Predoctoral and postdoctoral training is provided in each research program area. REAC/TS, the Radiation Emergency Assistance Center/Training Site, is maintained for the U.S. Department of Energy as a treatment and decontamination facility and a demonstration and training site.

Technical assistance is provided by the MHSD to DOE in Occupational Medicine (OM) by physician-conducted reviews of OM programs and technical assistance in addressing OM problems. The MHSD Center for Human Reliability Studies provides technical assistance to DOE in the assessment of human reliability. Through MHSD staff located in Washington, DC, the Division provides technical assistance to the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC). CIRRPC is charged with coordinating radiation matters between agencies, evaluating radiation research and providing advice for formulation of radiation policy.

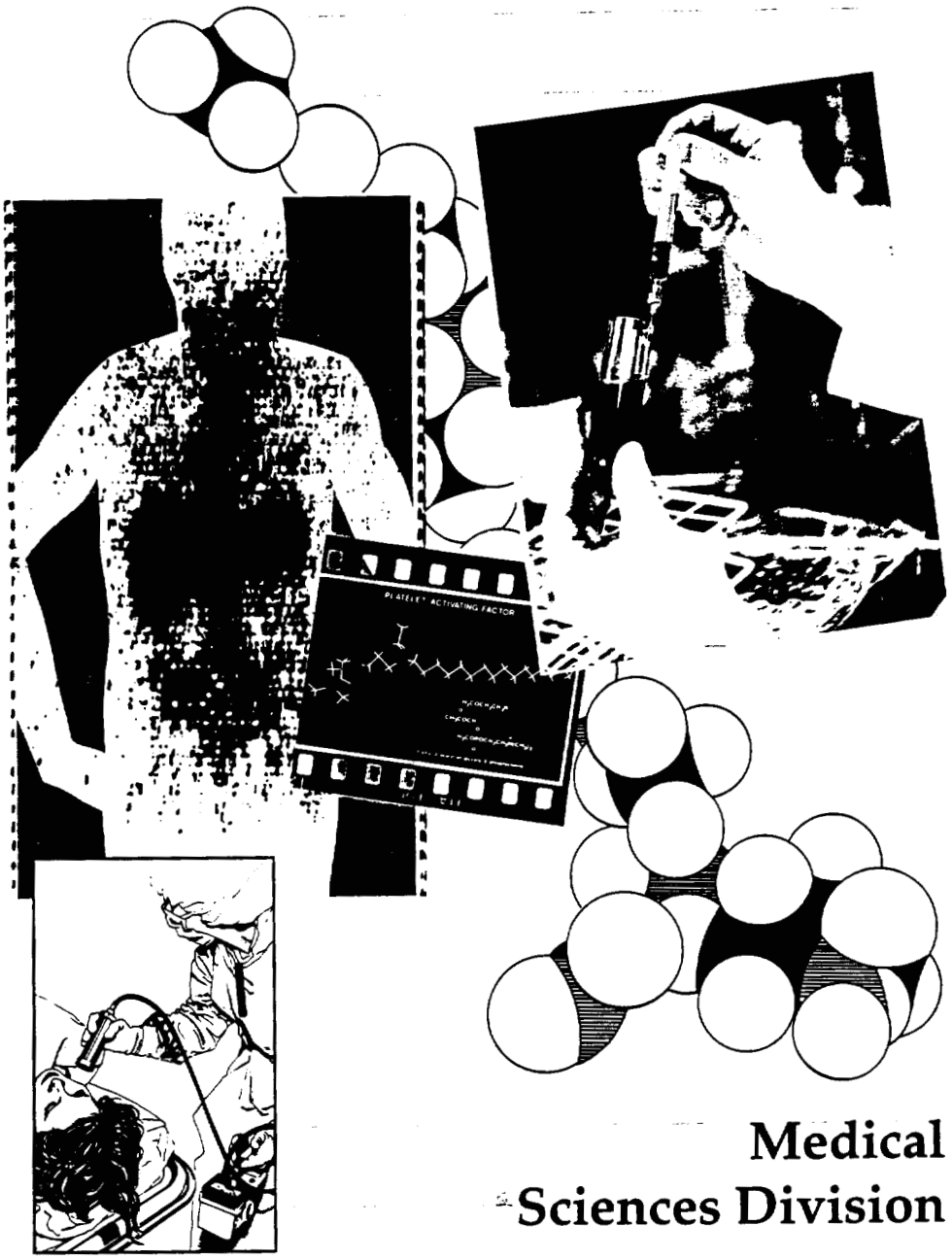
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1987/1988

1028802

Research Report 1987-1988



Medical Sciences Division

Oak Ridge Associated Universities

1028803

NOTICES

The opinions expressed herein do not necessarily reflect the opinions of the sponsoring institutions of Oak Ridge Associated Universities.

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Available to DOE and DOE contractors from the Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831; prices available from (615) 576-8401, FTS 626-8401.

Available to the public from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161.

Price: Printed Copy ORAU/89-E-148
Microfiche A01

1028804

Research Report 1987-1988

Medical Sciences Division



Oak Ridge
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ORAU/89-E-148

1028805

Preface

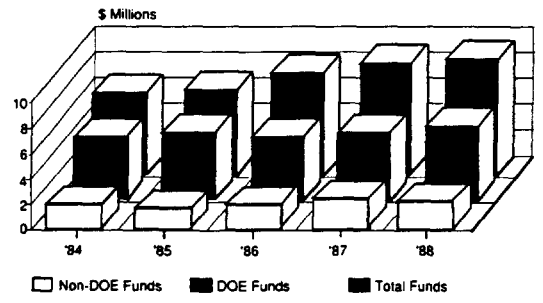
1028806

This report describes current research, service, and training activities at the Medical Sciences Division of Oak Ridge Associated Universities during Fiscal Year 1988. The extensive publications of significant research results in peer-reviewed journals and the important service and training contributions geared to meet the special needs of DOE and other federal agencies are positive indicators of a highly successful year. Continued growth of these programmatic activities is reflected in the accompanying illustration of the division's budgets over the past several years.

Many of our programs are well recognized internationally, as indicated by invitations for our staff to lecture at major international conferences and congresses throughout the world. In addition, a number of our scientists serve on national and international advisory panels and provide advice worldwide on medical problems associated with radiation accidents involving humans.

We believe this report offers a brief, yet representative view of ongoing programs. However, the citations in the publications section give many more details and we also are available to provide any additional information about our division.

Medical Sciences Division Funding
1984-1988



W. W. Burr, Jr.
Chairman

Fred Snyder
Vice Chairman

1028807

Table of Contents

1028808

Scope of Programs

Biochemistry 1
Preclinical and Clinical Nuclear Medicine 2
Cytogenetics 4
Microbiology 5
Marmoset Research 6
Radiation Emergency Assistance Center/Training Site 7
Center for Epidemiologic Research 8
Committee on Interagency Radiation Research and Policy Coordination 10
Occupational Medicine 11
Center for Personnel Security Assurance, Research and Analysis 11

Publications, Abstracts, and Invited Lectures

Full-length Journal Articles 12
Chapters, Review Articles, Government Reports, and Other Publications 14
Abstracts (Published) of Papers Presented at Meetings 15
Submitted Papers 16
Invited Lectures Presented at National and International Meetings 17

Collaborative Research 18

Training Programs 19

Major Awards, Honors, and Appointments 20

Patents Granted and Patents Pending 21

Non-DOE Research Funding 21

Medical Sciences Division Staff 22

Administration and Committees 25

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Scope of Programs

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Biochemistry Program

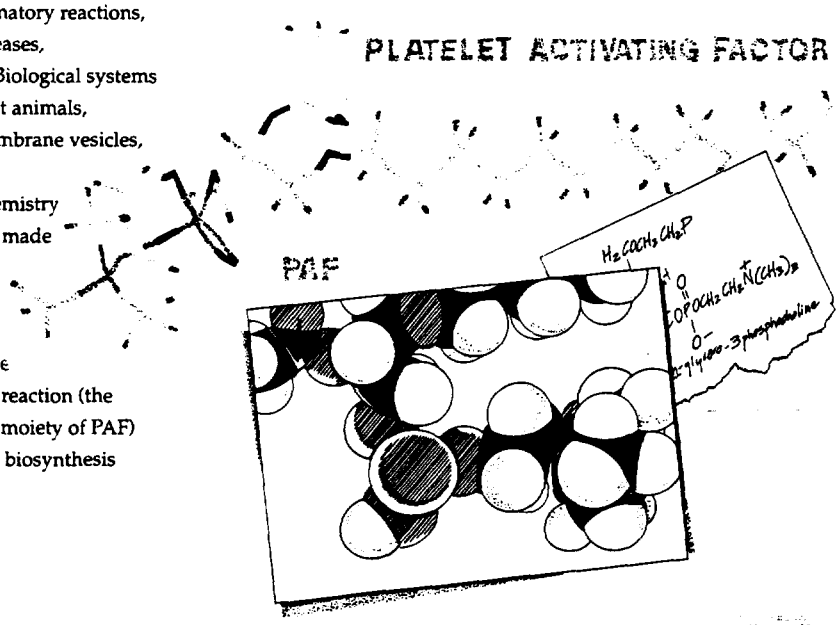
Program Director:
Fred Snyder

Lipids, essential components of all mammalian cells, display a variety of chemical structures that attest to their diverse functional roles. The ability of lipids to serve as signalling molecules, intrinsic structural components of membranes, required nutritional factors for growth, and stored sources of energy are well documented. Moreover, new concepts regarding the role of lipid cellular mediators in radiation injuries and toxicant-related inflammatory reactions are beginning to emerge. It is now clear that potent bioactive lipids such as platelet-activating factor and prostaglandins or other eicosanoid mediators are produced in excessive amounts in response to radiation and toxic substances. These lipid mediators, when present in excess, seriously impair respiratory, hematopoietic, cardiovascular, and renal functions.

The lipid biochemistry program centers on the structural and functional biology, metabolism, and regulation of lipids in mammalian cells, with an emphasis on platelet-activating factor (PAF), a potent bioactive phospholipid of great importance. A primary aim of our studies is to understand how an inert structural component of lipids can be converted to a group of cellular mediators that can cause a great variety of biological responses. Mediators such as PAF, prostaglandins, and leukotrienes are involved in controlling both physiological and pathological processes. The research efforts directly relate to relevant biomedical problems associated with cancer, inflammatory reactions, radiation-toxicant injury, pulmonary diseases, cardiovascular disorders, and nutrition. Biological systems used in these investigations include intact animals, cultured cells, subcellular organelles, membrane vesicles, and enzyme preparations.

Significant achievements in the biochemistry program during the past fiscal year were made in the following areas:

- Experiments with permeabilized platelets have provided direct evidence that identifies the cytidylyltransferase reaction (the enzyme source of the phosphocholine moiety of PAF) as the rate limiting step in the de novo biosynthesis of PAF.
- In an ongoing investigation, results obtained with phospholipase A_2, cyclooxygenase, and lipoxygenase inhibitors strongly implicate the existence of a phospholipase A_2 that is specific for polyunsaturated fatty acids at the *sn*-2 position of the stored precursor of PAF, alkylacylglycerophosphocholines. These findings explain why arachidonic acid (20:4) supplementation of cultured HL-60 cells stimulate the production of PAF by severalfold over unsupplemented control cells.
- A recently completed project has shown that the HL-60 cell line develops specific PAF receptors that are functionally active after they are chemically induced to differentiate into mature granulocytes. These results indicate the degree of cellular differentiation is extremely important in the biological response of cancer cells to PAF.
- A project on the selective antitumor activity of unnatural PAF analogs revealed that HL-60 cells become resistant to these analogs when they are differentiated into a granulocytic form. Also, inhibition of choline uptake was seen only in the undifferentiated cells treated with the methoxy analog, which indicates that choline uptake is a useful index for measuring the degree of the cytotoxic response elicited by the antitumor phospholipid agents. Since future experimentation can now be done with sensitive and



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resistant forms of the same cell line, the HL-60 system should prove an ideal model for investigating the biochemical mechanisms responsible for the antitumor response.

- Key factors were identified that influence the contribution of different types of acylation reactions in the transfer of fatty acids between membrane phospholipids. Transacylase activity mainly accounts for the transfer of polyenoic acids from diacylphospholipids to ether-linked phospholipids, whereas acyl-CoA-dependent reactions convert lyso-phospholipids to more saturated forms. Substrate concentrations, protein levels, incubation times, and pH all have pronounced effects on the extent and the type of acylation reaction catalyzed (that is, acyl-CoA-dependent versus CoA-independent) and therefore, caution is required in the interpretation of the physiological significance of the quantitative contributions of different acylation mechanisms.
- Membrane phospholipids of P388D$_1$ cells (a macrophage cell line) can be drastically modified by culturing these cells with marine oil fatty acids (n-3 type) that exhibit both anti-inflammatory properties and protection against cardiovascular disease. The modified cells display both a higher content and turnover of the plasmalogens that contain 22:6 (n-3) fatty acids than any other molecular species. Results from these experiments indicate membrane-associated ethanolamine plasmalogens are closely associated with the metabolism of the n-3 fatty acids.

Future projects in this program involve a number of new areas, but the main emphasis will continue to be on the regulatory mechanisms in the de novo and remodeling pathways of PAF biosynthesis. We expect to examine the metabolism and functional role of PAF in brain, since our earlier work has demonstrated that brain possesses high activities of all the enzymes required for synthesis of PAF by the de novo route and others have shown neurotransmitters stimulate this pathway in neuronal cells. Upcoming collaborative arrangements with a group at the University of California are expected to provide new information about differences in membrane phospholipids between healthy human brains and those afflicted with Alzheimer's disease.

Recently, cultured myocytes from neonatal rat hearts have been grown in our laboratory as a cell model for investigating the metabolism of PAF and its regulation in cardiac tissue. Moreover, the myocyte system will be used in our renewed efforts in an NCI-sponsored project to delineate the biosynthetic route(s) for choline plasmalogens, since myocytes obtained from neonatal rats contain significant quantities of this important subclass of membrane phospholipids. Also, preliminary studies have been initiated to determine the nutritional significance of glyceryl ethers in the diet and their relationship to the metabolism of polyunsaturated fatty acids (n-3 marine oils and the usual n-6 types) and cholesterol.

Whereas some of the new projects will broaden the scope of the biochemistry program, the primary focus continues to be on the metabolism, regulation, and function of membrane lipids. The ether-linkages are of special importance in these studies, since they not only serve as a group of diverse potent autocoids but also represent essential membrane constituents that are highly enriched reservoirs of arachidonate and other polyunsaturated fatty acids. These polyunsaturates can also be converted to extremely potent biologically active products.

In addition to the fundamental biochemical mechanistic studies, future work will examine some of the anti-PAF and anti-prostaglandin receptor antagonists now available in experimental clinical trials. Such drugs could have potential beneficial effects in treating radiation injuries and toxicant-induced inflammatory responses triggered by lipid mediators.

Preclinical and Clinical Nuclear Medicine

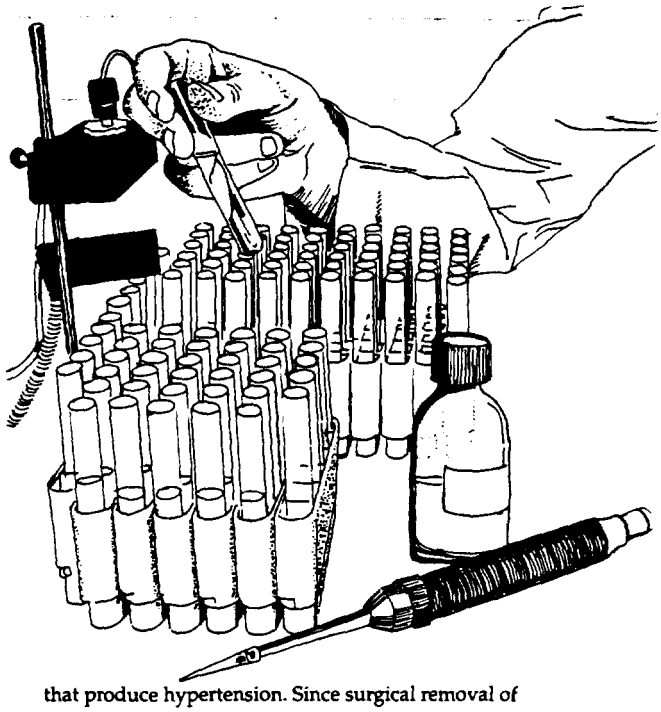
Program Director:
James E. Crook

The major objectives of the preclinical and clinical nuclear medicine programs are to develop new radiopharmaceutical agents and to evaluate them in phase I and phase II clinical studies. The work involves identifying medical needs, designing and synthesizing potential agents for radiodiagnosis and radiotherapy, testing these substances in animals for effectiveness and toxicity, and conducting clinical efficacy trials in humans according to U.S. Food and Drug Administration guidelines.

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Some recent achievements in nuclear medicine include:

- Preclinical development of radiolabeled monoclonal antibodies for diagnosis and therapy of cancer continues to be a major research interest. The National Cancer Institute grant, "Radiolabeled Monoclonal Antibodies for Cancer Therapy," was recently renewed for an additional three years of support with increased funding. This study investigates the ability of radiolabeled monoclonal antibodies to tumor-associated antigens to specifically deliver tumoricidal dosages of therapeutic radionuclides to malignant sites. Monoclonal antibodies are labeled with metal ion radionuclides via bifunctional chelate techniques in which a chelating agent is covalently coupled, under mild conditions, to the monoclonal antibody molecule. We compared six bifunctional chelate techniques and determined that using p-isothiocyanatobenzyl-DTPA and its analogs, supplied by Dr. O. A. Gansow, National Cancer Institute, results in the most stable binding in vivo with the beta-emitting radionuclide, yttrium-90.
- CO17-1A, a monoclonal antibody with specificity for colorectal and pancreatic carcinomas, was developed and supplied by Dr. Zenon Steplewski, a collaborator at the Wistar Institute in Philadelphia, Pennsylvania. ^{90}Y-labeled CO17-1A was shown to be taken up selectively by the tumors of nude mice bearing xenografts of human colorectal carcinoma cell line SW 948. In an experimental radiotherapy study in nude mice bearing SW 948 human colorectal carcinoma xenografts, a dose of 200 μCi of ^{90}Y-labeled CO17-1A per 25 grams of body weight caused up to an 80% reduction in the initial tumor volume. Clinical studies with this agent are planned.
- Based on a series of promising preliminary experiments with BR55-2, a monoclonal antibody to human colon carcinoma with activity toward the tamarin colon carcinoma, we received additional funding from DOE to develop radiolabeled monoclonal antibodies to tamarin colon cancer. We will collaborate with Dr. Zenon Steplewski, Wistar Institute, who will develop the monoclonal antibody to be radiolabeled and administered by us to tamarins with colon cancer.
- Additional experiments were conducted with the New England Deaconess Hospital rat bearing subcutaneously implanted pheochromocytomas. Pheochromocytomas are tumors of the adrenal medulla



that produce hypertension. Since surgical removal of the tumor is possible, this represents a potentially curable form of hypertension. Thus, detection and early surgical or other medical treatment is desirable. Experiments to determine the number of progesterone receptor binding sites present on tumors transplanted into male and female recipients were finalized. Although statistical significance was not achieved using the Student's t-test ($p > 0.07$), there was a trend toward a greater number of binding sites in females (106 ± 7) versus males (81 ± 9 fmol/mg cytosol protein). Since the clinical literature is at variance as to whether a gender predilection exists, this information provides additional insight.

Future plans will continue to emphasize positron emission tomography (PET), since it is a powerful tool that can produce high-resolution, three-dimensional images as well as act as a noninvasive probe of biochemical function. Specifically, applications for PET include detecting metabolic alterations associated with disease processes such as cardiovascular and neoplastic diseases. In-house availability of short-lived, positron-emitting radionuclides is anticipated in the very near future through our collaboration with ORBIT, Inc., headed by Dr. John Neiler. ORBIT, a small Oak Ridge corporation, obtained phase II support from DOE's Small Business Innovation Research

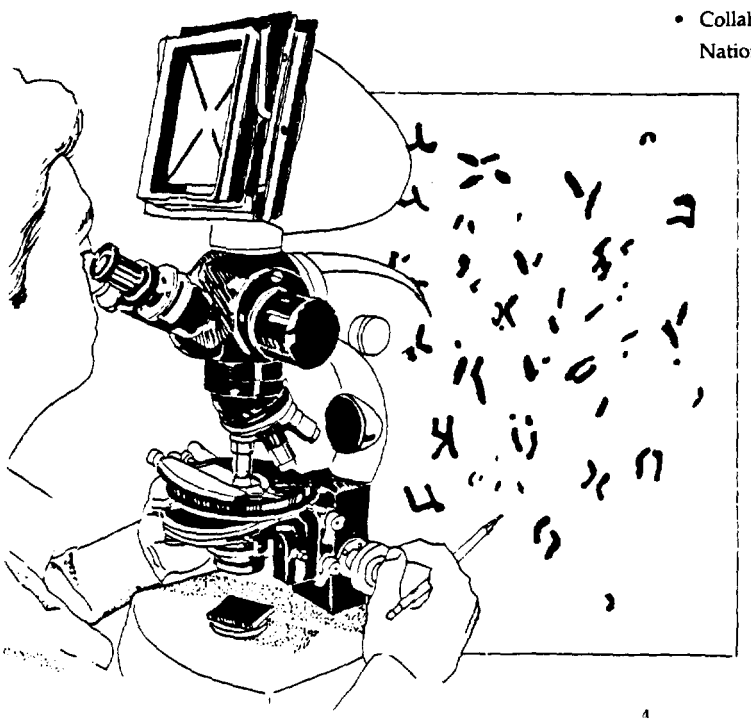
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Program to construct a small, proton-only, low-energy (8 MeV) cyclotron and to test its utility as a source of clinically important radionuclides for positron emission tomography. We envision that the small cyclotron and ECAT II™ positron tomographic scanner will be used for preclinical development and clinical feasibility studies of new positron-emitting radiopharmaceuticals. We are now exploring the possibilities for collaborative work with interested investigators from ORAU member institutions.

Cytogenetics

Program Director:
L. Gayle Littlefield

The cytogenetics program conducts basic research to further define the mechanisms involved in the induction, repair, and expression of chromosome damage caused by ionizing radiation and various types of DNA-damaging chemicals. In parallel studies, we develop and apply cytogenetic methods as biological indicators of dose in persons having real or suspected radiation overexposures. In addition, we conduct systematic cytogenetic evaluations in highly selected cohorts of patients having whole-body or partial-body radiation exposures many years ago.



Objectives of the follow-up studies are to determine whether the survival of lymphocytes carrying radiation-induced chromosome aberrations varies as a function of radiation dose, region of the body exposed, or number of years since exposure. Studies in animal models answer specific questions regarding the cytogenetic and cellular toxicity resulting from exposure to ionizing radiation and chemicals. Such data provide a basis for predicting and interpreting the cytogenetic consequences of accidental human exposures to genotoxic agents and for determining the usefulness of cytogenetic techniques for quantifying levels of recent or previous human exposures to mutagenic or carcinogenic agents.

Significant achievements in the cytogenetics program during the past year are highlighted here:

- Studies were continued using dimethyl sulfoxide (DMSO) as a model radioprotective chemical to quantify the role of OH radicals in producing DNA lesions involved in chromosome aberration induction by radiation of differing energies. These studies demonstrated that radioprotection by DMSO against the induction of chromosome aberrations varies as a function of neutron energy, and that while OH radical attack on DNA accounts for 50% of the dose dependency for dicentrics induced by low level x-radiation, such radiochemical damage contributes only 40% and 15% respectively of the dose dependency for chromosome damage induced by monoenergetic 6.0 and .44 MeV neutrons.
- Collaborative studies with scientists at Los Alamos National Laboratory demonstrated that a chromosome-specific DNA probe recently developed at that laboratory allows rapid identification of the centromere regions of chromosomes and accurate scoring of chromosome damage induced in human lymphocytes by radiation as well as by radiomimetic chemicals.
- Staff succeeded in establishing and propagating a continuous cell-line derived from a biopsy of cancer tissue from a tamarin having a spontaneous adenocarcinoma of the colon. The cell-line, which has been named TAC-1, will provide unique opportunities for studies of the molecular genetics of a spontaneously occurring primate adenocarcinoma and for numerous other basic studies in tumor cell biology.

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We will continue to employ in vitro irradiations of human lymphocytes as a model system for investigating radiochemical mechanisms involved in the induction of DNA lesions that lead to chromosome aberrations. We plan to extend our studies of the modulation of response by DMSO to include evaluations in human lymphocytes exposed to monoenergetic neutrons ranging in energy from 0.34 to 14 MeV. Our ultimate aim is to determine whether a descriptive model can be developed to predict the rate of DNA lesion induction in human cells by prompt ionizations versus radiochemical processes as a function of neutron energy and track average LET. Cytogenetic evaluations in a cohort of cancer patients receiving 14 MeV neutron therapy for various malignancies will provide a basis for determining the relative effectiveness of high energy neutrons in inducing chromosome damage in a human cell system in vivo.

Microbiology

Program Director:
Howard I. Adler

This newly formed group focuses on mechanisms used by bacteria to protect themselves against oxygen and related reactive species. These species include the superoxide anion (O_2^-), the OH radical, and hydrogen peroxide. All of these are formed during aerobic metabolism and during exposure of organisms to ionizing radiation. Evidence exists linking these reactive species to the induction of mutation and, in higher life forms, cancer.

The group will attempt to improve understanding of the ways in which cells prevent or repair damage resulting from oxygen and products derived from oxygen. Several strictly anaerobic bacterial species, as well as organisms that can grow aerobically or anaerobically, are being used in our research. A recently developed technique for producing anaerobic conditions in bacteriological media provides a novel methodology for this work. The technique uses sterile, oxygen-reducing fragments isolated from the cytoplasmic membranes of bacteria.

We are concentrating on a collection of recently isolated mutants of the bacterium *Escherichia coli*. These mutants have all lost the ability to produce macro colonies when incubated aerobically on the surface of agar. Previous work has established that the mutants can be assigned to three phenotypic classes that represent mutation at not less than six genetic loci.

Experiments performed since the creation of the microbiology group have concentrated on two of these mutants, Oxy^s-6 and Oxy^s-2. These organisms have undergone an alteration at 8.4 minutes on the *E. coli* chromosome map. They are deficient in catalase, peroxidase, and the inducible superoxide dismatase. Liquid cultures of these mutants, if exposed to air, undergo approximately four to five divisions and then achieve a static condition in which cells neither divide nor die. This stasis can be maintained for at least 36 hours at 37° C. At the end of this period, rapid cell division resumes if the cells are returned to an oxygen-free environment.

Significant research accomplishments of the microbiology group during the year include:

- We have determined that oxygen-sensitive mutants of the bacterium *E. coli* can undergo limited growth (approximately 4–5 divisions) under aerobic conditions if very small inocula are put into complex, organic medium. This phenomenon is not observed if a large inoculum is used or if a synthetic medium is substituted for the complex one.
- During limited aerobic growth in complex medium, oxygen-sensitive mutants of *E. coli* produce an extracellular substance that is toxic for the organisms producing it. This substance is present in filter sterilized medium harvested after the limited growth period. It is stable in air and does not degrade if stored at 4°C.
- The extracellular substance produced by these mutants is specifically toxic for these mutants when they attempt to grow under aerobic conditions. It does not inactivate them under anaerobic growth conditions. It does not inactivate the wild-type parental culture under either aerobic or anaerobic growth conditions. The substance, as yet unidentified, is at least partially destroyed by heat.
- One of the oxygen-sensitive mutants, Oxy^s-6, produces a variant clone after long exposures to air. This variant has a longer generation time than Oxy^s-6, is somewhat more oxygen-tolerant than Oxy^s-6, and forms very small colonies when incubated for several days on nutrient agar under aerobic conditions. No reversions to the oxygen resistance level of the wild-type parental culture have been observed. The available evidence suggests that the variant represents a mutation at a locus other than 8.4 minutes on the genetic map of *E. coli*.

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Future plans include continuing the detailed characterization of the collection of *E. coli* mutants, because this should contribute to our understanding of defense and repair mechanisms for oxygen insults. In addition, we will use our newly developed techniques for isolating naturally occurring anaerobic bacteria from unusual environments. These freshly isolated organisms should also prove valuable in expanding our appreciation of how oxygen produces damage and how cells cope with this damage. In addition, we anticipate that some of the newly isolated organisms will be useful in the degradation of environmental toxic wastes. In collaboration with colleagues at the University of Tennessee and Knoxville College, we will determine if the new isolates can degrade chlorinated organic compounds. We also intend to begin experiments to determine why the solvent-producing anaerobe *Clostridium acetobutylicum* rapidly degenerates to a non-solvent producing organism. This work will be done in collaboration with investigators at Rice University.

Marmoset Research

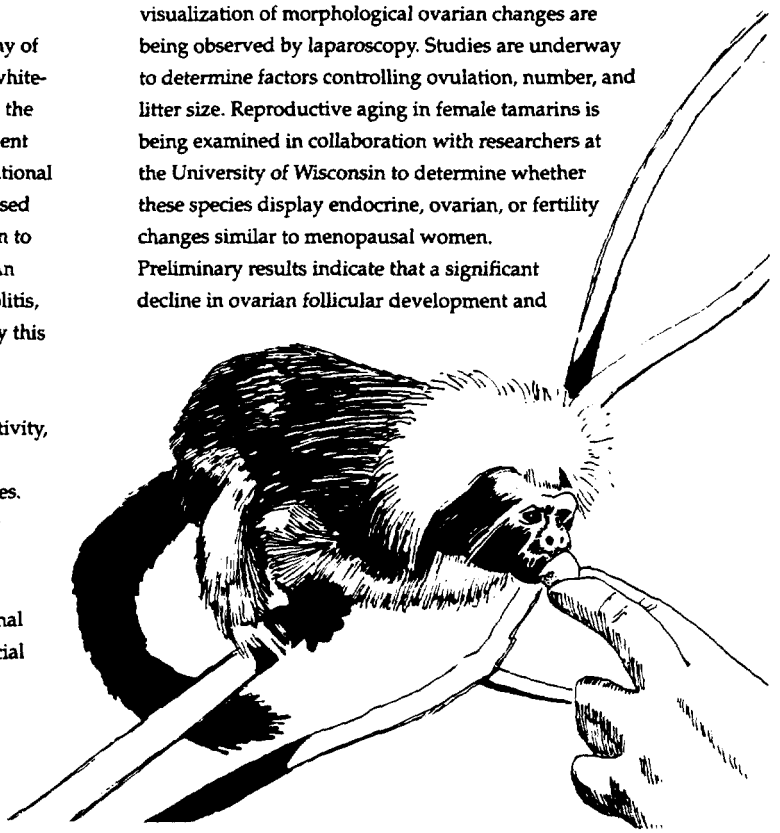
Program Director:
Neal K. Clapp

ORAU's marmoset research center houses a colony of the endangered cotton-top tamarin, along with the white-lipped tamarin and the common marmoset. Broadly, the aims of the marmoset program are to develop sufficient scientific interest to fully utilize this valuable international resource. Among ORAU scientists, research has focused on the unique susceptibility of the cotton-top tamarin to high incidences of spontaneous colonic carcinoma. An extremely interesting inflammatory disease, acute colitis, occurs in all three species and offers a model to study this devastating human disease under controlled environmental conditions.

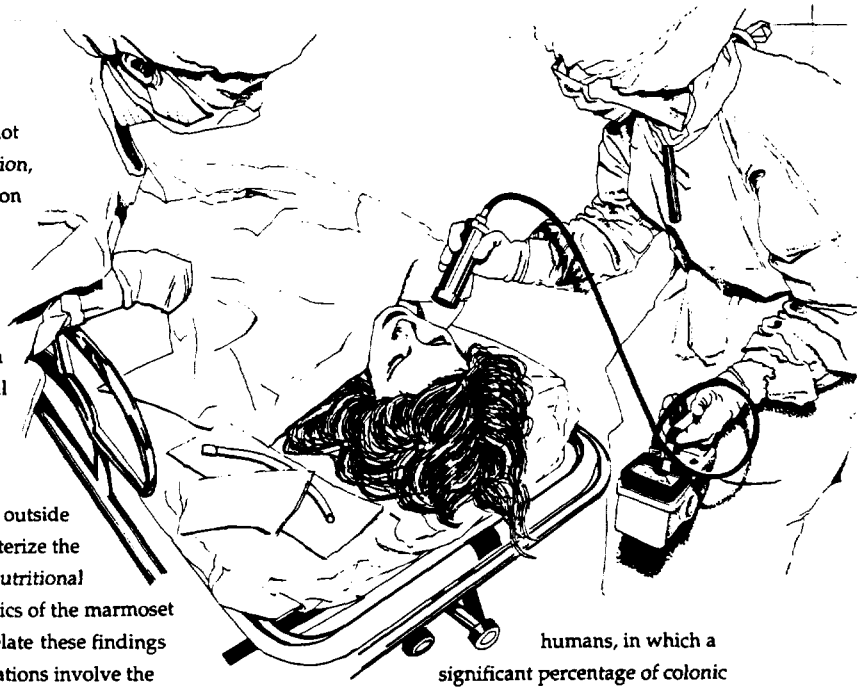
Because of the need to breed these animals in captivity, much of our work has involved studies of the reproductive behavior and physiology of these species. Outside investigators have collaborated both in pilot studies and in submitting funding requests to the National Institutes of Health (NIH) to adequately underwrite and staff the overall program. The National Foundation for Ileitis and Colitis continued its financial support of the colony during the past year.

Work this year includes:

- The continued effort to study the pathogenesis (both time course and disease process) of colitis has shown that colitis can be identified in animals before clinical symptoms are detectable. This longitudinal study has identified an early stage of the disease at which therapy may be more effective. We are now exploring with pharmaceutical firms and gastroenterologists new compounds whose efficacy can be tested in this model system. Also being explored are new technologies that may be used to progressively evaluate visualized changes and correlate them with histopathological changes.
- Research concerned with the behavioral and endocrine determinants of reproductive competence in the cotton-top tamarin was conducted through support from NIH. The initial objective was to define the importance of the social aspects of rearing infant siblings on the subsequent maternal behavior of females. Combined studies of behavior and the relation of demographic parameters to reproductive performance have enhanced our ability to select competent breeders. Demographic analyses have also been used to identify other areas for future research into tamarin reproductive function. In collaboration with investigators at the University of Tennessee, visualization of morphological ovarian changes are being observed by laparoscopy. Studies are underway to determine factors controlling ovulation, number, and litter size. Reproductive aging in female tamarins is being examined in collaboration with researchers at the University of Wisconsin to determine whether these species display endocrine, ovarian, or fertility changes similar to menopausal women. Preliminary results indicate that a significant decline in ovarian follicular development and



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reduced fertility occurs with age in tamarin females, but an absolute cessation of follicle production has not been seen in aging animals. In addition, the aged tamarin has greater variation in ovarian hormone levels and a greater chance of being acyclic.

- A second edition of an international studbook for the endangered cotton-top tamarin was published in August 1988. The studbook is crucial to the formation of sound conservation and research planning and use of this endangered species.
- Collaborative research projects with outside investigators are designed to characterize the biology, biochemistry, physiology, nutritional aspects, and behavioral characteristics of the marmoset as an experimental animal and to relate these findings to disease processes. These investigations involve the following studies: genetic diversity of species, genetic epidemiology and familial relationships in tamarin colon cancer, and idiopathic ulcerative colitis in colon cancer susceptibility and expression. Other projects are investigating the effect in colonic marmoset diseases of fecal mutagens and fecal microflora, coronaviruses, lectin binding, fecal toxins, fecal bile acids, and serum enzymes. The results of these studies are being reported in part in a book to be published by CRC Press titled *The Cotton-Top Tamarin: A Primate Model for the Study of Colon Carcinogenesis.*
- Extensive collaborations are underway with researchers in the Laboratory of Comparative Carcinogenesis at Frederick Cancer Research Facility, National Cancer Institute. Concerted efforts have attempted to identify changes in the fecal contents (both potential fecal mutagens and microbial flora) with mutagenicity and carcinogenesis. Identification of specific mutagenic agents has been largely unsuccessful to date.
- A collaborative research relationship has continued with the Harbor-UCLA School of Medicine through an NIH-funded center for the study of inflammatory bowel disease. By comparing the onset of colon carcinoma in colony-born cotton-top tamarins that have colon cancer in their family history and animals that have no cancer in their history, we have found that this unique species susceptibility appears to be pan-specific rather than familial. This contrasts with observations in humans, in which a significant percentage of colonic neoplasms are familial. We are trying to determine if leukotrienes are present in spontaneous marmoset colitis as they are in some other induced models in other species. This could suggest further similarities between the disease process in different species.

Major changes were made in the operation of the marmoset program this year. Plans were developed to establish a consortium under the administration of ORAU and the University of Tennessee to manage, market, and use the marmoset colony. This consortium arrangement will consist of interested universities plus several private companies that have expressed the need for using these unique animals in collaborative research activities.

Radiation Emergency Assistance Center/Training Site

Program Director:
Robert C. Ricks

The Radiation Emergency Assistance Center/Training Site (REAC/TS) continued to provide support for medical management of radiation accidents which includes on-site emergency medical assistance and consultation services.

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REAC/TS also continued to work closely with the International Atomic Energy Agency (IAEA) and with the World Health Organization (WHO) in the role of the Western Hemisphere Collaboration Center for radiation accident management.

Specific accomplishments during the past year are listed below:

- REAC/TS continued to assist the Brazilian government and the IAEA in following up the 1987 cesium-137 accident in Goiania. Staff members consulted with Brazilian physicians regarding the continuing medical needs of patients with local radiation injury. In May 1988, REAC/TS and ORAU radiation medicine staff assisted in drafting the official IAEA report of the accident. In July 1988, staff attended an international conference on the accident during which the final draft of the official IAEA report was prepared.
- At the request of the National Research Council, Office of International Affairs, REAC/TS staff met with a representative of the Soviet All Union Scientific Centre of Radiation Medicine. The purpose of this meeting was to establish initial contact regarding Soviet plans for a follow-up of the Chernobyl reactor accident, and ORAU's capabilities for epidemiological follow-up of exposed populations and for radiation accident management and medical related training.
- REAC/TS continued to coordinate the clinical use of DTPA for the evaluation of individuals known or suspected to be internally contaminated with plutonium or americium. Whole-body counting and radioassay evaluations are used to determine the efficacy of therapy and to determine a need for continued use of DTPA in contaminated persons. Data on the use of DTPA are incorporated as part of the REAC/TS registry.
- During the past year, REAC/TS continued to offer training courses in medical management of radiation accidents to medical, paramedical, and health physics personnel. This year, 79 physicians, 66 nurses, 35 health physicists, and 79 others attended courses at the REAC/TS facility. Since June 1976, a total of 108 training courses have been offered at REAC/TS, with 2,207 health professionals trained. The primary support for these radiation accident management training courses continues to be provided by the Federal Emergency Management Agency (FEMA).
- This year, REAC/TS expanded its efforts to assist DOE by conducting radiation accident management training courses at defense program or defense program contractor hospitals. Participants in these specialized training courses included physicians, nurses, paramedical support personnel, EMT/paramedics, and other first responders. Simulated radiation accident drills were included in each training session and participants were evaluated for their effectiveness as responders to radiation accidents.
- More than 200 physicians and scientists from around the world who are involved in responding to radiation accidents or managing and treating accidentally irradiated patients attended the REAC/TS sponsored Second International Conference on the Medical Basis for Radiation Accident Preparedness. Medical personnel from 26 states and 18 foreign countries were in attendance. Physicians from the Soviet Union and Brazil shared information on their experiences from the Chernobyl and Goiania accidents. Other topics covered in the three-day conference included radiation accident follow-up studies, new approaches to better diagnosis and therapy, and preparedness planning for radiation emergencies.

In addition to training and consultation activities, future directions for REAC/TS include continuing our work with the government of Brazil in following up the Goiania accident. In addition, we will continue to expand our training efforts at defense program and defense program contractor hospitals. Also, in light of information gained from Soviet colleagues during the international conference, we plan to revise the REAC/TS registry of radiation accidents. Revision of the registry is necessary since actual numbers of individuals with significant radiation exposures are lower than once thought.

Center for Epidemiologic Research

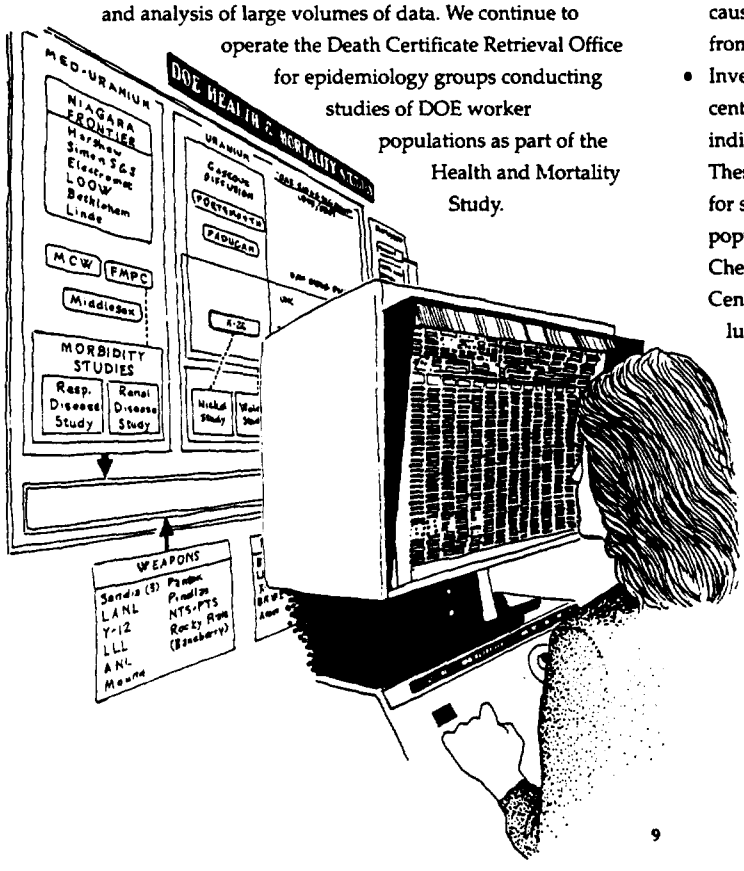
Program Director:
Shirley A. Fry

The epidemiology program carries out interdisciplinary research to examine the relationships between occupational exposure to low levels of ionizing radiation and the subsequent health and mortality of nuclear industry workers. The health effects of chemical toxicants, particularly uranium and other toxic metals, also are being

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studied as primary exposures and/or potentially confounding or modifying factors of any radiation effect. Our research is based on ongoing epidemiologic studies of 11 populations identified among a total of approximately 200,000 employees of DOE and DOE contractors and their predecessors. The goal is to identify any untoward effects of their employment at some time since 1942 at designated facilities nationwide. These studies are part of the comprehensive study of health and mortality among the total DOE work force, estimated to be 600,000, ever employed at the more than 70 active and closed sites nationwide.

Research and technical support activities involved: developing, testing, and applying new and improved statistical methods, including probabilistic modeling, Poisson regression, and Bayesian mathematics, for analyzing epidemiological data; critically evaluating methods and results of studies by other investigators among populations exposed to ionizing radiation and other industrial toxicants; advancing methodologies in data collection, records management, assessment of occupational hazards, and development of measures of exposure for use in epidemiologic analyses; and developing, testing and applying improved computer software systems to ensure the high quality and validity of the data bases and to facilitate processing, manipulation, and analysis of large volumes of data. We continue to operate the Death Certificate Retrieval Office for epidemiology groups conducting studies of DOE worker populations as part of the Health and Mortality Study.



Major accomplishments last year include:

- We completed studies of mortality among workers at DOE's Oak Ridge Y-12 and Savannah River plants. Among both study populations, overall mortality due to all causes and all cancers was less than or similar to that expected among the general population. No association was found between occupational exposure to radiation from external and internal sources and the greater than expected number of deaths due to brain cancer among white males who were employed at the Y-12 Plant between 1947 and 1974 and followed through 1979. This corroborates the findings of a parallel case control study of brain cancer among workers at DOE's Oak Ridge plants. Lung cancer deaths were also elevated in the Y-12 population and are included in an ongoing case control study of lung cancer among uranium processing workers. An as yet unexplained increase in leukemia mortality was identified among a subgroup of hourly white male employees hired at the Savannah River Plant before 1955 and who worked at the plant for 5 to 15 years. Further studies of Savannah River Plant workers are planned to evaluate relationships between their occupational exposures to radiation from external and internal sources and other toxic agents and overall and cause specific mortality, including their risk of dying from leukemia.
- Investigators have used methods developed at our center to derive uranium exposure measures for individual workers from a variety of monitoring data. These methods provide individual exposure estimates for studies of lung cancer among four worker populations at the Oak Ridge Y-12, the Mallinckrodt Chemical Works, and the Feed Material Production Center uranium processing plants. With almost 800 lung cancer deaths, and an equal number of matched control subjects from the same sites, this study will be one of the largest to date assessing the pulmonary hazard of protracted internal exposure to alpha radiation and uranium dust.
  - An innovative reanalysis of the Colorado uranium miner data indicates that the odds ratio of lung cancer versus other causes of death increases only for exposure rates over 15 working level months per year and for five or more years underground. Using the Bayesian

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methodology, this analysis included for the first time data on the miners' exposures to radon daughters in other hard rock mines prior to working in the uranium mines.

- We began a study for Martin Marietta Energy Systems, Inc. of mortality and morbidity among former gas centrifuge process workers at the Oak Ridge Gaseous Diffusion Plant. Gas centrifuge workers were at risk of exposure to certain chemicals suspected of being carcinogenic in animals. Site or tissue specific cancers of the gastrointestinal, genitourinary, and lymphopoietic systems; liver disease; skin tumors; and infertility are end points of interest in this telephone interview study. The data necessary for identifying the study population were drawn from the Health and Mortality Study computerized data files that we have developed and are maintaining on the newly installed, program-dedicated IBM 9370 mainframe. These resources enable us to respond rapidly to concerns about the health of DOE worker populations.
- We are collaborating with other DOE contractor epidemiologists on the combined analyses of data pooled from several DOE plant specific populations that have already been studied individually. Also, under the auspices of the World Health Organization's Institute for Research on cancer, we are collaborating with British and Canadian investigators to analyze combined mortality data obtained for nuclear industry workers in the U.S., the United Kingdom, and Canada. An objective of these efforts is to construct study populations of sufficient size and collective radiation dose to obtain direct estimates of the cancer risks associated with protracted exposure to low doses of radiation.
- The results of our recently completed multivariate analysis of mortality among workers employed at DOE's Oak Ridge plants during World War II indicate that plant, birth year, and socioeconomic status are stronger determinants of this population's overall mortality experience than the other risk factors considered, including radiation exposure. Initial results of the ORAU, Los Alamos National Laboratory, and Hanford Environmental Health Foundation/Pacific Northwest Laboratories collaborative DOE epidemiology group's combined analysis of mortality among the ORNL, Rocky Flats, and Hanford worker populations show a marked healthy worker effect overall. To date there is no evidence among these populations of an association between an increased risk of site or type specific cancer and level of external radiation exposure, except for multiple myeloma in the Hanford population. The collaborative group is also using this data set to improve and develop strategies for combined population analyses of nuclear worker populations.

Future directions for this program include continued surveillance of plant specific worker populations by periodically updating analyses of their mortality experience. We will complete ORAU-based multiple population studies, that is, the studies of all DOE Oak Ridge employees, workers who received ≥50 mSv/year while employed at DOE facilities, and studies of lung cancer among uranium processing workers. There will be an increasing emphasis on collaborative studies among DOE facilities of multiple populations of nuclear workers exposed to radiation externally. In addition, we will emphasize international study of combined nuclear worker populations. Finally, we will explore opportunities to expand the scope of the program to include studies of workers in other industries and to address epidemiologic questions in collaboration with clinical and experimental investigators.

Committee on Interagency Radiation Research and Policy Coordination

Program Director:
Anthony H. Ewing

The White House Office of Science and Technology Policy created the Committee on Interagency Radiation Research and Policy Coordination (CIRRPC) in 1984, chartering it under the Federal Coordinating Council for Science, Engineering, and Technology. CIRRPC coordinates radiation matters among 18 federal agencies, evaluates radiation research, and provides advice on the formulation of radiation policy. Seventeen of the agencies have designated policy representatives to CIRRPC, and fourteen of these agencies have designated senior scientists as members of CIRRPC's Science Panel. ORAU provides technical assistance for CIRRPC.

During its fourth year, CIRRPC completed the following activities:

1028820

- Set forth guidelines for use of the National Institutes of Health (NIH) radioepidemiological tables in adjudicating radiation injury claims by veterans.
- Outlined the extent to which the recommendations of the Science Subpanel on Radon Protection and Health Effects are being addressed by federal agencies concerned.
- Confirmed and augmented the agricultural implications of a major nuclear war as outlined in SCOPE 28, Volume II.
- Sponsored a comprehensive account by the National Council on Radiation Protection and Measurements of the radiation exposure of the U.S. population from all sources.
- Compiled a compendium of major U.S. radiation protection standards and guides including both legal and technical facts.
- Provided an overview of the FY85 federally sponsored radiation research addressing the biological effects of low levels of exposures.
- Responded to an Environmental Protection Agency (EPA) request for a definition of issues on EPA's proposed transuranic guidance. Because of the inability of the Science Panel to agree on the report by the Science Subpanel on Effects of Nonionizing Radiation, its draft report, along with comments, was forwarded to EPA.

Work in progress is expected to appear in the following reports next year:

- A report on the proposed increase in the neutron quality factor.
- The CIRRPC-sponsored BEIR V report by the National Research Council of the National Academy of Sciences on the biological effects of low levels of ionizing radiation.
- A document on the development of a research program that will provide a basis for more precisely determining the biological effects of neutron radiation.
- A report addressing the question of advance planning for health effects research following a major nuclear accident.
- A document on the feasibility of coordinating a federal position on ionizing radiation risk assessment.

Occupational Medicine

Program Director:
T. A. Lincoln

The occupational medicine program provides technical and administrative support to the DOE Office of the Deputy Assistant Secretary for Safety, Health, and Quality Assurance; the Human Health and Assessment Division of Health and Environmental Research; the Division of Quality Verification; and the DOE Oak Ridge Operations Office.

Services vary from year to year depending on both immediate and long-term needs of the DOE offices and divisions. Assistance this year included:

- The completion of detailed reviews of occupational medical programs at facilities of several DOE contractors.
- Submission of recommendations to DOE regarding procedures to use in notifying former DOE or contractor employees of possible earlier exposures to beryllium.
- Development of a quality assurance program to guarantee adequate health protection of human subjects involved in DOE biomedical research.

ORAU's occupational medical personnel also participate in the REAC/TS program training and service activities.

Center for Personnel Security Assurance, Research and Analysis

Center Director:
W. W. Burr, Jr.

Program Director:
G. R. Eisele

The Center for Personnel Security Assurance, Research and Analysis, formerly the Center for Human Reliability Studies, is the Department of Energy's Center of Excellence for the Personnel Security Assurance Program. The program uses established criteria and methodology to

1028821

assure that personnel in certain sensitive positions meet and maintain the highest standards of reliability. A structured system of review and evaluation allows for a continuous determination of an individual's reliability and judgment to prevent unacceptable damage to the national security.

The center has produced a number of documents in support of the Personnel Security Assurance Program:

- Legal Issues in the Human Reliability Program.
- Duty of Physician in Employer's Medical Department to Examinees.
- The Human Reliability Program of the Department of Energy—Program Orientation.
- An Overview of the Personnel Security Assurance Program.
- Department of Energy Personnel Security Assurance Program.
- A Guide to Developing an Implementation Plan for the Personnel Security Assurance Program.

Future work will continue on the development of education and training materials, including specific training courses for employees, supervisors, and managers on how to implement and conduct the program. Plans for workshops, seminars and site specific training are major tasks for the coming year. Research and analysis needed to improve or enhance personnel security will continue to be a significant activity of the center.

Publications, Abstracts, and Invited Lectures

Full-length Journal Articles

Aoyagi, M., Agranoff, B. W., Washburn, L. C., and Smith, Q. R.
Blood-brain barrier transport of 1-aminocyclohexanecarboxylic acid, a nonmetabolizable amino acid for in vivo studies of brain transport
J. Neurochem. (in press)

Bender, M. A., Awa, A. A., Brooks, A. L., Evans, H. J., Groer, P. G., Littlefield, L. G., Pereira, C., Preston, R. J., and Wachholz, B. W.
Current status of cytogenetic procedures to detect and quantify previous exposures to radiation
Mutation Research 196, 103–159, 1988

Blank, M. L., Lee, Y. J., Cress, E. A., and Snyder, F.
Stimulation of the de novo pathway for the biosynthesis of platelet activating factor (PAF) via cytidylyltransferase activation in cells with minimal endogenous PAF production
J. Biol. Chem. 263, 5656–5661, 1988

Blank, M. L., Lee, J., Smith, Z., and Snyder, F.
Effects of eicosapentaenoic and docosahexaenoic acid supplements on phospholipid composition and plasmalogen biosynthesis in P388D_1 cells
Arch. Biochem. Biophys. (in press)

Carpenter, A. V., Flanders, W. D., Frome, E. L., Tankersley, W. G., and Fry, S. A.
Chemical exposures and central nervous system cancers: A case-control study among workers at two nuclear facilities
Am. J. Ind. Med. 13, 351–362, 1988

Checkoway, H., Pearce, N. E., Crawford-Brown, D. J., and Cragle, D. L.
Radiation doses and cause-specific mortality among workers at a nuclear materials fabrication plant
Am. J. Epidemiol. 127, 255–266, 1988

Clapp, N. K., Henke, M. A., Lushbaugh, C. C., Humason, G. L., and Gangaware, B. L.
Effect of various biological factors on spontaneous marmoset and tamarin colitis: A retrospective histopathologic study
Dig. Dis. Sci. 33, 1013–1019, 1988

Clapp, N. K., Henke, M. A., McArthur, A. H., Tardif, S. D., and Carson, R.
Associations between development of acute colitis and colon cancer in cotton-top tamarins
Gastroenterology (in press)

1028822

Cragle, D. L., McLain, R. W., Qualters, J. R., Hickey, J. L. S., Wilkinson, G. S., Tankersley, W. G., and Lushbaugh, C. C.
Mortality among workers at the Savannah River Plant
Am. J. Ind. Med. 14, 279–401, 1988

Eisele, G. R., Bernard, S. R., and Nestor, C. W., Jr.
Gastrointestinal absorption of americium-241 by orally exposed swine: comparison of experimental results with predictions of metabolic models
Radiat. Res. 112, 62–73, 1987

Gavras, I., Mulinari, R., Gavras, H., Higgins, J., Reeves, R., Zawada, E., Crook, J., Halperin, A., and Garrett, B.
Antihypertensive effectiveness of the nifedipine gastrointestinal therapeutic system
Am. J. Med. 83, 20–23, 1987

Gilbert, R. O., Engel, D. W., Smith, D. D., Shinn, J. H., Anspaugh, L. R., and Eisele, G. R.
Transfer of aged Pu to cattle grazing on a contaminated environment
Health Phys. 54, 323–335, 1988

Jamison, C. S., and Adler, H. I.
Mutations in *Escherichia coli* that affect sensitivity to oxygen
J. Bacteriol. 169, 5087–5094, 1987

Kawasaki, T., and Snyder, F.
Synthesis of a novel acetylated neutral lipid related to platelet activating factor by acyl-CoA:1-*O*-alkyl-2-acetyl-*sn*-glycerol acyltransferase in HL-60 cells
J. Biol. Chem. 263, 2593–2596, 1988

Lambremont, E. N., Malone, B., and Snyder, F.
Platelet activating factor acetylhydrolase in insects: Identification and partial characterization of a 1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine acetylhydrolase in a cell-free system of *Heliothis*
Arch. Insect Biochem. Physiol. 7, 37–45, 1988

Lawless, D., Lee, Y-C. C., Crook, J. E., and Clapp, N.
An ELISA assay for colon cancer
J. Clin. Lab. Anal. 2, 35–38, 1988

Lawless, D., Lee, Y-C. C., Crook, J. E., and Clapp, N.
Colon cancer cells in peripheral blood of cancerous tamarins
Clin. Immunol. Immunopathol. 48, 338–342, 1988

Lee, T-c., Malone, B., and Snyder, F.
Formation of 1-alkyl-2-acetyl-*sn*-glycerols via de novo biosynthetic pathway for platelet activating factor: Characterization of 1-alkyl-2-acetyl-*sn*-glycero-3-phosphate phosphohydrolase in rat spleens
J. Biol. Chem. 263, 1755–1760, 1988

Lee, Y-C. C., Lawless, D., Crook, J. E., and Clapp, N.
Analysis of T lymphocyte subsets in tamarins with colitis and colon cancer
Am. J. Med. Sci. (in press)

Littlefield, L. G., Joiner, E. E., Colyer, S. P., Sayer, A. M., and Frome, E. L.
Modulation of radiation-induced chromosome aberrations by DMSO–An OH radical scavenger. I. Dose response studies in human lymphocytes exposed to 220kV x-rays
Int. J. Radiat. Biol. 53, 875–890, 1988

Lloyd, D. C., Edwards, A. A., Prosser, J. S., Barjaktarovic, N., Brown, J. K., Horvat, D., Ismail, S. R., Koteles, G. J., Almassy, Z., Krepinsky, A., Kucerova, M., Littlefield, L. G., Mukherjee, U., Natarajan, A. T., and Sasaki, M. S.
A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation
Mutat. Res. 179, 197–208, 1987

Lushbaugh, C. C., Fry, S. A., and Ricks, R. C.
Medical and radiobiological basis of radiation accident management
Br. J. Radiol. 60, 1159–1163, 1987

Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. F., and Shelby, M.
Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells
Mutat. Res. 189, 157–165, 1987

Preston, R. J., San Sebastian, J. R., and McFee, A. F.
The in vitro human lymphocyte assay for assessing the clastogenicity of chemical agents
Mutat. Res. 189, 175–183, 1987

Qian, C., Lee, T-c., and Snyder, F.
Metabolism of platelet activating factor (PAF) and related ether lipids by neonatal rat myocytes
J. Lipid Mediators (in press) 1988

Tardif, S. D., and Carson, R. L.
Relation of intra-group aggression to reproductive strategies in captive cotton-top tamarins
Intl. J. Primatol. (in press)

Tardif, S. D., Clapp, N. K., Henke, M. A., Carson, R. L., and Knapka, J. J.
Maintenance of cotton-top tamarins fed an experimental pelleted diet versus a highly diverse sweetened diet
Lab Animal Sci. 38, 588–591, 1988

Tardif, S. D., Harrison, M. L., and Simek, M. A.
Infant care in marmosets and tamarins
Intl. J. Primatol. (in press)

1028823

Vallari, D. S., Smith, Z. L., and Snyder, F.
HL-60 cells become resistant towards antitumor ether-linked phospholipids following differentiation into a granulocytic form
Biochem. Biophys. Res. Commun. 156, 1–8, 1988

Washburn, L. C., Lee, Y-C. C., Sun, T. TG. H., Byrd, B., Crook, J. E., Stabin, M. G., and Steplewski, Z.
Preclinical assessment of ^{90}Y-labeled antibody CO17-1A, a potential agent for radioimmunotherapy of colorectal carcinoma
Int. J. Radiat. Appl. Instrum. [B] 15, 707–711, 1988

Washburn, L. C., Lee, Y-C. C., Sun, T. TG. H., Byrd, B. L., Crook, J. E., and Steplewski, Z.
WR-2721 as an adjunct to radioimmunotherapy with Y-90-labeled monoclonal antibodies
J. Nucl. Med. (in press)

Washburn, L. C., Sun, T. T. H., Byrd, B. L., and Crook, J. E.
Synthesis of [^{18}F]7-fluoroheptylamine and tissue distribution studies in rats
J. Labeled Compd. Radiopharm. (in press)

Chapters, Review Articles, Government Reports, and Other Publications

Blank, M. L., Byrd, B. L., Cress, E. A., Washburn, L. C., and Snyder, F.
Use of liposomal DTPA in heavy metal detoxification
In *Liposomes as Drug Carriers: Recent Trends and Progress,* (Gregoriadis, G., ed.), pp. 497–506, John Wiley & Sons Ltd., Chichester, England, 1988

CIRRPC
Fourth Annual Report,
Office of Science and Technology Policy, Executive Office of the President, Washington, D.C., June 1988

CIRRPC
Seventeenth Quarterly Report,
Office of Science and Technology Policy, Executive Office of the President, Washington, D.C., September 1988

Clapp, N. K. (ed.)
Uniscience Monograph
The Cotton-Top Tamarin: A Primate Model for the Study of Colon Carcinoma, CRC Press, Boca Raton, Florida (in press)

Clapp, N. K., Henke, M. A., McArthur, A. H., and Carson, R.
Colonic epithelial changes associated with acute colitis and the development of colon carcinoma in tamarins
In *International Symposium on Future Research Approaches in IBD: Mechanisms of Chronic Infection and Inflammation,* pp. 713–718, Elsevier Science Publishers, Amsterdam, The Netherlands, 1988

DuMouchel, W., and Groer, P. G.
A Bayesian methodology for combining radiation studies
In *The Effects on Populations of Internally Deposited Alpha-Emitting Radionuclides,* 1987, Committee on the Biological Effects of Internal Radiations, National Academy Press, Washington, D.C. (in press)

Frome, E. L.
Statistical analysis of cytogenetic dose-response curves
In *Statistical Methods in Toxicological Research,* (Krewski, D., and Franklin, C., eds.), Gordon & Breach Science Publications, New York (in press)

Fry, S. A., Carpenter, A. V., Cragle, D. L., Dupree, E. A., Groer, P. G., Lushbaugh, C. C., Checkoway, H., Crawford-Brown, D. J., Shy, C. M., Watson, J. E., and Frome, E. L.
Studies of mortality among populations of U.S. nuclear industry workers
In *Proceedings of the International Conference, Health Effects of Low Dose Ionizing Radiation—Recent Advances and Their Implications,* London, England, May 11–14, 1987, Organized by the British Nuclear Energy Society and co-sponsored by the European Nuclear Society, 1988

Groer, P. G., and De B. Pereira, C. A.
Calibration of a radiation detector: Chromosome dosimetry for neutrons
In: *Probability and Bayesian Statistics,* (Viertl, R., ed.), pp. 225–232, Plenum Publishing Corporation, New York, New York, 1987

Lawless, D., Lee, Y-C. C., Crook, J. E., and Clapp, N.
Antibodies in the diagnosis of colon cancer
In [Proceedings] *Immunology and Immunopathology of the Alimentary Canal, 11th International Convocation on Immunology,* (in press)

Lee, T-c., and Snyder, F.
An overview of the biosynthesis and catabolism of platelet-activating factor
In *PAF in Health and Disease,* (Barnes, P. S., Page, C. P., and Henson, P. M., eds.) Blackwell, Oxford (in press)

Lee, Y-C. C., Lawless, D., Crook, J. E., and Clapp, N.
A murine immunoglobulin-G for simian colon cancer
In [Proceedings] *Immunology and Immunopathology of the Alimentary Canal, 11th International Convocation on Immunology* (in press)

Lincoln, T. A.
Educational opportunities in occupational medicine
In *Handbook of Occupational Medicine,* (McCunney, R. J., ed.), pp. 410–419, Little, Brown & Co., Boston, Massachusetts, 1988

1028824

Littlefield, L. G., Kleinerman, R. A., and Machado, S.
Applications and limitations of cytogenetic evaluations as an index of recent or previous radiation exposures
In *Report of ASA Conference on Radiation and Health, Coolfont, VI*, p. 15, American Statistical Association, Alexandria, Virginia, 1988

Littlefield, L. G., Joiner, E. E., and Hübner, K. F.
Cytogenetic techniques in biological dosimetry: Overview and example of dose estimation in 10 persons exposed to gamma radiation in the 1984 Mexican ^{60}Co accident
In *Medical Management of Radiation Accidents*, (Mettler, F. A., Jr., Kelsey, C. A., and Ricks, R. C., eds.), CRC Press, Boca Raton, Florida (in press)

Littlefield, L. G. [Contributor] State of the art of cytogenetic procedures to detect and quantify previous exposures to radiation.
Report to the National Cancer Institute based on work performed under contract No. DE-AC05-76OR00033 between the U.S. Department of Energy and Oak Ridge Associated Universities as DOE assistance to the National Cancer Institute through DOE Interagency Agreement No. 40-849-85, NCI No. Y01-CP-50512, August 1987

Lushbaugh, C. C.
Radiation
In *Trauma*, (Mattox, K. L., Moore, E. E., and Feliciano, D. V., eds.), pp. 691–696, Appleton & Lange, Norwalk, Connecticut, 1988

Lushbaugh, C. C.
Some examples of failure in X-ray safety
Adv. X-Ray Anal. 30, 569–573, 1987

Lushbaugh, C. C., Ricks, R. C., and Fry, S. A.
A U.S. perspective on Chernobyl
In *International Conference on Non-Military Radiation Emergencies*, 1986, pp. 31–37, Pan American Health Organization, Washington, D. C., 1987

Mills, W. A., Flack, D. S., Arsenault, F. J., and Conti, E. F.
A compendium of major U.S. radiation protection standards and guides: Legal and technical facts, ORAU 88/F-111, July 1988

Mossman, K. L., Flack, D. S., Geiger, L. F., and Mills, W. A.
The federal ionizing radiation research agenda related to low level biological effects: FY 1985, *ORAU7C-68*, March 1988

Snyder, F.
Biochemistry of platelet activating factor
In *Proceedings: Platelet Activating Factor, Pulmonary Hyperreactivity and Asthma*, (Holme, G., and Morley, J., eds.), L'Esterel, Quebec, Canada, 1986, Academic Press, New York (in press)

Snyder, F.
Biochemistry of platelet activating factor: A unique class of biologically active phospholipids
Proc. Soc. Exp. Biol. Med. (in press)

Snyder, F.
Metabolism of platelet activating factor and related ether lipids: enzymatic pathways, subcellular sites, regulation, and membrane processing
In *Aberration in Membrane Structure and Function*, (Karnovski, M., Leaf, A., and Bolis, C. L., eds.), Raven Press, New York, 1988

Snyder, F., Record, M., Smith, Z., Blank, M. L., and Hoffman, D. R.
Selective cytotoxic action of ether-lipid analogs of PAF: Mechanistic studies related to their metabolism, subcellular localization, and effects on cellular transport systems
In *Die Zellmembran als Angriffspunkt der Tumortherapie*, (Unger, C., Eibl, H., Nagel, G.A., eds.), Proceedings of Symposium, December 6–7, 1985, in Gottingen, FRG. Aktuel. Onkol. 34, 19–26, 1987

Snyder, F., Lee, T-c., and Blank, M. L.
Metabolism and function of bioactive ether lipids
In *Proceedings of an International Meeting on Neurochemical Aspects of Phospholipid Metabolism*, Perugia, Italy, 1988, Liviana Press, Padova-Springer Verlag, New York (in press)

Snyder, F., Lee, T-c., and Blank, M. L.
Metabolism of the ether analogs of phosphatidylcholine, including platelet activating factor
In *Phosphatidylcholine Metabolism*, (Vance, D. E., ed.), CRC Press Inc., Boca Raton, Florida (in press)

Tardif, S. D., and Colley, R.
International Cotton-Top Tamarin Studbook, Second Edition
Oak Ridge Associated Universities

Abstracts (Published) of Papers Presented at Meetings

Bales, L., Brooks, G. M., Richardson, V. B., Littlefield, L. G., and Colyer, S. P.
Cytogenetic evaluations in human lymphocytes exposed to methyl acetimidate: A lysine-specific protein crosslinking agent
Proceedings, NIH Centennial MBRS-MARC Symposium, October 1–3, 1987, Arlington, Virginia, p. 16.

Blank, M. L., Lee, Y., Cress, E. A., and Snyder, F.
Stimulation of the de novo pathway for the biosynthesis of platelet activating factor (PAF) via cytidylyltransferase activation in cells
FASEB J. 2, A1376, 1988

1028825

Clapp, N. K., Henke, M. A., McArthur, A. H., Tardif, S. D., Carson, R. L.
Associations between development of acute colitis and colon cancer in cotton-top tamarins
Gastroenterol. 94, A70, 1988

Frome, E. L., and DuFrain, R. J.
Analysis of cytogenetic dose-response data using a model derived from the theory of dual radiation action
Biometrics (in press)

Harrison, M. L., and Tardif, S. D.
Species differences in responses to nonspecific intruders in cotton-top tamarins and common marmosets
Am. J. Primatol. (in press)

Harrison, M. L., Tardif, S. D., and Henke, M. A.
Testes weight in a callitrichid primate (*Saguinus oedipus*)
Intl. J. Primatol. (in press)

Joiner, E. E., Littlefield, L. G., Colyer, S. P., and Frome, E. L.
DMSO concentration dependent modulation of X-ray induced aberrations in lymphocytes of two mammalian species
Environ. Molec. Mutagen. 11, 50, 1988

Kawasaki, T., Blank, M. L., and Snyder, F.
Arachidonic acid (AA) regulates platelet activating factor (PAF) synthesis in HL-60 cells
FASEB J. 2, A1376, 1988

Lee, T-c., Malone, B., Blank, M. L., Fitzgerald, V., and Snyder, F.
Regulation of the metabolism of platelet activating factor (PAF) and 1-alkyl-2-acetyl-sn-glycerols (G) by rabbit platelets
J. Cell. Biochem., Supplement 12 E, 49, 1988

Lee, Y-C., Crook, J. E., Clapp, N. K., Lawless, B. D., Fuhr, J. E., and Steplewski, Z.
Detection of circulating colon carcinoma cells in peripheral blood of tamarins with colon cancer
FASEB J. 2, A1409, 1988

Littlefield, L. G., Frome, E. L., Joiner, E. E., and Colyer, S. P.
ARA-C potentiation of chromosome damage induced by "direct" vs "indirect" radiation action
Environ. Molec. Mutagen. 11, 61, 1988

Littlefield, L. G., Frome, E. L., Joiner, E. E., and Colyer, S. P.
Modulation of radiation-induced chromosome aberrations by DMSO-preliminary comparisons in three mammalian species
Proceedings of DOE Contractor's Workshop, Cellular and Molecular Aspects of Radiation Induced DNA Damage and Repair, DOE CONF-8703182 UC-48, p. 100, 1987

Littlefield, L. G., Joiner, E. E., Frome, E. L., Colyer, S. P., and Sayer, A. M.
Modification of X-ray induced chromosome aberrations—radioprotection by DMSO
In *Radiation Research*, Proceedings of the 8th International Congress of Radiation Research, Edinburgh, 1987, (Fielden, E. M., Fowler, J. F., Hendry, H. J., and Scott, D., eds.), p. 146, Taylor & Francis, London, England, 1987

Lowe, K. W., and McFee, A. F.
Cytogenetic radioprotective effect of dimethyl sulfoxide in vivo
Environ. Molec. Mutagen. 11, 63, 1988

Qian, C., Lee, T-c., and Snyder, F.
Metabolism of platelet activating factor (PAF) and related ether lipids by neonatal rat myocytes
FASEB J. 2, A1376, 1988

Roberts, M. G., and McFee, A.
Influence of the solvent used on chemical induction of SCEs in mouse bone marrow
Environ. Molec. Mutagen. 11, 88, 1988

Sayer, A. M., Littlefield, L. G., Frome, E. L., Kholeif, S. F., and Ismail, S. R.
Dose response for X-ray-induced micronuclei in cytokinesis-blocked human lymphocytes
Environ. Molec. Mutagen. 11, 91, 1988

Snyder, F., Lee, T-c., Blank, M., Malone, B., Kawasaki, T., and Vallari, D.
Biosynthesis and regulation of platelet activating factor (PAF)
FASEB J. 2, A1739, 1988

Snyder, F., Lee, T-c, Blank, M., Malone, B., Kawasaki, T., and Vallari, D.
Metabolism and regulation of platelet activating factor (PAF)
J. Cell. Biochem., Supplement 12E, 40, 1988

Vallari, D., and Snyder, F.
CA^{2+} regulation of the metabolism of alkylacetylglycerol and platelet activating factor (PAF) in HL-60 cells
FASEB J. 2, A1376, 1988

Submitted Papers

Checkoway, H., and Hickey, J. L. S.
Estimating the potential disease rate reduction under varying conditions of combined effects
Int. J. Epidemiol. (submitted)

Freud, S. L., Durakovic, A., and Steele, J.
The psychological consequences of disasters: A review of the literature
Psychol. Rev. (submitted)

1028826

Hinck, A. P., Dasher, W. E., Washburn, L. C., and Byrd, B. L.
Synthesis of N-alkylethylenediamine-N,N′, N′-triacetates and biodistribution studies of the ^{68}Ga-labeled complexes in rats
Int. J. Radiat. Appl. Instrum. [B] (submitted)

Kasama, K., Blank, M. L., and Snyder, F.
Identification of l-alkyl-2-acyl-3-(2',3'-diacylglycerol) glycerols, a new type of lipid class, in Harderian gland tumors of mice
J. Biol. Chem. (submitted)

Kleinerman, R. A., Littlefield, L. G., Machado, S. G., Blettner, M., Peters, L. J., and Boice, J. D., Jr.
Radiation dose and chromosome aberrations in patients treated for cancer of the cervix.
Radiat. Res. (submitted)

Lee, T-c., Blank, M. L., Fitzgerald, V., and Snyder, F.
Factors that affect the CoA-dependent and CoA-independent acylation of lyso-phospholipids and the molecular species produced
Biochem. Biophys. Acta (submitted)

Littlefield, L. G., Sayer, A. M., and Frome, E. L.
Comparisons of dose response parameters for radiation-induced acentric fragments and micronuclei observed in cytokinesis-arrested lymphocytes.
Mutagenesis. (submitted)

Lowe, K. W., and McFee, A. F.
An unusual occurrence of supernumerary chromosomes in the mouse, *MUS MUSCULUS*
Chromosoma (submitted)

McFee, A. F.
Genotoxic potency of three quinoline compounds evaluated *in vivo* in mouse marrow cells
Environ. and Molecu. Mutagenesis. (submitted)

McFee, A. F., and Lowe, K.
Caprolactam and benzoin: Tests for induction of chromosome aberrations and SCEs in mouse bone marrow
Mutat. Res. (submitted)

Meyne, J., Littlefield, L. G., and Moyzis, R. K.
Labeling of human centromeres using an alphoid DNA consensus sequence: Application to the scoring of chromosome aberrations.
Mutat. Res. Letters. (submitted)

Invited Lectures Presented at National and International Meetings

Fry, S. A.
Review of studies published to date by ORAU/UNC on mortality among cohorts of DOE workers
Presented at the meeting of Working Group on Epidemiological Studies of Cancer Risk Among Nuclear Workers at the International Agency for Research on Cancer, Lyon, France, June 7–10, 1988

Fry, S. A.
Epidemiology of groups occupationally exposed to radiation
Presented at the 14th L. H. Gray Conference on Low Dose Radiation: Biological Bases of Risk Assessment, Oxford, England, September 11–16, 1988

Groer, P. G.
Radiation risk analysis
Presented at the American Occupational Medical Association meeting, New Orleans, Louisiana; April 1988

Groer, P. G., and De B. Pereira, C. A.
Lung cancer in Colorado plateau uranium miners: A reanalysis
Presented at the International Workshop on Radiological Protection in Mining, Darwin, Australia, April 1988

Groer, P. G., and De B. Pereira, C. A.
Prediction of lung cancer risk after exposure in Rn-Daughters
Presented at the 7th IRPA Congress, Sydney, Australia, April 1988

Groer, P. G.
Weight of evidence analysis of lung cancer in Colorado Plateau uranium miners
Presented at the 14th L. H. Gray Conference on Low Dose Radiation: Biological Bases of Risk Assessment, Oxford, England, September 11–16, 1988

Groer, P. G.
^{222}Rn-Daughter measurements with an instant working level meter
Presented at the Austrian Nuclear Research Center, Seibersdorf, Austria, September 19–20, 1988

Snyder, F.
Metabolism and regulation of platelet-activating factor (PAF), related metabolites, and precursor pools
Presented at the UCLA Symposia on Molecular and Cellular Biology. Symposium on Cell Activation and Signal Initiation: Receptor and Phospholipase Control of Inositol Phosphate, PAF, and Eicosanoid Production, Keystone, Colorado, April 1988

1028827

Snyder, F.
Biosynthesis and regulation of platelet-activating factor
Presented at the Federation of American Societies for Experimental Biology, Symposium on Biochemical Functions of Ether-Linked Lipids, Las Vegas, Nevada, May 1988

Snyder, F.
Metabolism and function of bioactive ether lipids
Presented at the Giuseppe Porcellati Foundation International Meeting on Neurochemical Aspects of Phospholipid Metabolism, Perugia, Italy, May 1988

Snyder, F.
Cellular regulation of enzymes in the metabolism of platelet-activating factor (PAF)
Presented at the XXII Congress of the International Society of Hematology, PAF Symposium on PAF and PAF Antagonists, Milan, Italy, August,1988

Snyder, F.
Transacylation of polyenoic fatty acids between phospholipids
Presented at an International Conference on Lipoproteins and Phospholipases: Present and Future, Paris, France, September 1988

Snyder, F.
Platelet-activating factor and related ether lipid mediators: biological activities, metabolism and regulation
Presented at the National Institute on Aging Conference on Calcium, Membranes, Aging, and Alzheimer's Disease, The University of California, Irvine, Calif., October 1988

Tankersley, W. G., Cragle, D. L., Ziegler, M. F., and Watson, J. E.
Comparison of assigned exposure indices and radiation monitoring data
Presented at the American Industrial Hygiene Conference, San Francisco, Calif., May 1988

Collaborative Research

H. I. Adler

Study of the ability of microorganisms to bind heavy metals. Oak Ridge National Laboratory, Chemical Technology Division

Study of anaerobic bacteria isolated from waste disposal sites. Knoxville College, Mary Brown

J. E. Crook

Development of software for quantitative positron tomography. EG&G ORTEC (Oak Ridge), R. M. Keyser

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp, Jr.

Clinical use of radiopharmaceuticals in human disease. The University of Tennessee Medical Center (Knoxville), Department of Radiology, K. F. Hubner

Flow cytometry and OKT4+/OKT8+ measurements. The University of Tennessee Medical Center (Knoxville), Department of Biology, J. Fuhr

Use of radiopharmaceuticals in animal models of human disease. The University of Tennessee (Knoxville), College of Veterinary Medicine, R. E. Gompf

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. Fordham University (New York), Department of Science, B. D. Lawless

Development of imaging techniques for positron emission tomography. Computer Technology and Imaging, Inc. (Knoxville), M. Andreaco

Evaluation of an 8 MeV proton cyclotron as a source of radionuclides for positron emission tomography. ORBIT, Inc. (Oak Ridge), J. H. Neiler

S. A. Fry

Morbidity among workers exposed to mercury at the Oak Ridge Y-12 plant. University of Michigan (Ann Arbor), Department of Environmental and Industrial Health, L. Fine

1028828

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Radiation Epidemiology Branch (Bethesda), R. Kleinerman, John Boice

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons. Lawrence Livermore National Laboratory (Livermore), L. Dobson, T. Straume, A. Carrano, and J. Minkler; Los Alamos National Laboratory (Los Alamos), L. Devan; Radiation Effects Research Foundation (Hiroshima), A. Awa

Coordinated research program on routine use of chromosomal aberration analysis in occupationally exposed radiation workers. International Atomic Energy Agency (Vienna), A. Bianco

Chromosome aberrations in human lymphocytes exposed to monoenergetic neutrons in the presence or absence of DMSO. Radiological Research Accelerator Facility, Columbia University (New York), Paul Goldhagen, Stephen Marino

The effect of methyl acetimidate on cellular proliferation and chromosome structure. Tuskegee University (Alabama), Velma B. Richardson

Application of centromere specific-DNA probes in radiation cytogenetics. Los Alamos National Laboratory (New Mexico), Julianne Meyne

Persistent radiation-induced aberrations in lymphocytes of Y-12 accident survivors: Comparisons of chromosome painting and classical scoring methods. Lawrence Livermore National Laboratory (California), Tore L. Straume

C. C. Lushbaugh and L. C. Washburn

Diethylenetriaminepentaacetate (DTPA) project. Department of Energy contractors, 45 co-investigators

A. F. McFee

In vivo mouse assay for chemically induced SCE and chromosomal aberrations. Brookhaven National Laboratory (New York), Dr. Raymond Tice

F. Snyder

Biological effects of unique phospholipid analogs. California State University (Northridge), Department of Chemistry, J. Hajdu

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill), Department of Medicinal Chemistry, C. Piantadosi

Cardiovascular and sympathetic effects of 1-O-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine. NIH and Uniformed Services University (Bethesda), Department of Neurology, G. Feuerstein

Synthesis and biochemical studies of analogs of platelet-activating factor. Queens College of the City University of New York (Flushing), Department of Chemistry, R. Bittman

L. C. Washburn

Short-lived alpha-emitting radionuclides for labeling monoclonal antibodies. Oak Ridge National Laboratory, Chemical Technology Division, J. E. Bigelow

Synthesis of labeled compounds using organoborane techniques. University of Tennessee (Knoxville), Department of Chemistry, G. W. Kabalka

Training Programs

Training programs at the division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. In addition, special short-term courses conducted by REAC/TS provide training for medical personnel and attorneys in matters related to nuclear accidents and associated radiation health problems.

Most graduate students doing Ph.D. dissertation research in our division have generally been affiliated with ORAU member universities. To receive the Ph.D., students must meet all of the academic requirements for the degree imposed by their own universities. Financial support for graduate studies has generally been available from the National Cancer Institute Training Grant administered by the Oak Ridge National Laboratory, the Science/Engineering Education Division at ORAU (sponsored by DOE), and individual research grants.

Postdoctoral training continues to be an important part of the division's research efforts. Over the years funds for support of postdoctoral fellows have been available from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health, American Cancer Society, the World Health Organization (International Agency for Research on Cancer), the

1028829

Fogarty International Center (U.S. Public Health Service), and Medical Sciences Division funds.

Summer research opportunities enable faculty and undergraduate students from colleges and universities throughout the United States to participate in our division's research programs. The DOE-sponsored summer program is administered through the Science/Engineering Education Division at ORAU.

Visiting scientists from various universities and other U.S. and foreign institutions also participate in division research programs. Some of these scientists are sponsored through the Research Participation program administered by ORAU's Science/Engineering Education Division. In some instances, funds for visiting scientists are awarded through national or international agencies, and occasionally, appointments are made directly from programmatic funds.

Physicians, health physicists, paramedical personnel, and attorneys attend short-term courses in the management of medical problems associated with radiation accidents offered at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of 11 REAC/TS courses were given during the past year.

Several members of the senior staff conduct special courses and provide specific lectures at the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee (Knoxville). These scientists usually hold joint appointments where they teach; some also advise graduate students who pursue their research studies at ORAU.

The individuals listed below participated in the training programs at the division during the past year:

Predoctoral students

Elizabeth Dupree Ellis (Epidemiology) (University of North Carolina, Chapel Hill)

Daniel Petersen (Biochemistry) (Rice University, Houston)

Postdoctoral Fellows

Yu-Chen C. Lee (Radiopharmaceuticals)

Kazuhiro Suga (Biochemistry)

David S. Vallari (Biochemistry)

M.S. Students

Janice Watkins (Epidemiology) (University of Tennessee, Knoxville)

Amie Fetcher (Epidemiology) (University of Tennessee, Knoxville)

Visiting Scientists

B. DeSales Lawless (Radiopharmaceuticals) (Fordham University, New York, New York)

Velma Richardson (Cytogenetics) (Tuskegee University, Tuskegee, Alabama)

Chen Deqing (Beijing, China)

College Student Research Participant

Ruthann Austinhirst (Biochemistry) (University of Puget Sound, Tacoma, Washington)

Major Awards, Honors, and Appointments

D. Cragle

Member of the International Nickel Speciation Research Project Steering Committee

S. A. Fry

Member of the National Council on Radiation Protection and Measurements Scientific Committee's Task Force on Uranium (SC57-15)

P. G. Groer

Member of the Cytogenetics Working Group for NCI in response to Public Law 98-542

Consultant to the National Council on Radiation Protection and Measurement (NCRP), Committee on Radiation Exposure and Potentially Related Injury

Consultant to CIRRPC Science Subpanel on Radioepidemiological Tables

Member of the National Academy of Sciences' BEIR IV Alpha Committee on Health Risks of Internally Deposited Alpha Emitters

Consultant to the Mining, Safety, and Health Administration on setting of new WL-standard for underground mining

T-c. Lee

Adjunct professor, University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences

L. G. Littlefield

Appointed as Adjunct Faculty Member, University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences, September 1988

1028830

C.C. Lushbaugh

Adjunct professor, School of Public Health (Epidemiology)

Member, Graduate Faculty, University of North Carolina

F. Snyder

Executive Editor and Member of the Editorial Committee, *Archives of Biochemistry and Biophysics*

Member of the Editorial Board, *Lipids*

Member of the Editorial Board, *Journal of Biological Chemistry*

Editor, *Handbook of Lipid Research*, A continuing series, Plenum Publishing Corporation, New York

Associate Editor, *Journal of Lipid Mediators*

Adjunct professorships at the University of North Carolina and the University of Tennessee–Oak Ridge Graduate School of Biomedical Sciences

Patents Granted

Fred Snyder and Merle Blank
1-Alkyl-2-acetyl-*sn*-glycerol and its use as an antihypertensive agent

James Crook
Copper-64 citrate as a myocardial imaging agent

Patents Pending

Fred Snyder and Merle Blank
Alkyl phospholipid antihypertensive agent

Gayle Littlefield and Shirley Colyer
Continuous cell-line tamarin adenocarcinoma of the colon (ORAU-TAC-1)

C. D. Scott and H. I. Adler
High-productivity biocatylst gel beads

Non-DOE Research Funding*

H. I. Adler
Fundamental Microbiological Research in Support of Technology Development. Martin Marietta Energy Systems, $10,000 (1 October 1988 to 30 September 1989)

H. I. Adler
PCB Degradation. Martin Marietta Energy Systems, $15,000 (1 September 1988 to 30 April 1989)

S. A. Fry
Death Data Retrieval Project. National Cancer Institute, $80,000 (October 1988 to September 1989)

S. A. Fry
Epidemiological Studies of Fernald Workers. Westinghouse Materials Company of Ohio, $80,000 (1 October 1988 to 30 September 1989)

S. A. Fry
Gaseous Centrifuge Worker Study. Martin Marietta Energy Systems, $61,000 (October 1988 to September 1989)

L. G. Littlefield
Studies of radiation-induced chromosome damage in humans. National Cancer Institute–U.S. Department of Energy Interagency Agreement, $156,141 (October 1988 to September 1989)

A. F. McFee
Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations. National Institute of Environmental Health Sciences–U.S. Department of Energy Interagency Agreement, $408,727 (October 1988 to September 1989)

R. C. Ricks
Decision making in nuclear power plant emergencies for local officials. Federal Emergency Management Agency, $20,000 (1 October 1988 to 30 September 1989)

R. C. Ricks
Radiological training courses for health physics and medical personnel. Federal Emergency Management Agency, $150,000 (1 October 1988 to 30 September 1989)

F. Snyder
Metabolism, regulation, and function of ether lipid mediators and related phospholipids in cancer cells and their antitumor analogs. American Cancer Society, $108,000 (January 1989 to December 1989)

1028831

F. Snyder
A novel class of biologically active phospholipids.
National Heart, Lung, and Blood Institute, $139,788 (April 1988 to March 1989)

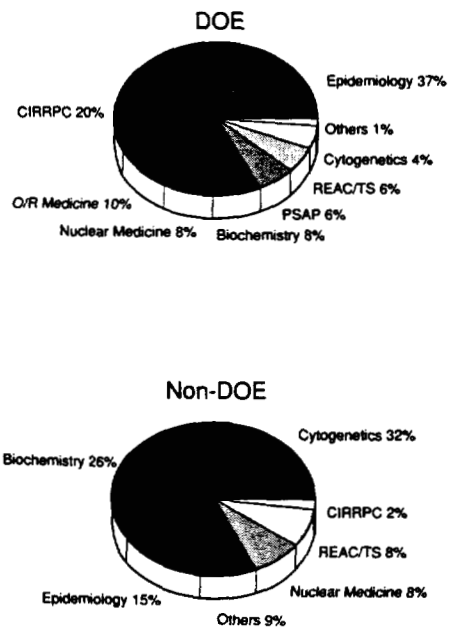
F. Snyder
An antihypertensive class of neutral lipids in kidney.
National Heart, Lung, and Blood Institute, $239,538 (December 1985 to November 1988)

F. Snyder
Plasmalogens and related ether lipids in cancer cells.
National Cancer Institute, $142,150 (July 1988–June 1989)

L. C. Washburn
Radiolabeled monoclonal antibodies for cancer therapy.
National Cancer Institute, $173,376 (1 September 1988 to 31 August 1989; first year of 3-year renewal award)

**Total costs, i.e., direct plus overhead*

Medical Division Programs Fiscal Year 1988



Medical Sciences Division Staff

Administration

Frances J. Banner
William W. Burr, Jr., M.D., Ph.D.
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Cheryl A. Fox
Susan J. Herrell[4]
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*Margeuriete A. Robinson, B.S.[5]
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Sandra D. Ridings[3]
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Lora H. Treece, C.P.S.
Louise C. Wyatt

Biochemistry

Merle L. Blank, B.S.
Edgar A. Cress, B.S.
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*Martha R. Kahl, B.S.[3]
*Tomio Kawasaki, M.D.
Ten-ching Lee, Ph.D.
*Lori McKamey
Boyd B. Malone, B.S.
Myra Perry[3]
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Fred L. Snyder, Ph.D.
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Kazuhiro Suga, M.D.[2]
Dorothy L. Vola
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Lillie I. Woods

Marmoset Research Center

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Karren D. Hyde, B.A.
Roy C. Rice
Suzette D. Tardif, Ph.D.

Microbiology

Howard I. Adler, Ph.D.

1028832

Center for Epidemiologic Research

*Peter C. Ashline[5]
Melinda J. Armes[3]
Susan C. Ball, B.S.
Sondra L. Butcher[6]
Donna L. Cragle, Ph.D.
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Patricia J. Deems, A.S.
Bradley W. Dolan, B.S.
Elizabeth D. Ellis, M.S.
Shirley A. Fry, M.B., B.Ch., M.P.H.
*Theresa M. Gray, B.A.
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Peter G. Groer, Ph.D.
B. Jolene Jones
Karen H. Kennedy[3]
Janet L. Kile
Carolyn A. Murphy
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Preclinical and Clinical Nuclear Medicine

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Cynthia D. Glasscock
Elizabeth C. Holloway, R.T.
Yu-chen C. Lee, Ph.D.[2]
Tan Tan Sun, M.S.
Lee C. Washburn, Ph.D.

Radiation Medicine

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Cytogenetics

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Anthony H. Ewing, B.S.
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Catherine M. Schafer
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*Mei Lee Strom, B.A.
Darlene S. Thompson

1028833

Radiation Emergency Assistance Center/ Training Site

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*Harold Hodges, B.S.
Martha H. Payne
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Ann H. Sipe, L.P.N.
Charles M. West, B.S.[4]

Center for Personnel Security Assurance Research and Analysis

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*Linda J. Froehlich
Steven Nessel, M.S.[5]
*Chandra Tolwani, B.A.[3]

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Martha R. Kahl, B.S.
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Animal Care

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Billy R. Kyle
Mack A. Ruffner
John H. Suttles

Custodial Care

Mark A. Brewer
Leonard Brown
Jimmy R. Duncan
Grace E. Foster
Douglas E. Herron
Frank D. Walls[3]

Electronics

Mark A. Brewer
William T. Pope

**Personnel no longer here*
[1]DOE Fellow
[2]Postdoctoral Research Associate
[3]Part-time Temporary
[4]Part-time Continuing
[5]Full-time Temporary
[6]VOE Student

Consultants

Preston S. Abbott, Ph.D. (CIRRPC)
Frank J. Arsenault, B.S. (CIRRPC)
Larry A. Blackwell, Ph.D. (CPSARA)
Don G. Bock (OM)
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James C. White, Ph.D. (CPSARA)
Steven Wing (CER)
Jill D. Wright, Ph.D. (CPSARA)
G. F. Zanolli, M.D. (OM)

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David E. Drum, M.D., Brigham & Women's Hospital, Boston, MA
A. Seaton Garrett, Jr., M.D., Oak Ridge National Laboratory
Karl F. Hübner, M.D., University of Tennessee Memorial Research Center and Hospital, Knoxville, TN
Roger E. Linnemann, M.D., University of Pennsylvania School of Medicine, Philadelphia, PA
James A. Martin, Jr., M.A., U.S. Nuclear Regulatory Commission, Washington, DC
Marilyn McLaughlin, B.S., Oak Ridge National Laboratory, Oak Ridge, TN
Fred A. Mettler, Jr., M.D., M.P.H., University of New Mexico School of Medicine, Albuquerque, NM
John W. Poston, Ph.D., Department of Nuclear Engineering, Texas A&M University, College Station, TX
William A. Pryor, B.S., U.S. Department of Energy, Oak Ridge, TN
C. E. Roessler, Ph.D., University of Florida, Gainesville, FL
Eugene L. Saenger, M.D., University of Cincinnati Hospital, Cincinnati, OH
George L. Voelz, M.D., Los Alamos National Laboratory, Los Alamos, NM
Niel Wald, M.D., University of Pittsburgh, Pittsburgh, PA

1028835

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Mississippi State University
University of Missouri
University of North Carolina
North Carolina State University
University of North Texas
University of Oklahoma
Oklahoma State University
University of Puerto Rico
Rice University
University of South Carolina
Southern Illinois University at Carbondale
Southern Methodist University
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Tennessee Technological University
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Texas Woman's University
Tuskegee University
Vanderbilt University
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Virginia Commonwealth University
Virginia Polytechnic Institute and State University
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College of William and Mary

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Oak Ridge Associated Universities (ORAU) is a private, not-for-profit association of 49 colleges and universities and a management and operating contractor of the U.S. Department of Energy (DOE). ORAU's mission is to foster, encourage, and engage in the identification and development of solutions to scientific, engineering, technical, medical, and human resource problems through the resources available to ORAU and its member universities. In support of this mission, ORAU provides diverse services (principally academic outreach, research, training and education, technical assistance, and technology transfer) for DOE, ORAU's member institutions, other colleges and universities, and other private and governmental organizations. Established in 1946, ORAU was one of the first university-based, science-related corporate management groups.

The **Medical Sciences Division** (MSD) conducts research on biomedical problems, offers training in conjunction with research programs, and develops and conducts scheduled training activities. MSD also provides technical assistance and support to DOE and other federal agencies.

Research emphasis is on cancer and other diseases related to occupational exposures to chemical toxicants and ionizing radiation involved in energy production. Specific programs in the division are focused on the following areas: (a) preclinical and clinical development of new radiopharmaceutical agents for in vivo diagnosis and treatment of diseases in humans, (b) biochemical aspects of cellular lipid mediators and membrane lipids in physiological and disease processes, (c) epidemiological evaluation of occupational exposures and health experience of workers in industries related to fuel and energy production, (d) microbial research on reactive oxygen species formed during aerobic metabolism by bacteria and during exposure of organisms to ionizing radiation, (e) cytogenetic investigations designed to assess exposure and possible genetic hazards associated with human exposure to environmental mutagens (radiation and chemicals), (f) human radiobiology and radiation medicine, and (g) the assessment of human reliability in sensitive positions within the DOE and its contractor organizations.

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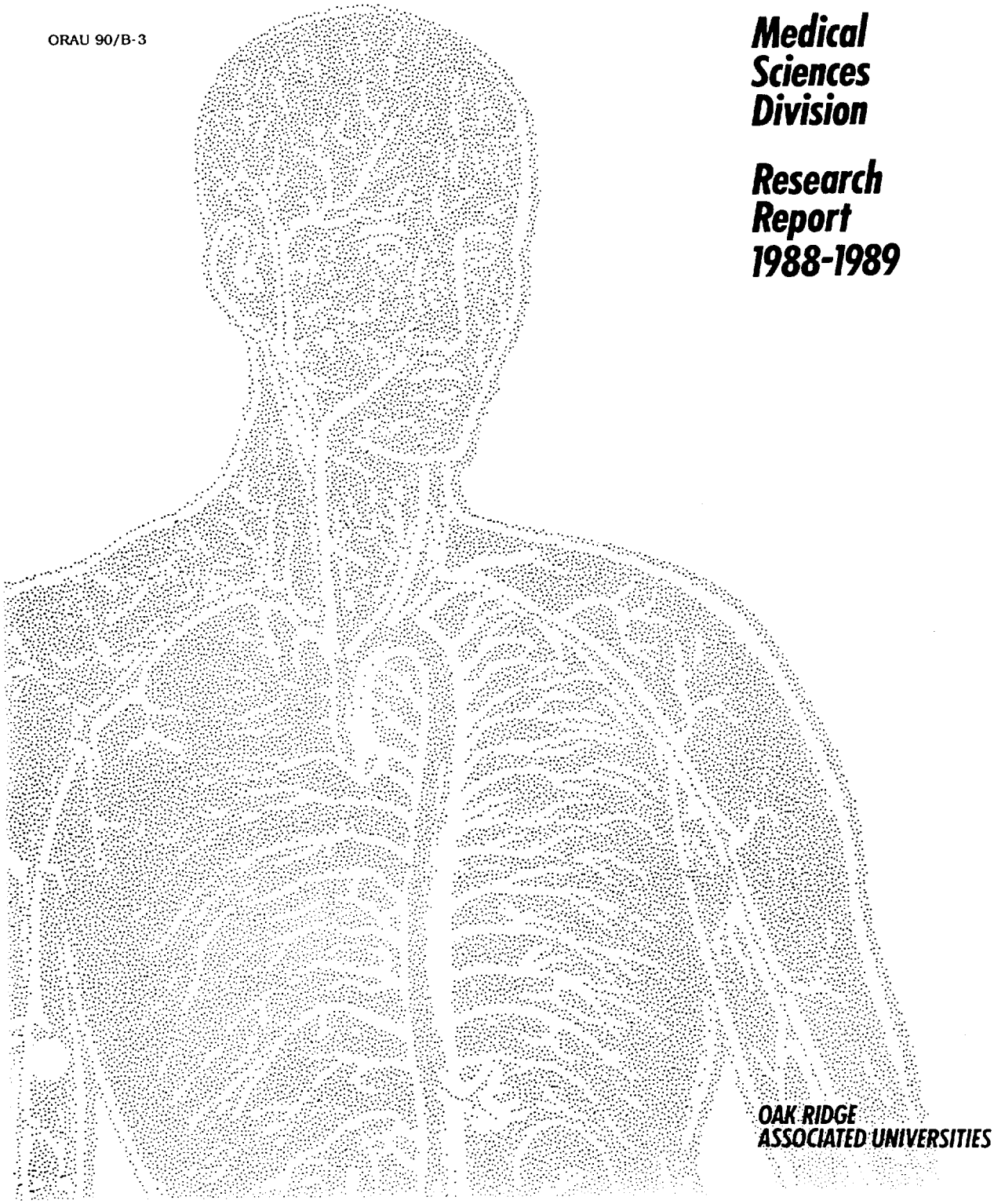
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Medical Sciences Division

Research Report 1988-1989



OAK RIDGE ASSOCIATED UNIVERSITIES

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Preface

We believe this brief report of the research, training, and service activities at the Medical Sciences Division of Oak Ridge Associated Universities during fiscal year 1989 provides a succinct summary of our accomplishments and a glimpse of the future directions of our various programs.

spite budgetary restraints, our staff has been very successful in making fiscal year 1989 a highly productive year in research, as is evident from the excellent quality of our publications listed in this report.

Our program enjoys considerable recognition in the international medical community, as indicated by invitations for our staff to lecture at major international symposia. In addition, many of our scientists serve on national and international editorial boards and advisory panels. Advice and guidance are provided on radiation-related medical problems throughout the world.

Please contact one of us or one of the program directors for more information about any of the programs, research, or publications described in this report.

W. W. Burr
Chairman

Fred Snyder
Vice Chairman

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Table of Contents

Scope of Programs

Biochemistry 1
Radiation Medicine 3
Nuclear Medicine 3
Cytogenetics 5
Radiation Emergency Assistance Center/Training Site 7
Radiopharmaceutical Internal Dose Information Center 8
Microbiology 10
Center for Epidemiologic Research. 11
Center for Personnel Security Assurance, Research and Analysis 12

Publications, Abstracts, and Invited Lectures

Full-length Journal Articles 14
Submitted Papers 16
Chapters, Review Articles, Government Reports, and Other Publications 17
Abstracts, Oral Presentations, and/or Posters 18
Invited Lectures at National and International Conferences 21

Collaborative Research 22

Training Programs 24

Major Awards, Honors, and Current Appointments 26

Patents Pending 28

Non-DOE Research Funding 28

Medical Sciences Division Staff 29

Administration and Committees 32

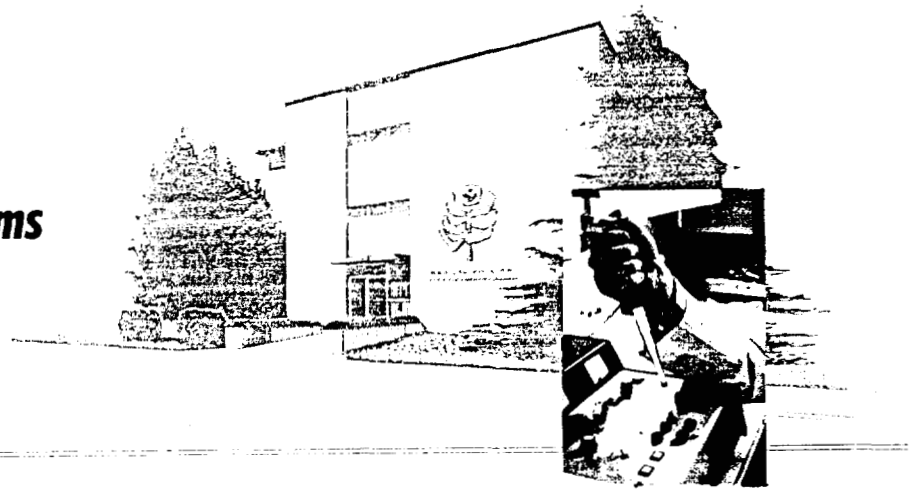
Member Institutions 33

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Scope of Programs

Biochemistry

Program Director: ***Fred Snyder***



Glycerolipids were once viewed solely as simple structural components of membranes and as an efficient storage form of body fuel. However, in recent years, our knowledge about the biological role and significance of lipids has greatly expanded. For example, minor cellular glycerolipids are known to serve as crucial anchors for various membrane proteins and as very potent mediators involved in modulating diverse cellular tivities. Lipid mediators such as platelet activating factor(s) and eicosanoid metabolites appear to trigger signal transduction mechanisms that are involved in such crucial events as the control of cell growth and cellular differentiation. Research in the Biochemistry Program centers on the function of lipids in a variety of physiological and pathological processes. Specific projects under investigation relate to the role of lipids and lipid mediators in cancer, immune responses, inflammatory reactions, renal physiology, pulmonary and cardiovascular disorders, radiation injury, and nutrition. A primary objective is to acquire basic knowledge about the biochemistry of phospholipids in mammalian cells, especially those that act as cellular mediators and as antitumor agents. Such information is expected to provide an understanding of the mechanism(s) responsible for the biological properties of these novel lipids with the hope that this knowledge will lead to improved human health through clinical applications.

Significant programmatic accomplishments during the past fiscal year are cited below.

• Major progress was made in the characterization and subcellular location of enzymes responsible for the de novo synthesis of platelet activating factor (PAF). Specific results in this project completed during FY 1989 are:

- Myocytes from neonatal rat heart, a cell model being utilized in both the lipid mediator and plasmalogen projects, metabolize PAF in a manner that is qualitatively but not quantitatively similar to hematopoietic cell types. These enzymatic results are the first to demonstrate the high degree of PAF catabolism by actively functioning cells isolated from heart tissue. Moreover, it appears these heart cells that continuously contract in cell culture will serve as an important model for investigating the role of PAF in cardiac function.

- An enzyme responsible for the conversion of 1-alkyl-2-acetyl-*sn*-glycerols (an immediate precursor of PAF and modulator of protein kinase C functions) to

1028842

alkylglycerols was partially characterized in a membrane fraction of Ehrlich ascites cells. Properties of this enzyme are strikingly different from those of other lipolytic enzymes such as diacylglycerol lipase, monoacylglycerol lipase, and PAF acetylhydrolase. The high degree of substrate specificity of this enzyme further emphasizes both the uniqueness and importance of the de novo pathway of PAF biosynthesis. In addition to this, acetylhydrolase appears to be a crucial regulatory enzyme in controlling the cellular levels of potent bioactive alkylacetylglycerols.

- Enzymes involved in the remodeling and de novo pathway of PAF synthesis (acetyltransferase and cholinephosphotransferase, respectively) were found to be located in the endoplasmic reticulum in undifferentiated HL-60 cells. Since this is similar to what has been reported in human polymorphonuclear leukocytes, it appears the subcellular localization of these enzymes is not altered in neoplastic cells.

- Results indicate de novo PAF synthesis in the renal inner medulla is the source of blood levels of PAF. Moreover, a single enzyme (dithiothreitol-insensitive cholinephosphotransferase) in the de novo route correlates with the decreased rate of PAF synthesis in the kidney medulla during the very early stages of development of experimentally induced renal necrosis.

• Preliminary studies were initiated on the role of PAF and related lipid mediators in radiation injury for two key enzymes (acetyltransferase and acetylhydrolase) responsible for controlling PAF levels in the spleen, skin, and blood of rats. Plans are to examine the production of PAF and eicosanoid metabolites in various animal tissues and cell culture systems during both early and later stages of radiation injury and repair.

• Ultracentrifugation studies of the subcellular distribution of alkylmethoxyglycerophosphocholine (an antitumor analog of PAF) have shown this unnatural phospholipid preferentially locates in the plasma membrane. These data further support our earlier concept that the chemotherapeutic actions of this novel lipophilic drug are directly targeted against specific biochemical systems associated with the surface membrane of tumor cells.

• A unique complex ether-linked glycerolipid structure (a *bis* type of glyceryl ether) was found to occur in tumor tissue but not in normal cells of the same origin. It is possible that this unusual ether lipid that contains two glycerol moieties could have potential as a diagnostic marker for certain types of cancer.

• Membrane modification of macrophages with omega-3 fatty acids (a major component of fish oil) caused marked changes in both the cellular and enzymatic aspects of lipid metabolism involving the ethanolamine plasmalogens. Cellular membranes that become highly enriched in the omega-3 fatty acids exhibit an increased capacity in their ability to convert alkyl phospholipids to the plasmalogens form via the Δ1-desaturase enzyme. These results indicate that specific lipid modifications can exert highly selective alterations in membrane functions, which could have clinical implications in the treatment of disease.

Future directions in the Lipid Biochemistry Program include continuation of the studies directed toward understanding the mechanism(s) by which physiological and pathological levels of PAF are regulated. Long-range plans in the PAF project will emphasize enzyme purification, gene cloning, and studies on regulatory control of gene expression of key enzymes involved in PAF metabolism. A new area of investigation is expected to focus on the combined role of dietary ether lipids and omega-3 fatty acids in cell mediator responses and nutritional-related health effects. Projects regarding the membrane-targeted antitumor phospholipids and the tumor-associated *bis*-glyceryl ether lipid are ongoing activities that will continue to be pursued.

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Radiation Medicine

Program Director: C. C. Lushbaugh

The recent radiation accidents in Russia (1986), Brazil (1988), and El Salvador (1989) underline the need for improved therapy for both high dose, local skin damage, and high dose, hematopoietic (bone marrow) damage. The Radiation Medicine program is endeavoring to meet these needs using two approaches: (1) retrospective analysis of the course of clinical events in radiation accidents and assessment of the clinical efficacy of new, cell-stimulating hormones, such as GMCSF and anti-prostaglandins, and (2) prospective experiments in animals and cell cultures, wherein the effects of radiation on cellular and subcellular systems are studied along with the antidotal remedial effects of anti-inflammatory substances from the arachidonic acid cascade and related lipid mediators.

The retrospective analyses of the clinical courses of the foreign radiation accident victims involve about 150 recently published case studies. Our analyses have been published and disseminated in several instances (see the "Publications, Abstracts, and Invited Lectures" section of this report).

The experimental approach concerning the cellular and subcellular events induced by irradiation and remedied by the anti-prostaglandins, aloe vera extracts, and subcellular hormones has proceeded this year by a thorough literature search which defined various experimental approaches that have been used to design preliminary trials to develop a cellular test system that can be used to study the molecular biology of radiation damage to growth processes.

Nuclear Medicine

Program Director: James E. Crook

The major objectives of the Nuclear Medicine program are to develop new radiopharmaceutical agents and to carry out phase I and phase II testing of such materials in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of potential radiopharmaceutical agents for diagnosis and therapy, tests of these substances in animals for effectiveness and toxicity, and efficacy trials in humans according to U.S. Food and Drug Administration (FDA) rules and guidelines.

Some recent achievements in nuclear medicine include the following:

•Preclinical development of radiolabeled monoclonal antibodies for cancer therapy is supported by a grant, now in its fifth year, from the National Cancer Institute (NCI). CO17-1A, a monoclonal antibody with specificity for colorectal and pancreatic carcinomas, was developed and supplied by Z. Steplewski, a collaborator from The Wistar Institute (Philadelphia, Pennsylvania). CO17-1A radiolabeled with the beta-emitting radionuclide, yttrium-90, via a series of new bifunctional chelate reagents was compared in nude mice bearing SW 948 human colorectal carcinoma xenografts. Site-specific conjugation using p-aminobenzyl-DOTA-3A, a bifunctional chelate reagent developed by O. A. Gansow and M. W. Brechbiel at the NCI, gave the highest tumor uptake and the greatest in vivo stability.

• In experimental radioimmunotherapy studies in nude mice bearing SW 948 human colorectal carcinoma xenografts, a

1028844

single dose of 200 μCi ^{90}Y-CO17-1A per 25 grams of body weight reduced the initial tumor size by ~95%. For the first time, we were able to effect total regression of the tumor in a few animals. Tumor regrowth in animals in which total tumor eradication was not effected was found to be controllable by reinjection of 150 μCi ^{90}Y-CO17-1A per 25 grams of body weight at intervals of six to seven weeks.

• A clinical protocol for phase II clinical investigations of ^{90}Y-CO17-1A in patients with disseminated colorectal carcinomas was developed in collaboration with J. J. Costanzi of the Thompson Cancer Survival Center (Knoxville, Tennessee). Toxicity studies in nude mice and guinea pigs were carried out in support of the planned submission of an amended Investigational New Drug (IND) application to the FDA.

• A collaborative study was initiated with T. J. MacVittie and M. L. Patchen of the Armed Forces Radiobiology Research Institute (Bethesda, Maryland) to investigate the potential of colony stimulating factors (CSF) as adjuncts to ^{90}Y-labeled monoclonal antibody therapy of cancer. Colony stimulating factors may be effective at stimulating hematopoiesis in radiation-damaged bone marrow.

• Following a successful pilot experiment in the nude mouse model, a grant application, "Colony Stimulating Factors – Use with Radioimmunotherapy," was prepared and submitted to the NCI. A study was also carried out in which the effect of 200 μCi ^{90}Y-CO17-1A per 25 grams body weight was compared, either alone or with adjunctive administration of G-CSF (3 mg/mouse/day) at days 1-14 or at days 8-14 after injection. The adjunctive use of G-CSF at days 1-14 gave the greatest survival rate. Hematological parameters also showed the beneficial effect of G-CSF on regeneration of the bone marrow following administration of ^{90}Y-CO17-1A.

• GA 22-2, a monoclonal antibody developed by Z. Steplewski and demonstrated to have selective affinity in vitro for cells of the spontaneous tamarin colorectal carcinoma, was radiolabeled with iodine-123 and used to successfully image tumor-bearing tamarins.

• In a collaborative study with G. Digenis and T. Elder of the University of Kentucky, [^{18}F]fluoride obtained from CTI, Inc. (Knoxville, Tennessee), was used to synthesize the ^{18}F-fluoride-labeled anti-inflammatory drug, flurbiprofen. After injection of this agent into a dog in which minor inflammation had been induced in one knee, images obtained using the Medical Sciences Division's ECAT-II positron emission tomograph showed selective accumulation of the agent in the area corresponding to the inflammation.

• Representatives from seven ORAU member institutions attended the ORAU-organized "Workshop on Positron Emission Tomography (PET)." A PET Steering Committee was formed to facilitate ORAU's coordination of PET research among interested member institutions.

Future plans for the Nuclear Medicine program will continue to focus on the development and application of radiolabeled monoclonal antibodies. An amended IND application for ^{90}Y-CO17-1A will be submitted and the phase I/II clinical investigative trials initiated following FDA approval. Preclinical biodistribution, radioimmunotherapy, and toxicity studies will be completed for ^{90}Y-CO17-1A prepared via the site-specific conjugation of (p-amino-benzyl) DOTA-3A. The potential of colony stimulating factors as adjuncts to radioimmunotherapy with ^{90}Y-labeled monoclonal antibodies will be investigated in detail. Also, pilot studies on the pharmacological modifications of radiolabeled monoclonal antibody distribution will be expanded. A workshop, "Radiolabeling Monoclonal Antibodies: A Practical Mini-Course," was inaugurated in December 1989, with future courses conducted as required to accommodate interests and demands. In collaboration with J. Neiler of ORBIT, Inc., the potential of the ORBIT, Inc., 8 MeV cyclotron will be evaluated as a source of clinically useful quantities of positron-emitting radionuclides.

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Cytogenetics

Program Director: L. Gayle Littlefield

The Cytogenetics program conducts basic research to develop and improve various assays for quantifying levels of chromosomal injury induced in somatic cells following human exposure to ionizing radiation or genotoxic chemicals. Such techniques are applied as biological indicators of radiation dose in persons having real or suspected overexposures and in "cytogenetic epidemiology" evaluations in highly selected cohorts of patients having whole-body or partial-body radiation exposures. The purpose of these latter studies is to gather information on the correlation between long-term survival of cells with chromosome damage and subsequent risk of late effects. Major research efforts continue using in vitro exposures of human cells or selected assays in animal models as surrogates for man to further define the molecular mechanisms involved in the induction, repair, and expression of chromosome damage resulting from exposure to ionizing radiation and various types of DNA damaging chemicals. Our overall goals are to define the immediate and long-term cytogenetic consequences of human overexposures to genotoxic agents.

Significant research accomplishments during the last year include:

• Long-term follow-up studies in a large cohort of women who received high-dose localized radiotherapy for cancer of the cervix revealed a direct relationship between radiation dose and stable chromosome aberrations detectable in circulating lymphocytes more than 17 years after radiotherapy.

• Cytogenetic evaluations in cultured lymphocytes of three men who sustained severe radiation overexposures in the 1989 San Salvador accident provided evidence that each man received exposures to over 90% of his body and that their doses ranged from >3 to >8 Gy. Evaluations in marrow samples obtained from the most heavily exposed patient provides evidence that, by about four months after exposure, the victim's marrow compartment had been primarily repopulated by a clone derived from a radiation damaged stem cell.

• We continued basic research studies using the human lymphocyte system and dimethyl sulfoxide as a model scavenger of OH radicals to further quantify chromosome damage induced by direct vs indirect mechanisms of radiation action. Chromosome aberrations in lymphocytes exposed to monoenergetic neutrons varied as a function of neutron energy, radiation dose, and presence or absence of radioprotector during radiation. In contrast, no differences in SCE frequencies were noted between any of the irradiated and nonirradiated cultures, which demonstrates that monoenergetic neutrons do not induce DNA lesions that lead to SCEs by either direct or indirect mechanisms.

• Studies of dose response relationships for chromosome aberration induction in human lymphocytes irradiated at cold temperature (4°C) in the presence or absence of DMSO suggest that the dose-modifying effects of cold temperature in reducing dicentric yields are primarily due to reductions in DNA damage mediated by indirect mechanisms.

• Test systems utilizing animal models have been expanded to include developing the capability for conducting micronucleus assays for chemical genotoxicity. This measure of chromosome breakage is accomplished with greater speed than classical aberration scoring and provides improved statistical accuracy since large numbers of cells are evaluated.

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• Collaborative efforts with scientists from Los Alamos National Laboratory have resulted in the development of an in situ hybridization probe for identifying centromeric heterochromatic regions in mouse chromosomes. Although mice are widely used for genotoxicity assays, they are a notoriously difficult species for cytogenetic evaluations due to the terminal location of the centromeric regions of their chromosomes. Our technique produces definitive identification and location of the centromeres and has potential for improving the ease and accuracy of chromosome evaluation in mice.

In continued collaboration with scientists at the Radiation Epidemiology Branch at NCI, we are evaluating the induction and persistence of chromosome aberrations in selected patients who received orthovoltage X-ray vs 14 MeV neutron therapy for various types of tumors. These data will allow us to derive estimates of the relative effectiveness of the two qualities of radiation in inducing chromosome damage in human cells in vivo and provide information on the relative life span of radiation-injured lymphocytes in cohorts of patients receiving the different modalities of therapy. In collaboration with scientists in the Nuclear Medicine program, we are evaluating the relative effectiveness of beta radiation emitted by ^{90}Y in inducing chromosome damage in human cells. Cytogenetic evaluations in persons receiving ^{90}Y-tagged monoclonal antibodies against specific tumors may provide a biological monitor for comparison between treatments. Utilization of the in vivo micronucleus assay will be expanded with special emphasis being placed on further evaluation of the merits of this assay, relative to others presently in use.



1028847

Radiation Emergency Assistance Center/Training Site (REAC/TS)

Program Director: **Robert C. Ricks**



The Radiation Emergency Assistance Center/Training Site (REAC/TS) continued to provide support to the Department of Energy (DOE), Federal Emergency Management Agency (FEMA), World Health Organization (WHO), and the International Atomic Energy Agency (IAEA) in the areas of medical management of radiation accidents as well as assistance in the training of medical and medical support personnel for radiation accident response. The REAC/TS program also provides medical support to the DOE's Accident Response Group (ARG) and the Federal Radiological Monitoring and Assessment Center (FRMAC) and is prepared to support DOE emergency response programs following major radiation accidents at DOE-Defense Programs contractor facilities or commercial nuclear power plants. The REAC/TS emergency response activities continued to include on-site emergency medical assistance and consultation services. These services included the following:

• An experienced emergency response team consisting of physicians, nurses, health physicists, coordinators, and necessary additional support personnel continued on 24-hour call to provide consultation or direct medical and radiological assistance in the event of a major radiation accident. Approximately 60 domestic requests for assistance were received in the past year. Since June 1976, REAC/TS has received over 1,100 calls for assistance, approximately one-half of which required consultation only. REAC/TS emergency response teams also continued to provide direct or consultative support to the WHO, IAEA, and the U.S. Department of State following accidents in foreign countries.

• In February 1989, REAC/TS provided assistance to the IAEA, WHO, and governments of El Salvador and Mexico, following accidental high dose irradiation by an unshielded ^{60}Co source used to sterilize medical equipment in San Salvador, El Salvador. Seven individuals were accidentally irradiated in the accident with three receiving whole body dose equivalents ranging from 3-8 Gy. The three high dose individuals experienced signs and symptoms of the acute radiation syndrome and were transferred to Mexico City, Mexico, for treatment. Two of the three individuals received very high doses to the lower extremities with dose estimates ranging from 200-500 Gy. In addition to on-site advice and consultation, REAC/TS conducted cytogenetic, histopathologic, and biochemical studies in Oak Ridge. All three seriously irradiated individuals recovered from their acute hematological crises, although two later required surgical amputations due to radiation induced tissue injury to the lower extremities. One patient subsequently died as a result of his accidental exposure. REAC/TS staff also provided assistance to the IAEA in generating a formal report regarding the El Salvador radiation accident. The official IAEA report of the Salvadoran accident is currently in press.

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• REAC/TS continued to coordinate the clinical use of DTPA for the evaluation of individuals known or suspected to be internally contaminated with plutonium or americium. Data from the DTPA Accident Registry contain information on approximately 485 individuals in the United States treated with DTPA following internal contamination with transuranics. Approximately 3,100 doses of DTPA have been administered to accidentally contaminated individuals with 55% having received only a single dose of chelator. Plutonium was the most frequently treated transuranic intake, and 70% of the plutonium exposures resulted from inhalation of airborne particles. Less than 3% of individuals treated with DTPA experienced minor transient side effects such as pain at the site of injection, allergic rashes, nausea, or diarrhea. No serious side effects have been reported following DTPA use.

• REAC/TS continues to work with the government of Brazil and otherwise monitor follow-up on the persons involved in the September 1987 cesium-137 accident in Goiania, Brazil.

• In FY 89, REAC/TS conducted an international conference entitled, "The Medical Basis for Radiation Accident Preparedness: II. Clinical Experience and Follow-up Since 1979." Two hundred twenty-five physicians and scientists from around the world attended the international conference. Conference attendees were from the United States and 18 foreign countries, including Brazil, China, Belgium, Australia, England, France, Norway, U.S.S.R., Canada, Holland, Indonesia, Hungary, Denmark, Japan, the Netherlands, Germany, Taiwan, and Argentina. All major radiation accidents worldwide since 1979 were discussed during this second international conference. Of special interest were in-depth discussions of the Chernobyl, U.S.S.R., and Goiania, Brazil, multi-casualty radiation accidents. The international conference marked the first time that Soviet physicians involved in direct patient care following Chernobyl discussed the accident in an open forum in the United States. Conference proceedings are currently in press.

• Through the REAC/TS Registry System, we continue to add to our medical data base of information on radiation accidents. Through our WHO Collaboration Center designation and closer ties with Soviet and Chinese physicians, we anticipate this registry data base will continue to expand and serve as a more thorough information base.

REAC/TS will continue to provide emergency medical response and training for radiation accident management in support of DOE/DOE contractor programs. As a follow-up to recent major radiation accidents, REAC/TS will conduct an International Conference, "Psychological Aspects of Radiation Accidents," in late 1990. In Fiscal Year 1991, REAC/TS will continue to work closely with the World Health Organization (WHO) to provide training and accident response assistance.

Radiopharmaceutical Internal Dose Information Center

Program Director: ***Evelyn E. Watson***

As a result of administrative changes at ORAU, the Radiopharmaceutical Internal Dose Information Center (RIDIC), which was established in 1971, is now a part of the Medical Sciences Division. The center provides radiation dose information to governmental agencies and contractors, nuclear medicine researchers, physicians, and others. In addition, the center's staff conducts research designed to improve internal radiation dosimetry. The radiation dose that a patient receives from a nuclear medicine procedure is critical to the decision of whether a radiopharmaceutical should be made available for routine diagnostic or therapeutic use. For diagnostic nuclear medicine studies, the radiation dose is a factor in evaluating the

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risks versus the potential benefits to be gained from the administration of the radiopharmaceutical. For radioactive materials used for therapy, the radiation dose is an essential component in treatment planning.

Nuclear medicine investigators test many new radiopharmaceuticals each year. These radioactive drugs require estimates of the patient's radiation dose. The internal dose center is the only group in the United States that routinely provides this information to those who need it.

Accomplishments of the center during the past fiscal year are summarized as follows:

• The center responded to more than 500 questions as a result of about 320 calls from nuclear medical centers, nuclear pharmacies, radiopharmaceutical manufacturers, the Department of Energy (DOE), DOE contractors, the Food and Drug Administration, the Nuclear Regulatory Commission, the National Institutes of Health, and others. The number of requests for information about the radiation dose from radiolabeled monoclonal antibodies has increased greatly in recent years.

• A bibliographic data base that is maintained by the center now has about 33,500 entries on information needed for radiation dose calculation, an increase of approximately 1,500 entries during the year.

• Mathematical models of the pregnant woman at the end of the first and third trimesters have been completed and Monte Carlo calculations of the energy absorbed by the fetus from radioactivity in the maternal organs and in the fetus itself have been done.

• Several projects are currently being pursued or will be initiated during the next fiscal year. The center is developing physiologically based pharmacokinetic models for interspecies scaling of radionuclide biodistribution data. Improved compart- [illegible]tal modeling of biological systems for use in radiolabeled [illegible]clonal antibody anticancer therapy is a primary goal of this study.

• Preparations for the Fifth International Symposium c Radiopharmaceutical Dosimetry have been initiated. This symposium, to be held in Oak Ridge, Tennessee, in the spring of 1991, will examine the applicability of traditional and microdosimetry techniques for new nuclear medicine procedures, such as the use of radiolabeled monoclonal antibodies and other radioactive materials for therapy. The proceedings of the symposium will be published to provide a resource to people interested in radiation dosimetry.

• The center will continue to be a source of radiation dose information for investigators, staff of governmental agencies, and others. Additions to the bibliographic data base will continue to be made, and plans include making the data base accessible to other facilities by phone line.

• A mathematical model of the pregnant woman at the end of the second trimester will be developed. This model plus those for the first and third trimesters should provide sufficient absorbed fractions of energy to calculate the radiation dose to the fetus over the course of the pregnancy. The center is also working to improve both the estimates of the energy absorbed by the marrow and the determination of the distribution and retention of radionuclides in the marrow.

In the future, the center plans to expand its services to include information on occupational dose estimation. In the next few years, employers will need to include the internal radiation dose as part of the exposure records of radiation workers. RIDIC's expertise will be valuable to those who have not performed the necessary calculations in the past. A great need exists for a concerted effort to collect and disseminate appropriate data for calculating radiation dose. The center is a logical group to spearhead such an initiative. RIDIC staff would work with research facilities to develop protocols that would result in the collection of data during controlled trials and also routine nuclear medicine procedures. The center would become a repository for the data and make it available to those who need it.

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Microbiology

Program Director: ***Howard I. Adler***

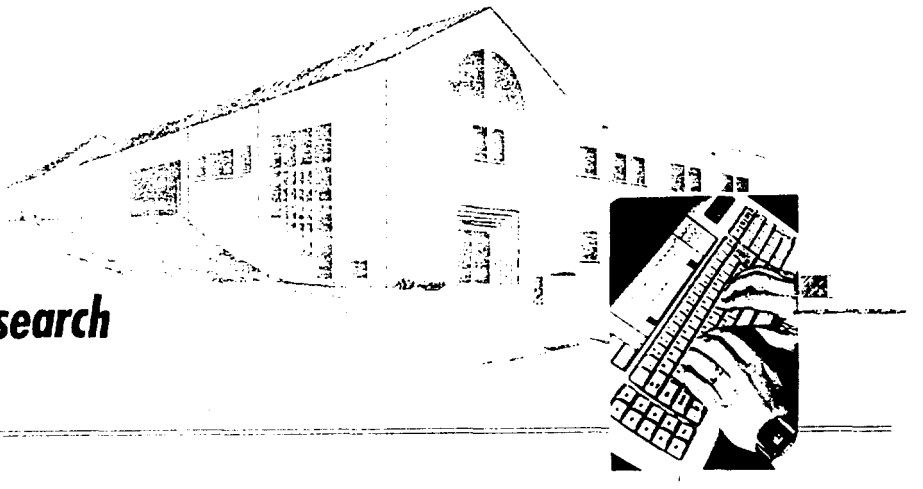
This group investigates the means by which oxygen produces harmful effects in microorganisms. It is well known that oxygen and the reactive species derived from it can interfere with DNA metabolism, enzyme activity, cell growth, and cell replication. Many microorganisms contain enzymes and other chemicals that protect against or repair oxygen-mediated damage, whereas other microorganisms do not. This latter group, known as anaerobes, contains organisms of interest in disease production, degradation of organic wastes, and other important processes. It is anticipated that an increased understanding of the interactions between oxygen and anaerobes will permit us to use these organisms more effectively in the future.

At this time, most of the group's work is being done using an oxygen sensitive mutant of Escherichia coli. This mutant, Oxys-6, was isolated after exposure of Escherichia coli AB1157, a well-characterized K-12 strain, to ultraviolet light. We have previously established that Oxys-6, when incubated aerobically in a complex growth medium, produces an extracellular material that prevents aerobic cell division but does not interfere with anaerobic growth. During the past year we have made progress regarding the nature and production of the material and have further defined the genetic locus responsible.

- The inhibitory material has a molecular weight of approximately 1,000 as judged by diffusion through ultrafilter membranes.
- This material is made by the mutant from some component found in the yeast extract used in the complex growth medium. The other ingredients of the medium (beef extract, peptone, glucose, sodium chloride) are not required.
- The precursor of the inhibitory material is a relatively low molecular weight compound (circa 1,000) that can be found in freshly prepared yeast extract as well as commercially available products.
- The parental organism, AB1157, does not make the inhibitory material under incubation conditions suitable for production by the mutant.
- The ability to grow aerobically can be restored to Oxys-6 by the replacement of a five kilobase DNA section located near 8.5 minutes on the Escherichia coli genomic map.

In the future, we intend to determine the molecular nature of the material that prevents aerobic growth. We will also determine how much of the five kilobase section of DNA is actually required for aerobic growth. Eventually the minimum DNA will be base sequenced, because this should provide important mechanistic information. The group will also initiate related work with other, naturally occurring anaerobes. Particular attention will be paid to organisms that contribute to the degradation of environmental contaminants. The basic information being developed with anaerobic Escherichia coli mutants should allow us to enhance the growth and metabolic activity of other anaerobes.

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Center for Epidemiologic Research

Program Director: ***Shirley A. Fry***

The Epidemiology program combines the science and technologies of epidemiology, biostatistics, health physics, industrial hygiene, and computer sciences in evaluating relationships between protracted exposure to low levels of ionizing radiations from external and internal sources and subsequent health and mortality among nuclear industry workers. The health effects of chemical toxicants also present in the work place are being evaluated as primary exposures and/or potential confounders or modifiers of any radiation effect. Ongoing follow-up studies are based on 11 populations, comprising about 250,000 of the estimated total of 600,000 persons ever employed by DOE and predecessor agencies and their contractors, some time since 1942 at facilities nationwide. By continually monitoring the health and mortality experience of these populations, the studies contribute to assuring occupational health protection of current and future DOE contractor employees and the scientific basis for radiation protection standards.

Related programmatic objectives include (1) developing and applying improved biostatistical methods, including probabilistic models, Poisson regression, and Bayesian mathematics for analyzing complex epidemiologic data involving time dependent factors, (2) advancing methods and technologies for systematically collecting, processing, managing, and analyzing large volumes of data for multiple populations while assuring a high level of quality control, and (3) critically evaluating methods and findings of studies of health effects among other populations exposed to radiation and other industrial toxicants. The program also continues to provide technological support to the DOE epidemiology contract groups at Los Alamos National Laboratory, Hanford Environmental Health Foundation/Pacific Northwest Laboratory, and elsewhere by operating the DOE Death Certificate Retrieval Office as the principal collection point for death data and the coordinating office between all DOE epidemiology groups and Vital Records Registrars in 50 states.

Major research achievements during the past year include the following:

- A study was completed on morbidity among 301 workers with potential for exposure to epoxy resins and solvents in the gas centrifuge process at the Oak Ridge Gaseous Diffusion Plant (ORGDP). The major finding was of five cases of bladder cancer among the centrifuge process workers versus none in the matched comparison group selected from workers at the same plant. Performance in this study clearly demonstrated the capabilities and resources developed by the Center for Epidemiologic Research enabling DOE and its contractors to rapidly respond to workers' health concerns, as well as benefiting workers of morbidity studies with earlier detection of increased disease risks than is possible when mortality is the end point.

- A study was completed as a doctoral thesis in the Department of Epidemiology at the University of North Carolina

1028852

that updated the mortality of uranium processing workers at the Linde Ceramics Plant by incorporating radiation exposure into the analysis. Among workers exposed to the highest level of uranium dust, an excess risk of digestive and respiratory tract cancers was observed. A statistically significant dose-response relationship was found for the former. These results led to the recommendation that case-control studies be undertaken for cancers of these two organ systems.

• Results have been obtained on use of new updated mean skeletal doses for the MIT ^{226}Ra-dial painter cohort in an exploratory cohort analysis of mortality due to osteosarcoma and head cancer. Aalen's nonparametric procedure to estimate the cumulative hazard as a function of mean skeletal dose was used to evaluate the adequacy of a simple exponential model in which the cumulative mean skeletal dose replaces time from first exposure to death due to bone tumor. This data shows that an exponential model is plausible with the new doses.

• Two papers accepted for publication were coauthored by CER researchers and other DOE contract epidemiologists on the results of combined mortality analyses of pooled data for cohorts of Oak Ridge National Laboratory, Rocky Flats, and Hanford workers and the study methodologies. No correlation was found between radiation exposure and all cancer or leukemia mortality; only multiple myeloma among the Hanford population was significantly correlated with radiation dose.

• Two major research support tasks were completed that are expected to provide high quality data for analysis and strengthen the scientific validity of the results of analyses in which they are used. One of the tasks is linking personnel radiation monitoring records with the work history data of individuals presently or formerly employed at DOE's Y-12 (from 1947 only), National Laboratory, or Gaseous Diffusion facilities in Oak Ridge. The second task led to the development of a computer software algorithm that uses vital status information from multiple sources and time periods to determine the most accurate vital status on a given date for individuals in a defined study population.

The ultimate programmatic goal of the Epidemiology program is to combine and analyze data for multiple populations, to the extent it is epidemiologically feasible and valid, in order to maximize the power of analyses to detect untoward effects if they exist and improve the precision of cancer risk estimates for low levels of radiation. Additionally, the data being prepared for these analyses provide a basis for continued surveillance of DOE workers' health and mortality experience and evaluation of the efficacy of occupational protection standards and practices. The data also will contribute to the proposed Comprehensive Epidemiologic Data Resource whereby epidemiologic data compiled by DOE will be made available for independent research projects. Opportunities will continue to be explored to expand the scope of the workers in other industries and to address epidemiologic questions at the molecular level in collaboration with clinicians and experimental scientists.

Center for Personnel Security Assurance, Research and Analysis

Program Director: G. R. Eisele

The Center for Personnel Security Assurance, Research and Analysis (CPSARA), is the Department of Energy's (DOE) Center of Excellence for the Personnel Security Assurance Program (PSAP). This program requires initial screening and

1028853

annual evaluation of employees who occupy certain special positions that are critical to the national security in order to detect the development of conditions that might result in an error in judgment or reliability. The Center provides support to the PSAP program by providing a standard for achieving programmatic consistency, technical assistance, and operational guidance to all aspects of human reliability and by conducting or providing research and analysis in areas related to personnel security assurance.

Significant accomplishments during the past year are listed below.

• The Center developed several training programs designed to prepare DOE and DOE contractor personnel for incorporation into the PSAP. The training programs stress the benefits of PSAP and include such items as clearance criteria, operational definitions, federal regulations, security concerns, program process, substance abuse glossary, and copies of all authorization and consent forms to be used by PSAP personnel.

• Training manuals were developed specifically for instructors who will be training PSAP participants. All training modules incorporate lecture and media-augmented presentations, including videotapes, group interaction, case scenarios, and question and answer sessions. These documents include: Train-the-Trainer Instructor Guide, Train-the-Trainer Student Information Packet, Supervisor Instructor Guide, Supervisor Training and Reference Guide, Employee Instructor Guide, and Employee Training Student Information Packet.

• CPSARA conducted two DOE-sponsored Implementation Planning workshops for 53 DOE facilities and 97 DOE contractors to introduce the training materials and various support documents developed to assist in implementing the program. A PSAP Cost Estimation Model program written for personal computer utilization was demonstrated for use in determining site-specific costs of the program. A subsequent PSAP Train-the-Trainer Workshop was conducted for 48 DOE and DOE contractor personnel.

• To augment the training process CPSARA developed a 15-minute video for employees in PSAP positions that describes various situations which might endanger the national security and demonstrates why this program is needed. The video addresses the "insider risk," a person who has authorized access to a DOE nuclear facility whose judgment and reliability may be impaired due to a number of situations or conditions. This video also can be used as an informational tool for audiences having no knowledge of the Personnel Security Assurance Program.

• In order to measure the success of the PSAP training program, CPSARA has developed a training evaluation component to determine the training program's effectiveness by measuring the following: (1) knowledge of the PSAP, (2) attitudes towards the PSAP, (3) understanding of why the PSAP is being implemented, and (4) understanding of how the PSAP functions. Four assessment instruments, designed to address the above, will be utilized before, during, and after training to determine whether a participant has an overall positive or negative attitude towards basic program concepts and adequate knowledge to understand the components and procedures of the PSAP.

Future CPSARA activities will focus on developing additional resource materials, evaluating legal principles and analyzing legal standards regarding reliability programs and their components, training personnel in the detection of unusual behavior in the work place, and publishing a newsletter for dissemination of information to DOE facilities affected by the PSAP. The Center will continually address the needs for research and analysis in personnel security. This will involve analyzing existing research in human reliability and reviewing new methodologies used to screen applicants for high risk or sensitive positions and their possible application to the evaluation of present adjudication processes relating to personnel security activities.

1028854

Publications, Abstracts, and Invited Lectures



Full-length Journal Articles

Blank, M. L., Smith, Z. L., Cress, E. A., and Snyder, F.
Characterization of the enzymatic hydrolysis of acetate from alkylacetylglycerols in the de novo pathway of PAF biosynthesis
Biochem. Biophys. Acta (in press)

Blank, M. L., Smith, Z. L., Lee, Y. J., and Snyder, F.
Effects of eicosapentaenoic and docosahexaenoic acid supplements on phospholipid composition and plasmalogen biosynthesis in P388D1 cells
Arch. Biochem. Biophys. 269, 603-611, 1989

Burson, Z. and Lushbaugh, C. C.
The (1983) Ciudad Juarez, Mexico, ^{60}Co Accident
In *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up Since 1979*. Elsevier Science Publishers, Inc., New York (in press)

Cornforth, M. N., Meyne, J., Littlefield, L. G., Bailey, S. M., and Moyzis, R. K.
Telomere staining of human chromosomes and the mechanism of radiation-induced dicentric formation
Radiat. Res. (in press)

Frome, E. L., and Morris, M. D.
Evaluating goodness of fit of Poisson regression models in cohort studies
Am. Statis. (in press)

Gilbert, E. S., Fry, S. A., Wiggs, L. D., Voelz, G. L., Cragle, D. L., and Petersen, G. R.
Analyses of combined data on workers at the Hanford Site, Oak Ridge National Laboratory, and Rocky Flats Nuclear Weapons Plant
Radiat. Res. 120, 19-35, 1989

Gilbert, E. S., Fry, S. A., Wiggs, L. D., Voelz, G. L., Cragle, D. L., and Petersen, G. R.
Methods for analyzing combined data from studies of workers exposed to low doses of radiation
Am. J. Epid. (in press)

Kasama, K., Blank, M. L., and Snyder, F.
Identification of 1-alkyl-2-acyl-3-(2',3'-diacylglycerol)-glycerols, a new type of lipid class in Harderian gland tumors of mice
J. Biol. Chem. 264, 9453-9461, 1989

Kleinerman, R. A., Littlefield, L. G., Machado, S. G., Blettner, M., Peters, L. J., and Boice, J. D., Jr.
Chromosome aberrations in peripheral lymphocytes and radiation dose to active bone marrow in patients treated for cancer of the cervix *Radiat. Res.* 119, 176-190, 1989

Lee, T-c., Malone, B., Woodard, D., and Snyder, F.
Renal necrosis and the involvement of a single enzyme of the de novo pathway for the biosynthesis of platelet-activating factor in rat kidney inner medulla
Biochem. Biophys. Res. Commun. 163, 1002-1005, 1989

1028855

Lee, Y-C. C., Lawless, D., Crook, J. E., and Clapp, N. K.
Analysis of T lymphocyte subsets in tamarins with colitis and colon cancer
Amer. J. Med. Sci. 296, 118-122, 1989

Littlefield, L. G., Sayer, A. M., and Frome, E. L.
Comparisons of dose response parameters for radiation-induced acentric fragments and micronuclei observed in cytokinesis-arrested lymphocytes
Mutagenesis. 4, 265-270, 1989

McFee, A. F.
Genotoxic potency of three quinoline compounds evaluated in vivo in mouse marrow cells
Environ. and Molecu. Mutagen. 13, 325-331, 1989

McFee, A. F., Jauhar, P. P., Lowe, K. W., MacGregor, J. T., Wehr, C. M., and Shelby, M. D.
ssays of three carcinogen/non-carcinogen chemical pairs for in vivo induction of chromosome aberrations, sister chromatid exchanges, and micronuclei
Environ. Molecu. Mutagen. (in press)

McFee, A. F., and Lowe, K.W.
Caprolactam and benzoin: Test for induction of chromosome aberrations and SCEs in mouse bone marrow
Mutat. Res. (in press)

Meyne, J., Littlefield, L. G., and Moyzis, R. K.
Labeling of human centromeres using an alphoid DNA consensus sequence: Application to the scoring of chromosome aberrations
Mutat. Res. 226:75-79, 1989

Murphy, P. A., Beasley, C. W., Moore, W. H., and Stabin, M. G.
Thallium-201 in human milk: observations and radiological consequences
Health Phys. 56 (4), 539-541, 1989

Powers, W. J., Stabin, M. G., Howse, D., Eichling, J. O. and Herscovitch, P.
Radiation absorbed dose estimates for oxygen-15 radiopharmaceuticals ($H_2{}^{15}O$, $C^{15}O$, $O^{15}O$) in newborn infants
J. Nucl. Med. 29:1961-1970, 1988

Qian, C., Lee, T-c., and Snyder, F.
Metabolism of platelet activating factor (PAF) and related ether lipids by neonatal rat myocytes
J. Lipid Med. 1, 113-123, 1989

Record, M., and Snyder, F.
Intracellular location of acetyltransferase in the remodeling pathway of PAF biosynthesis in undifferentiated human leukemic cells
J. Lipid Med. (in press)

Ricks, R. C.
EMS response to radiation accidents
Emergency Magazine February 1989, 28

Snyder, F., Lee, T-c., and Blank, M. L.
Platelet activating factor and related ether lipid mediators: Biological activities, metabolism, and regulation
Annals of the New York Acad. Sci. (in press)

Stabin, M. G.
Radiation dosimetry in radionuclide hysterosalpingography with Tc-99m
J. Nucl. Med. 30 (3), 415-416, 1989

Tice, R. R., Ormiston, B. G., and McFee, A. F.
The effect of agent dose and treatment time on the intercellular distribution of sister chromatid exchanges induced by genotoxic agents in mouse bone marrow cells in vivo
Mutat. Res. (in press)

1028856

Vallabhajosula, S., Zimmerman, R. E., Picard, M., Stritzke, P., Mena, I., Stabin, M.G., Morgan, R.A., and Goldsmith, S.J.
Technetium-99m ECD: A new brain imaging agent: In vivo kinetics and biodistribution studies in normal human subjects
J. Nucl. Med. 30, 599-604, 1989

Vallari, D. S., Austinhirst, R., and Snyder, F.
Development of specific functionally-active receptors for platelet-activating factor in HL-60 cells following granulocytic differentiation
J. Biol. Chem. (in press)

Vallari, D. S., Record, M., Smith, Z., and Snyder, F.
O-Alkyl-O-methylglycerophosphocholine, an antineoplastic lipid, undergoes spontaneous redistribution between biological membranes prepared from HL-60 cells
Biochim. Biophys. Acta (in press)

Vallari, D. S., Record, M., and Snyder, F.
Conversion of alkylacetylglycerol to platelet-activating factor in HL-60 cells: the subcellular localization of the mediator
Arch. Biochem. Biophys. (in press)

Washburn, L. C., Sun, T. T. H., Byrd, B. L., and Crook, J. E.
Synthesis of [^{18}F]7-fluoroheptylamine and tissue distribution studies in rats
J. Labelled Compd. Radiopharm. 26:219-221, 1989

Weber, D. A., Makler, P. T., Jr., Watson, E. E., Coffey, J. L., Thomas, S. R., and London, J.
Radiation absorbed dose from Tc-99m labeled bone imaging agents. MIRD Dose Estimate Report No. 13
J. Nucl. Med. 30, 1117-1122, 1989

Submitted Papers

Dobson, R. L., Straume, T., Carrano, A. V., Minkler, J. L., Deaven, L. M., Littlefield, L. G., and Awa, A. A.
Biological effectiveness of neutrons from Hiroshima bomb replica: Results of a collaborative cytogenetic study
Radiat. Res.

McFee, A. F., and Tice, R. R.
Influence of treatment-to-sacrifice time and the presence of BrdUrd on chemically-induced aberration rates in mouse marrow cells
Mutat. Res.

Suga, K., Kawasaki, T., Blank, M. L., and Snyder, F.
An arachidonoyl (polyenoic)-specific phospholipase A_2 (PLA_2) activity regulates the synthesis of platelet activating factor (PAF) in granulocytic HL-60 cells
J. Biol. Chem.

Zuoyuan, W., Boice, J. D., Jr., Luxin, W., Beebe, G. W., Yongru, Z., Kaplan, M. M., Zufan, T., Maxon, H. R., III, Shouzhi, Z., Schneider, A. B., Bingde, T., Wesseler, T. A., Deqing, C., Ershow, A. G., Kleinerman, R. A., Littlefield, L. G., and Preston, D.
Thyroid nodularity and chromosome aberrations among women in areas of high background radiation in China
JNCI.

1028857

Chapters, Review Articles, Government Reports, and Other Publications

Adler, H. I.
The Use of Microbial Membranes to Achieve Anaerobiosis. C.R.C. Press (in press)

Fry, S. A., and Littlefield, L. G.
Cytogenetic studies in cultured lymphocytes of Y-12 and UT-AEC accident victims
In *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up Since 1979.* Elsevier Science Publishers, Inc., New York (in press)

Lee, T-c., Malone, B., and Snyder, F.
Comparative studies on the biosynthesis of platelet activating factor through de novo pathway in rat kidneys and rabbit platelets
n *Proceedings of the Taipei Satellite Symposium on Platelet Activating Factor* (Hicks, N., ed.), *Excerpta Medica*, Hong Kong (in press)

Lee, T-c., and Snyder, F.
Overview of PAF biosynthesis and catabolism
In *Platelet Activating Factor and Human Disease* (Barnes, P.S., Page, C.P., and Henson, P.M., eds.) 1-22. Blackwell, Oxford. 1989

Littlefield, L. G., Joiner, E. E., and Hubner, K. F.
Cytogenetic techniques in biological dosimetry: Overview and example of dose estimation in 10 persons exposed to gamma radiation in the 1984 Mexican ^{60}Co accident
In *Medical Management of Radiation Accidents*, CRC Press, Inc., Boca Raton (in press)

Littlefield, L. G., and Lushbaugh, C. C.
Cytogenetic dosimetry for radiation accident victims– "The good, the bad, and the ugly"
In *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up Since 1979.* Elsevier Science Publishers, Inc., New York (in press)

Lushbaugh, C. C.
Radiation Accidents. Part I. Review
Ala. J. Med. Sci. 25(4), 460-465, 1988

Lushbaugh, C. C.
Radiation Accidents. Part II. Triage
Ala. J. Med. Sci. 25(4), 466-469, 1988

Lushbaugh, C. C.
Radiation Accidents World-Wide: An Additional Decade of Experience
In *The Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up Since 1979.* Elsevier Science Publishers, Inc., New York (in press)

Lushbaugh, C. C., Ricks, R. C., and Fry, S. A.
Radiological Accidents – An Historical Review of Sealed Sources Accidents
In *Proceedings of IAEA International Conference on Radiation Protection in Nuclear Energy*, Sydney, Australia, October 1988

Mettler, F. A., Kelsey, C. A., and Ricks, R. C. (eds.)
Medical Management of Radiation Accidents. CRC Press, Inc., Boca Raton, (in press)

Ostrouchov, G., and Frome, E. L.
A model search procedure for hierarchical log-linear models
In *1989 Proceedings of the Statistical Computing Section of the American Statistical Association* (in press)

1028858

[illegible], R. C., and Fry, S. A. (eds.)
[illegible] Medical Basis for Radiation Accident Preparedness II: Clinical Experience and Follow-up Since 1979. Elsevier Science Publishers, Inc., New York (in press)

Snyder, F.
Biochemistry of platelet-activating factor
In Perspectives in Asthma, Vol. 3, PAF in Asthma, (Holme, G. and Morley, J., eds.), pp. 21-33, Academic Press, New York, 1989

Snyder, F.
Biochemistry of platelet-activating factor: A unique class of biologically active phospholipids
Proc. Soc. Exp. Biol. Med. 190, 125-135, 1989.

Snyder, F., Lee, T-c., and Blank, M. L.
Metabolism of ether analogues of phosphatidylcholine, including platelet activating factor
In *Phosphatidylcholine Metabolism* (Vance, Dennis E., ed.), pp. 143-164, CRC Press, Inc., Boca Raton, Florida, 1989

[illegible]der, F., Lee, T-c., and Blank, M. L.
[illegible]abolism and function of bioactive ether lipids
In *Neurochemical Aspects of Phospholipid Metabolism* (Freysz, L., Hawthorne, J.N., and Toffano, G., eds.), pp. 151-160, Springer-Verlag, New York, 1989

Snyder, F., Vallari, D. S., Smith, Z. L., and Blank, M. L.
Membrane-targeted biochemical effects and the role of cellular differentiation in the selective antitumor actions of alkylmethoxyglycerophosphocholine
In *Prostaglandins, Leukotrienes, and Cancer, 1st International Conference on Eicosanoids and Bioactive Lipids in Cancer and Radiation Injury,* Kluewer Academic Publishing Co. (in press)

Watson E. E.
Macro vs. Microdosimetry
In [Proceedings] *Biology of Radionuclide Therapy,* American College of Nuclear Physicians, pp. 196-215, 1989

Abstracts, Oral Presentations, and/or Posters

Abbott, M. G., and McFee, A. F.
In vivo genotoxicity of chemicals when administered in different solvent/carriers
Environ. Molecu. Mutagen. 14(Suppl. 15):4, 1989

Adler, H. I.
Oxygen sensitivity of an *Escherichia coli* mutant
Abstracts of the 89th Annual Meeting of the American Society for Microbiology, New Orleans, Louisiana, May 14-18, 1989

Blank, M. L., Smith, Z. L., Lee, Y. J., and Snyder, F.
Effects of eicosapentaenoic and docosahexaenoic acid supplements on phospholipid composition and plasmalogen biosynthesis in P388D$_1$ cells
FASEB J. 3, A705 (Abstr. 2774), 1989

Blank, M. L., Smith, Z. L., Lee, J., and Snyder, F.
Effect of eicosapentaenoic and docosahexaenoic acid supplements on phospholipid composition and plasmalogen biosynthesis in P388D$_1$ cells
Presented at the XXIII Annual Regional Lipid Conference, Cashiers, North Carolina, 1988

1028859

Bushe, H. S., Leppo, J. A., Eckerman, K., Stabin, M., and Brill, A. B.
Radiation dosimetry and pharmacokinetics of In-111 antimyosin.
J. Nucl. Med. 30 (5), 832, 1989

Crook, J. E.
Effect of a calcium channel blocker, verapamil on the tissue distribution of a radiolabeled monoclonal antibody
In *Abstracts, Fourth International Symposium on Calcium Antagonists: Pharmacology and Clinical Research*, Florence, Italy, p. 53, 1989

Crook, J. E., Washburn, L. C., Lee, Y-C. C., Byrd, B. L., Sun T. T. H., and Steplewski, Z.
Tissue distribution of ^{90}Y-labeled monoclonal antibody CO17-1A in young and old athymic mice
In *Abstracts, Fourth International Conference on Monoclonal Antibody Immunoconjugates for Cancer*, San Diego, California, p. 127, 1989

Crook, J. E., Washburn, L. C., Lee, Y-C. C., Sun, T. T. H., Byrd, B. L., Holloway, E. C., Clapp, N. K., and Steplewski, Z.
Imaging and radiolabeling studies of tamarin anticolon carcinoma monoclonal antibody BR55-2 with indium-111
In *Proceedings, Fourth Asia and Oceania Congress of Nuclear Medicine*, Taipei, Taiwan, R.O.C., pp. 260-261, 1988

Eckerman, K. F., Ryman, J. C., Watson, E. E., and Stabin, M. G.
Use of patient-specific kinetics and S-values in radioimmunotherapy dosimetry
Current Clinical Role of Nuclear Medicine: Comparison with Other Modalities (McCartney, W.H., ed.), p. 4, Southeastern Chapter of the Society of Nuclear Medicine, 1988

Gray, J. W., Lucas, J., Straume, T., Littlefield, L. G., Tenjin, T., and Pinkel, D.
Human chromosome translocation analysis using fluorescence *in situ* hybridization with chromosome-specific probes
In *Abstracts of Papers for Thirty-Seventh Annual Meeting of the Radiation Research Society*, p. 156, Seattle, Washington, 1989

Joiner, E. E., Littlefield, L. G., Colyer, S. P., and Frome, E. L.
Temperature effects on radiation-induced chromosomal aberrations in the presence or absence of dimethyl sulfoxide
Environ. Molecu. Mutagen. 14 (Suppl. 15), 96, 1989

Lawless, D., Lee, Y-C. C., Crook, J. E., and Clapp, N. K.
Autoantibodies in the diagnosis of colon cancer
Immunol. Invest. 18, 617, 1989

Lee, Y-C. C., Crook, J. E., Clapp, N. K., Lawless, B. D., Fuhr, J. E., and Steplewski, Z.
Immunodiagnosis of tamarin colon cancer
In *Proceedings, Fourth Asia and Oceania Congress of Nuclear Medicine*, Taipei, Taiwan, R.O.C., pp. 224-225, 1988

Lee, Y-C. C., Lawless, D., Crook, J. E., and Clapp, N. K.
A murine immunoglobulin-G for simian colon cancer
Immunol. Invest. 18, 618, 1989

Lee, Y-C.C., Washburn, L. C., Sun, T. T. H., Byrd, B. L., Crook, J. E., and Steplewski, Z.
Experimental radioimmunotherapy of human colorectal carcinoma xenografts using Y-90 CO17-1A with unlabeled CO17-1A or mouse IgG as coinjection
In *Abstracts, Society of Nuclear Medicine, 36th Annual Meeting*, June 1989

1028860

Lee, Y-C. C., Washburn, L. C., Sun, T. T .H., Byrd, B. L., ook, J. E., and Steplewski, Z.
.adiotherapy of human colorectal carcinoma xenografts using Y-90-labeled CO17-1A
In [Proceedings] *Fourth International Conference on Monoclonal Antibody Immunoconjugates for Cancer*, San Diego, California, p. 132, 1989

Littlefield, L. G., and Frome, E. L.
Cytogenetic indices of direct vs indirect radiation action
To be presented at UCLA Symposia on Molecular and Cellular Biology: Ionizing Radiation Damage to DNA Molecular Approaches, Lake Tahoe, California, January 1990

Littlefield, L. G., Frome, E. L., Joiner, E. E., and Colyer, S. P.
Lack of induction of SCEs in human lymphocytes exposed to monoenergetic neutrons
Environ. Molecu. Mutagen. 14 (Suppl. 15), 116, 1989

Littlefield, L. G., Frome, E. L., Joiner, E. E., and Colyer, S. P.
LET dependency for radioprotection of chromosome aberrations by DMSO
bstracts of Papers for Thirty-Seventh Annual Meeting of the Radiation Research Society, p. 200, Seattle, Washington, 1989

McFee, A. F., and Lowe, K. W.
Cytogenetic evaluation of the in vivo genotoxicity of three quinoline compounds
Environ. Molecu. Mutagen. 14 (Suppl. 15) 363, 1989

Richardson, V. B., Dunmore, T., Littlefield, L. G., and Sayer, A. M.
Chromosome abnormalities in MTS-arrested human leukocytes exposed to therapeutic imidates and X-irradiation
Proceedings of the Seventeenth NIH-MBRS Symposium, p. 21, 1989

Richardson, V. B., Littlefield, L. G., and Colyer, S. P.
Inhibition of cellular proliferation and induction of abnormal metaphase configurations in human lymphocyte cultures exposed to therapeutic alkyl imidates
Presented at ASCB Summer Research Conference: Chromosome Structure and Segregation, Airlie, Virginia, 1989

Robertson, S. D. and McFee, A. F.
Cytogenetic evaluation of the in vivo genotoxicity of superlethal doses of dioxin
Environ. Molecu. Mutagen. 14 (Suppl. 15), 477, 1989

Snyder, F.
Platelet activating factor and related acetylated ether phospholipids as diverse cellular mediators
Presented at Western Carolina University, Cullowhee, North Carolina, 1989

Suga, K., Kawasaki, T., Blank, M. L., and Snyder, F.
The effect of arachidonate and differentiation on PAF production in HL-60 cells
Presented at the XXIII Annual Regional Lipid Conference, Cashiers, North Carolina, 1988

Suga, K., Kawasaki, T., Blank, M. L., and Snyder, F.
Phospholipase A2 (PLA2) regulates the increase of platelet activating factor (PAF) synthesis in arachidonic acid (AA)-supplemented differentiated HL-60 cells
FASEB J. 3, A1224 (Abstr. 5974), 1989

Sun, T. T. H., Washburn, L. C., Lee, Y-C. C., Byrd, B. L., Crook, J. E., and Steplewski, Z.
Effect of carrier IgG on biodistribution of Y-90 labeled monoclonal antibody Co17-1A in tumor-bearing nude mice
J. Nucl. Med. (in press)

1028861

Vallari, D. S., Austinhirst, R., and Snyder, F.
Identification of platelet-activating factor receptors in HL-60 cells following granulocytic differentation
FASEB J. 3. A1223 (Abstr. 5793). 1989

Vallari, D. S., Smith, Z. L., and Snyder, F.
HL-60 cells become resistant towards antitumor ether-linked phospholipids following differentation into a granulocytic form
Presented at the XXIII Annual Regional Lipid Conference. Cashiers, North Carolina. 1988

Washburn, L. C., Sun, T. T. H., Lee, Y-C. C., Byrd, B. L., Crook, J. E., Stabin, M. G., Gansow, O. A., Brechbiel, M. W., and Steplewski, Z.
Comparison of five bifunctional chelate techniques for radiolabeling monoclonal antibody CO17-1A with Y-90
J. Nucl. Med. 30 (5). 763. 1989

shburn, L. C., Sun, T. T. H., Lee, Y-C. C., Byrd, B. L., Crook, J. E., Stabin, M. G., Gansow, O. A., Brechbiel, M. W., and Steplewski, Z.
Comparison of Five Bifunctional Chelate Techniques for Radiolabeling Monoclonal Antibody CO17-1A with Y-90
Presented at the Society of Nuclear Medicine 36th Annual Meeting, June 1989

Invited Lectures at National and International Conferences

Lushbaugh, C. C.
An Assessment of Therapy of Chernobyl Victims
Presented at Technico Southwest, Inc./Defense Nuclear Agency Symposium on "The Effects of Combined Injury on Mortality," January 1989

Lushbaugh, C. C.
Causes of Death in the Recent Radiation Accidents in Chernobyl and Brazil
Presented at American Registry of Pathology/Armed Forces Institute of Pathology Seminar on The Pathological Effects of Radiation, Bethesda, Maryland, September 11-13, 1989

Lushbaugh, C. C.
Consensus and Confusion in Radiation Dosages
Presented at the First Consensus Development Conference on The Treatment of Radiation Injuries sponsored by the Medical Radiobiology Advisory Team (MRAT) of the Armed Forces Radiobiology Research Institute (AFRRI), May 1989

Lushbaugh, C. C.
The Role of Forensic Pathology in the SL-1 Reactor Accident
Presented at American Registry of Pathology/Armed Forces Institute of Pathology Seminar on The Pathological Effects of Radiation, Bethesda, Maryland, September 11-13, 1989

Lushbaugh, C. C., and Fry, S. A.
Classification and Incidence of Radiation Accidents
Presented at IBC Technical Services Ltd. Symposium, "The Control and Management of Accidental Radioative Releases to the Environment," London, November 1988

Lushbaugh, C. C., Fry, S. A., Sipe, A., and Ricks, R. C.
An Historical Perspective of Human Involvement in Radiation Accidents
Presented at Annual Meeting of the National Council on Radiation Protection and Measurements, April 1989

Snyder, F.
Platelet Activating Factor and Related Ether Lipid Mediators: Biological Activities, Metabolism, and Regulation
Presented at the National Institutes of Health meeting on Calcium, Membranes, Aging, and Alzheimer's Disease, Irvine, California, 1988

1028862

Snyder, F., Lee, T-c., Blank, M. L., Suga, K., and Malone, B.
Regulatory Features of PAF Metabolism and Hypersensitivity Responses
Presented at the XIV Congress of the European Academy of Allegology and Clinical Immunology, Berlin, West Germany, 1989

Snyder, F., Suga, K., Kawasaki, T., and Blank, M. L.
Metabolic Coupling of PAF and Polyenoic Acids
Presented at the 3rd International Conference on Platelet-Activating Factor and Structurally Related Ether Lipids, Tokyo, Japan, 1989

Stabin, M. G.
Radiolabeled Antibody Bone Marrow Dosimetry: Calculation Methods I
Presented at the symposium on Radiolabeled Antibody Bone Marrow Dosimetry, held at the annual meeting of the American Society of Therapeutic Radiologists, New Orleans, Louisiana, October 9, 1988. (Proceedings to be published in Antibody, Immunoconjugates and Radiopharmaceuticals)

Stabin, M. G.
Workshop on MIRD Technique
Presented at the Frontiers in Nuclear Medicine Joint Symposium on Dosimetry of Administered Radionuclides, Washington, D.C., September 21-22, 1989 (Proceedings to be published by American College of Nuclear Physicians)

Watson, E. E.
Mathematical Models of the Pregnant Woman at the End of the First and Third Trimesters
Presented at the Frontiers in Nuclear Medicine Joint Symposium on Dosimetry of Administered Radionuclides, Washington, D.C., September 21-22, 1989 (Proceedings to be published by American College of Nuclear Physicians)

Watson, E. E.
Radiolabeled Antibody Bone Marrow Dosimetry: Calculation Methods IV
Presented at the symposium on Radiolabeled Antibody Bone Marrow Dosimetry, held at the annual meeting of the American Society of Therapeutic Radiologists, New Orleans, Louisiana, October 9, 1988 (Proceedings to be published in Antibody, Immunoconjugates and Radiopharmaceuticals)

Collaborative Research

H. I. Adler

Research on the characterization of DNA responsible for the ability to grow aerobically carried out in collaboration with Richard Mural, Biology Division, Oak Ridge National Laboratory.

Study of anaerobic bacteria isolated from waste disposal sites carried out with students and faculty at Knoxville College (Knoxville, Tennessee).

J. E. Crook

Phase I/II clinical investigation of ^{90}Y-labeled monoclonal antibody CO17-1A in patients with disseminated colorectal carcinoma. Thompson Cancer Survival Center (Knoxville, Tennessee). J. J. Costanzi

1028863

Development of software for quantitative positron tomography. EG&G ORTEC (Oak Ridge, Tennessee), R. M. Keyser

Collaborative studies in radiopharmaceutical development. Oak Ridge National Laboratory, Health and Safety Research Division, F. F. Knapp, Jr.

Use of radiopharmaceuticals in animal models of human disease. The University of Tennessee (Knoxville, Tennessee), College of Veterinary Medicine, R. E. Gompf

Development of imaging techniques for positron emission tomography. Computer Technology and Imaging, Inc. (Knoxville, Tennessee), M. Andreaco

Evaluation of an 8 MeV proton cyclotron as a source of radionuclides for positron emission tomography. ORBIT, Inc. (Oak Ridge, Tennessee), J. H. Neiler

L. G. Littlefield

Studies on radiation-induced chromosome damage in humans. National Cancer Institute, Radiation Epidemiology Branch (Bethesda, Maryland), R. Kleinerman, John Boice

Evaluations of radiation-induced lesions in human lymphocytes exposed to low energy fission spectrum neutrons. Lawrence Livermore National Laboratory (Livermore, California), L. Dobson, T. Straume, A. Carrano, and J. Minkler; Los Alamos National Laboratory (Los Alamos, New Mexico), L. Devan; Radiation Effects Research Foundation (Hiroshima, Japan), A. Awa

Coordinated research program on Routine Use of Chromosomal Aberration Analysis in Occupationally Exposed Radiation Workers. International Atomic Energy Agency (Vienna, Austria), A. Bianco

The effect of methyl acetimidate on cellular proliferation and chromosome structure. Tuskegee University (Alabama), Velma B. Richardson

Application of centromere specific-DNA probes in radiation cytogenetics. Los Alamos National Laboratory (New Mexico), Dr. Julianne Meyne

Persistent radiation-induced aberrations in lymphocytes of Y-12 accident survivors – Comparisons of chromosome painting and classical scoring methods. Lawrence Livermore National Laboratory (California), Dr. Tore L. Straume

C. C. Lushbaugh and L. C. Washburn

Diethylenetriaminepentaacetate (DTPA) project. Department of Energy contractors, 45 co-investigators

A. F. McFee

In vivo mouse assay for chemically induced SCE and chromosomal aberrations. Brookhaven National Laboratory (New York), Dr. Raymond Tice

Development of an in situ hybridization probe for identification of mouse centromeric heterochromatic regions. Los Alamos National Laboratory, Dr. Julianne Meyne

1028864

. Snyder

Biological effects of unique phospholipid analogs. California State University (Northridge, California), Department of Chemistry, J. Hajdu

Synthesis of lipid analogs and their precursors. University of North Carolina (Chapel Hill, North Carolina), Department of Medicinal Chemistry, C. Piantadosi

Biochemical studies of analogs of platelet-activating factor. Queens College of the City University of New York (Flushing, New York), Department of Chemistry, R. Bittman

Blood pressure and selective antitumor activities of structural analogs of platelet-activating factor. University of Ottawa (Canada), Biochemistry Department, Morris Kates

Platelet-activating factor and other bioactive lipid in infants with respiratory distress syndrome and related disease. University of Tennessee, Department of Pediatrics, Vichien Lorch and Mark Saylor

L. C. Washburn

Radiolabeled monoclonal antibodies for cancer diagnosis and therapy. The Wistar Institute (Philadelphia, Pennsylvania), Z. Steplewski

Colony stimulating factors as adjuncts to radioimmunotherapy with ^{90}Y-labeled monoclonal antibodies. Armed Forces Radiobiology Research Institute (Bethesda, Maryland), T. J. MacVittie and M. L. Patchen

New bifunctional chelate techniques for radiolabeling monoclonal antibodies with metal ion radionuclides. National Cancer Institute (Bethesda, Maryland), O. A. Gansow and M. W. Brechbiel, and Albert Einstein College of Medicine (New York, New York), K. K. Bhargava

Pharmacological studies using positron emission tomography. University of Kentucky (Lexington, Kentucky), G. Digenis

Miniature thermoluminescent dosimeters for measuring the radiation dosimetry of ^{90}Y-labeled monoclonal antibodies. George Washington University Medical Center (Washington, D.C.), B. W. Wessels

E. E. Watson and M. Stabin

Development of mathematical models of the pregnant woman at the end of the first, second, and third trimesters. Oak Ridge National Laboratory, Metabolism and Internal Dosimetry Group

Training Programs

Training programs at the Medical Sciences Division involve postdoctoral and predoctoral fellows, visiting scientists, college undergraduate students, university faculty, and high school vocational trainees. In addition, special short-term courses conducted by REAC/TS provide training for medical personnel and attorneys in matters related to nuclear accidents and associated radiation health problems.

1028865

Graduate students doing Ph.D. dissertation research in our division generally are affiliated with ORAU member universities. To receive the Ph.D., students must meet all of the academic requirements for the degree imposed by their own universities. Financial support for graduate studies has generally been available from the National Cancer Institute Training Grant administered by the Oak Ridge National Laboratory, the Science/Engineering Education Division at ORAU (sponsored by DOE), and individual research grants.

Postdoctoral training continues to be an important part of the division's research efforts. Over the years funds for support of postdoctoral fellows have been available from a variety of sources: Damon Runyon-Walter Winchell Cancer Fund, Andrew W. Mellon Foundation, National Cancer Institute Training Grant (at Oak Ridge National Laboratory), National Institutes of Health, American Cancer Society, the World Health Organization (International Agency for Research on Cancer), the Fogarty International Center (U.S. Public Health Service), and Medical Sciences Division funds.

Summer research opportunities enable faculty and undergraduate students from colleges and universities throughout the United States to participate in our division's research programs. The DOE-sponsored summer program is administered through the Science/Engineering Education Division at ORAU.

Visiting scientists from various universities and other U.S. and foreign institutions also participate in division research programs. Some of these scientists are sponsored through the Research Participation program administered by ORAU's Science/Engineering Education Division. In some instances, funds for visiting scientists are awarded through national or international agencies, and, occasionally, appointments are made directly from programmatic funds.

Physicians, health physicists, paramedical personnel, and attorneys attend short-term courses in the management of medical problems associated with radiation accidents offered at regular intervals by REAC/TS. Invited lecturers from throughout the United States augment the ORAU teaching staff in this program. A total of 11 REAC/TS courses were given during the past year.

During FY 89, 180 participants received instruction in one of four different training courses offered in the REAC/TS facility. Included in this number were 60 physicians, 52 nurses, 34 health physicists, and 34 others. One hundred twenty-three courses have been offered at REAC/TS with 2,387 health professionals trained. The Federal Emergency Management Agency (FEMA) continues to provide the primary support for these radiation accident management training courses. A new course, entitled "Occupational Health in Nuclear Facilities," was designed and implemented in FY 89. The course was extremely successful, and we anticipate offering the course two or three times beginning in FY 91.

The REAC/TS training programs were also available for off-site presentation, and training assistance was provided to the American Medical Association, the American College of Emergency Physicians, the American Association of Critical Care Nurses, as well as numerous state, regional, or national medical/health physics associations. In FY 89, REAC/TS responded to a request from the Westinghouse Corporation to develop a hospital-based training course in support of the Waste Isolation Pilot Plant (WIPP) in Carlsbad, New Mexico. A one-day course was developed and scheduled for presentation at selected hospitals in Idaho, Utah, Wyoming, Colorado, and New Mexico. Completion date for this scheduled training is FY 90.

1028866

Several members of the senior staff conduct special courses and provide specific lectures at the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences and at the University of Tennessee (Knoxville, Tennessee). These scientists usually hold joint appointments where they teach; some also advise graduate students who pursue their research studies at ORAU.

The staff of the Radiopharmaceutical Internal Dose Information Center participates in the ORAU Professional Training and REAC/TS training activities by lecturing on various topics related to radiation dose, radiation risk, medical uses of radionuclides, etc. In addition, during the past fiscal year, the center's staff conducted a three-day course on Radiation Dose Calculational Techniques.

The individuals listed below participated in the training programs at the division during the past year:

Predoctoral Fellow

Latresia A. Wilson (RIDIC)
University of Florida

Postdoctoral Fellows

Hsin-Shan Julia Ju (Nuclear Medicine)

Yu-Chen C. Lee (Nuclear Medicine)

Kazuhiro Suga (Biochemistry)

David S. Vallari (Biochemistry)

Visiting Scientists

Dr. Velma Richardson (Cytogenetics) Tuskegee University, Tuskegee, Alabama

Daniel Billen (Microbiology) University of Tennessee

Dennis Duggan (Microbiology) Department of Microbiology, University of Florida, Gainesville

Major Awards, Honors, and Current Appointments

D. Cragle

Member of the International Nickel Speciation Research Project Steering Committee

Consultant to the Thompson Cancer Survival Center

S. A. Fry

Member of the National Council on Radiation Protection and Measurements Scientific Committee's Task Force on Uranium (SC57-15)

P. G. Groer

Consultant to the National Council on Radiation Protection and Measurement (NCRP), Committee on Radiation Exposure and Potentially Related Injury

1028867

L. G. Littlefield

Member, National Council for Radiation Protection, Scientific Committee Number 40 on Biological Aspects of Radiation Protection Criteria (1978-Present)

U.S. Representative IAEA Coordinated Research Program on Use of Chromosomal Analysis in Radiation Protection (1983-Present)

Editorial Board, *Environmental and Molecular Mutagenesis* (1987-Present)

Adjunct Faculty Member, University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences (1988-Present)

C. C. Lushbaugh

The C.C. Lushbaugh Lecture on Controversial Radiation ncepts, presented by Eugene Saenger, M.D., during the econd International REAC/TS Conference on Radiation Accident Preparedness, October 1988

Science Subpanel on Pre-Disaster Planning for Human Health Effects Research

A. F. McFee

Member of the Editorial Board of Mutation Research

F. Snyder

Executive Editor and Member of the Editorial Committee, Archives of Biochemistry and Biophysics

Member of the Editorial Board, Lipids

Member of the Editorial Board, Journal of Biological Chemistry

Member of the Editorial Board, Biochimica Biophysica Acta

Editor, Handbook of Lipid Research, a continuing series, Plenum Publishing Corporation, New York

Associate Editor, Journal of Lipid Mediators

Member of the Advisory Committee for the First International Conference on Eicosanoids and Bioactive Lipids in Cancer and Radiation Injury

Member of International Advisory Committee for 7th International Conference on "Prostaglandins and Related Compounds"

Member of the International Advisory Board for the International Congresses on Inflammation

Adjunct professorships at the University of North Carolina and the University of Tennessee-Oak Ridge Graduate School of Biomedical Sciences

M. Stabin

Certified Health Physicist, 1989

Chairman, Public Information Committee, East Tennessee Chapter of the Health Physics Society

Secretary, East Tennessee Chapter of the Health Physics Society

Member of the American Association of Physicists in Medicine (AAPM) Task Group on Antibody Dosimetry

Member of the Medical Internal Radiation Dose (MIRD) Committee Task Group on Urinary Bladder Dosimetry

1028868

E. Watson

Member of the National Council on Radiation Protection and Measurement (NCRP) Scientific Committee 57-14 on Placental Transfer of Radionuclides

Member of the Society of Nuclear Medicine Medical Internal Radiation Dose (MIRD) Committee

Member of the Steering Committee, American Association of Physicists in Medicine (AAPM) Task Group on Antibody Dosimetry

Editor, Newsletter, Southeastern Chapter of the Society of Nuclear Medicine

Patents Granted and Pending

F. Snyder and M. L. Blank

Alkyl phospholipid antihypertensive agent

L. G. Littlefield and S. Colyer

Continuous cell-line tamarin adenocarcinoma of the colon (ORAU-TAC-1)

C. D. Scott and H. I. Adler

High-productivity biocatalyst gel beads

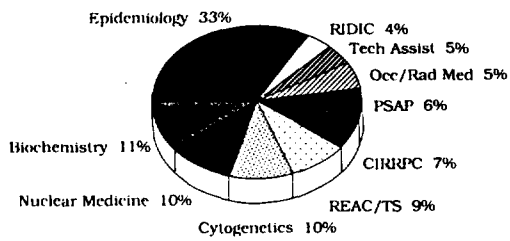
Non-DOE Research Funding

S. A. Fry

Death Data Retrieval Project, National Cancer Institute, $95,800 (October 1988 to September 1989)

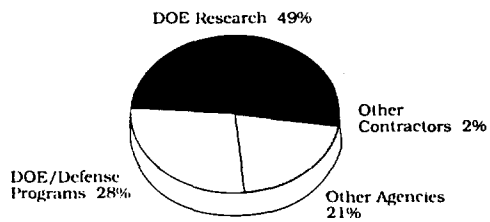
Gaseous Centrifuge Worker Study, Martin Marietta Energy Systems, $61,305 (October 1988 to September 1989)

Medical Sciences Division Programs
Fiscal Year 1989



Medical Sciences Division
Funding Sources

Total Funding — $9.7M **103 Employees**



1028869

Epidemiological Studies of Fernald Workers. Westinghouse Materials Company of Ohio. $80,000 (October 1988 to September 1989)

L. G. Littlefield

Studies of radiation-induced chromosome damage in humans
National Cancer Institute-U. S. Department of Energy Interagency Agreement. $156,141

C. C. Lushbaugh

Assessment of Protracted Radiation Dose Effects on Performance after Therapy and Radiation Accidents. Defense Nuclear Agency. $25,000

A. F. McFee

Evaluation and application of an in vivo mouse assay for chemically induced sister chromatid exchanges and chromosome aberrations
National Institute of Environmental Health Sciences-U. S. Department of Energy Interagency Agreement. $420,990

F. Snyder

Metabolism, Regulation, and Function of Ether Lipid Mediators and Related Phospholipids in Cancer Cells and Their Antitumor Analogs
$86,400 (direct) January 1989 to December 1989 (ACS)

A New Class of Biologically Active Phospholipids
$100,799 (direct) April 1989 to March 1990 (NHLBI) (ends in 1991)

Plasmalogens and Related Ether Lipids in Cancer Cells
$102,673 (direct) July 1989 to June 1990 (ends in 1991)

Platelet-Activating Factor and the Respiratory Distress Syndrome Premature Infants
Regional Neonatal Associates. $21,000 (August 1989 to July 1990)

L. C. Washburn

Radiolabeled Monoclonal Antibodies for Cancer Therapy
National Cancer Institute. $118,292 (1 September 1989 to 31 August 1990; second year of 3-year renewal award)

E. E. Watson

Interagency agreement between the DOE and the FDA for the internal dose center to provide radiation dose information in response to requests from radiopharmaceutical users and conduct research to improve accuracy of dose estimates. $134,000

Medical Sciences Division Staff

Administration

Frances J. Banner
William W. Burr, Jr., Ph.D., M.D.
Richard S. Bufturini, M.S., C.P.A.
Cynthia D. Glasscock
Billie P. Ryan
Fred L. Snyder, Ph.D.
Lora H. Treece, C.P.S.
Louise C. Wyatt

Animal Care

Billy R. Kyle
Mack A. Ruffner
John H. Suttles

1028870

Biochemistry

Merle L. Blank, B.S.
Bernice Corn
Edgar A. Cress, B.S.
Veronica L. Fitzgerald, B.A.
Ten-ching Lee, Ph.D.
Boyd B. Malone, B.S.
Sally T. Shipley
Zigrida L. Smith, M.L.S.
Fred L. Snyder, Ph.D.
David S. Vallari, Ph.D.
Kazuhiro Suga, M.D.
Jenny L. Wagner
Lillie I. Woods

Center for Epidemiologic Research

Melinda J. Armes
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Sondra L. Butcher
Donna L. Cragle, Ph.D.
Deborah A. Davies
Dennis L. Davis, B.A.
Patricia J. Deems
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Peter G. Groer, Ph.D.
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Linda R. Dry, B.S.
Barbara Gangaware, B.A.
Eugene E. Joiner, M.S.
L. Gayle Littlefield, Ph.D.
Alfred F. McFee, Ph.D.
C. Kaye McGuffin, B.A.
Josephine R. Outlaw, B.S.
Anne M. Sayer, M.S.
Dianne P. Wilson

Center for Personnel Security Assurance Research and Analysis

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Myra S. Perry

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Mark A. Brewer
William T. Pope

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Rana Yalcintas, M.S.

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Bruce Suttle, B.S.

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Billy L. Byrd, B.S.
James E. Crook, M.D., Ph.D.
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Elizabeth C. Holloway, R.T.
Yu-Chen C. Lee, Ph.D.
Tan Tan Sun, M.S.
Lee C. Washburn, Ph.D.

Radiation Emergency Assistance Center/Training Site

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Martha H. Payne
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Radiation Medicine

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Marsha A. Henke, M.S.
Clarence C. Lushbaugh, Ph.D., M.D.

1028811

Radiopharmaceutical Internal Dose Information Center

Audrey T. Schlafke-Stelson, B.A.
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Michael G. Stabin, M.E.
James B. Stubbs, Ph.D.
Evelyn E. Watson, B.A.

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Tommy F. McCraw, B.S.

VOE Students

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Erin Barger (RIDIC)
Diana R. Russell (CER)
Jenny L. Wagner (BCL)

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aquata Fredericks (CER)
Robert T. Givens (CER)
Peggy P. Harris (OM)
Susan J. Herrell (Admin.)
Howard Hovagimian (CER)
Karen H. Kennedy (CER)
Barbara Knight, B.S. (CYTO)
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Ingrid Reese (CER)
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Carolyn F. Russell (CER)
Therese I. Savage (CER)
Charles M. West (CER-REAC/TS)

Consultants

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G.F. Zanolli, M.D. (OM)

Abbreviation Key

Admin.	-	Administration
BCL	-	Biological Chemistry Laboratory
CER	-	Center for Epidemiologic Research
CPSARA	-	Center for Personnel Security Assurance Research and Analysis
CYTO	-	Cytogenetics
NM	-	Nuclear Medicine
OM	-	Occupational Medicine
REAC/TS	-	Radiation Emergency Assistance Center/Training Site
IDIC	-	Radiopharmaceutical Internal Dose Information Center
RM	-	Radiation Medicine
SS	-	System Safety and Operations

1028872

Administration and Committees

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Naomi Harley, Ph.D., New York University
Theodore Holford, Ph.D., Yale University
Roy Shore, Ph.D., New York University Medical Center (Chairman)

Extramural REAC/TS Faculty

James T. Alexander, B.S., U.S. Department of Energy, Oak Ridge, Tennessee
Bryce D. Breitenstein, Jr., M.D., Brookhaven National Laboratory, Upton, New York
Jane Parker-Conrad, Ph.D., Occupational Health Consultant, Knoxville, Tennessee
Daniel E. Conrad, M.D., Martin Marietta Energy Systems, Oak Ridge, Tennessee
Scott L. Davis, B.S., U.S. Department of Energy, Oak Ridge, Tennessee
Howard Dickson, M.S., Bechtel National, Inc., Oak Ridge, Tennessee
A. Seaton Garrett, Jr., M.D., Oak Ridge National Laboratory, Oak Ridge, Tennessee
James E. Henry, M.D., Methodist Medical Center, Oak Ridge, Tennessee
Roger E. Linnemann, M.D., University of Pennsylvania, Philadelphia, Pennsylvania
Peter Loper, M.D., Duke Power Company, Charlotte, North Carolina
James A. Martin, Jr., M.A., U.S. Nuclear Regulatory Commission, Washington, D.C.
Fred A. Mettler, Jr., M.D., M.P.H., University of New Mexico, Albuquerque, New Mexico
Enrico L. Quarantelli, Ph.D., University of Delaware, Newark, Delaware
Charles E. Roessler, Ph.D., University of Florida, Gainesville, Florida
Eugene L. Saenger, M.D., Cincinnati General Hospital, Cincinnati, Ohio
Robert N. Sawyer, M.D., Environmental and Technical Services, Inc., Troy, New York
John H. Spickard, M.D., Lawrence Livermore National Laboratory, Livermore, California
George L. Voelz, M.D., Los Alamos National Laboratory, Los Alamos, New Mexico
Niel Wald, M.D., University of Pittsburgh, Pittsburgh, Pennsylvania

1028873

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University of Alabama
at Birmingham
University of Arkansas
Auburn University
Catholic University of America
Clark Atlanta University
Clemson University
University of Delaware
Duke University
Emory University
Fisk University
University of Florida
University of Central Florida
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University of Houston
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Louisiana State University
University of Louisville
University of Maryland
Meharry Medical College
Memphis State University
University of Miami
University of Mississippi
Mississippi State University
University of Missouri
University of North Carolina
North Carolina State Univ.
University of North Dakota
University of North Texas
University of Oklahoma
Oklahoma State University
University of Puerto Rico
Rice University
University of South Carolina
Medical University of
South Carolina
Southern Illinois University
at Carbondale
Southern Methodist Univ.
University of Tennessee
Tennessee Technological Univ.
Texas A & M University
University of Texas at Austin
Texas Christian University
Texas Woman's University
Tuskegee University
Vanderbilt University
University of Virginia
Virginia Commonwealth Univ.
Virginia Polytechnic Institute
and State University
West Virginia University
College of William and Mary

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Oak Ridge Associated Universities *is a consortium of 55 colleges and universities and a contractor to the U.S. Department of Energy that explores opportunities, solves problems, and seeks to make increasingly positive contributions to society through science and technology. ORAU operates in four major areas: medical sciences, science and engineering education, training and management systems, and energy and environment systems.*

The ***Medical Sciences Division*** *(MSD) carries out basic research; develops beneficial applications to human health from science and technology; develops techniques to avoid, moderate, or reverse negative health impacts; analyzes the health risks and outcomes to society from applications of technologies; and provides information systems and management primarily for the U.S. Department of Energy, federal and international agencies, and industries such as utilities and pharmaceutical companies.*

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oak ridge associated universities

medical sciences division research report 1989-1990



40th anniversary 1949–1989

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preface

This year's research report summarizes the fortieth year of research, training, and service activities of the Medical Sciences Division (MSD) of Oak Ridge Associated Universities. The diversity of programs covered in this report emphasizes how this division has expanded from its beginnings as strictly a center for developing clinical applications of radioisotopes to its current pursuits, ranging from basic studies in biochemistry, nuclear medicine, cytogenetics, and microbiology to applied research in epidemiology and risk assessment. The format of this report for fiscal year 1990 is identical to our other recent research reports; it is purposely designed to provide a concise bird's-eye view of the scope and accomplishments of the various scientific programs in the MSD. More comprehensive information about specific topics can be obtained from the Chairman's Office and from the published papers listed elsewhere in this brochure.

Fiscal year 1990 has continued to be a rewarding year for most of our programs, even though tight budgetary constraints at the national level have limited an expansive mode. The year has also been one of considerable change with respect to key personnel. Dr. Glenn Davis became the new Chairman of MSD in May; he is the fifth chairman since the division was formed in 1949 under Dr. Marshall Brucers's leadership. Dr. William Burr, the former Chairman, now devotes full time to directing the Occupational Medicine Support Program. During the year, Doctors C. C. Lushbaugh and Thomas Lincoln retired from their positions as program directors for Radiation Medicine and Occupational Medicine, respectively. Also, Dr. James Crook,



Dr. Marshall Brucer (second from left), first Chairman of MSD, and a group of consultants (1950).

Director of the Nuclear Medicine Program, accepted a position elsewhere. A new addition to the senior staff in the Radiation Medicine Program and REAC/TS is Dr. Fun Fong, a physician trained in emergency preparedness and certified in Emergency Medicine.

Although today's budgetary uncertainties for future planning are faced by most all biomedical research organizations, we believe the strengths and international recognition of our programs in epidemiology, REAC/TS, biochemistry, and cytogenetics provide a solid base and healthy outlook for meeting upcoming challenges. Certainly the strong commitment of MSD staff for excellence will be a great asset in shaping the future directions of this division. Because of the diversity and flexibility of our programs, we anticipate an expanded leadership role for the MSD in responding to the needs of DOE and other agencies in solving problems of national concern related to human health and disease.

Glenn Davis
Chairman

Fred Snyder
Vice Chairman

1028878

significant events in the history of the medical sciences division

1950
- Construction of the clinical facility was completed and the first patient with thyroid cancer admitted.
- Training, including short courses, was initiated for visiting students and scientific/clinical staff.

1951
- An efficient cobalt-60 teletherapy machine was designed and operated.
- First patient admitted to hospital for treatment for thyroid cancer using radioactive iodine.

1952
- Teletherapy evaluation project was launched.
- Contributed to the development and design of brachytherapy devices.

1953
- A radioiodine uptake calibration program was initiated using mannequins and calibrated mock iodine sources.

late 1950s
- Collaborative efforts between the Medical Sciences Division and Oak Ridge National Laboratory led to the development and testing of a single-channel analyzer for measuring iodine-131 uptake in the thyroid and radioisotope scanners for mapping the distribution of radioisotopes throughout the body.
- Preclinical studies of rare earth elements (e.g., cerium) led to the discovery of the "rare earth fatty liver."

1958
- Program in lipid biochemistry was established.
- Oak Ridge Y-12 plant irradiation accident occurred; the five victims were treated for radiation injuries at the Medical Sciences Division.

1960
- The first total body irradiation facility was constructed for use in clinical studies of bone marrow transplantation in patients with leukemia and lymphoma.
- A program in immunology was established with basic studies done in the marmoset as an animal model.
- A Microbiology Program was initiated and laminar air-flow sterile rooms were installed.



Former chairmen of the Medical Sciences Division are; top left, Marshall Brucer (1949-1961); top right, Gould Andrews (1962-1975); second row left, C.C.Lushbaugh (1975-1984); above right, William W. Burr (1984-1990). At left is J. Glenn Davis, present chairman of MSD since 1990.

early 1960s
- The first computer facilities were set up at the Medical Sciences Division; they were also made available to other groups at ORAU.
- A whole-body counting facility was developed and constructed.

1964
- A Cytogenetics Program was established to study effects of radiation in inducing chromosome damage in radiotherapy patients.

1028879

1967

- A low exposure total body irradiation (LETBI) facility was designed and constructed for clinical trials to determine the efficacy of whole-body radiation in treatment of systemic blood diseases.

1968

- The affinity of gallium-67 for soft-tissue tumors was discovered.
- A program in electron microscopy and cell biology was initiated.
- A unique group of ether-linked lipids (glyceryl ether diesters) was discovered as a marker in both animal and human tumors. Enzymes that form the ether bond in lipids were discovered during the subsequent few years.

early 1970s

- An ORAU-led cooperative clinical study demonstrated the utility of gallium-67 citrate for tumor imaging and resulted in expeditious approval for widespread use.

1975

- The Radiation Emergency Action/Training Site (REAC/TS) Program was created.

1976

- First cytogenetic dose estimate provided for REAC/TS patient with suspected radiation exposure.
- The first REAC/TS course was completed.

1977

- An emission computerized axial tomography (ECAT) scanner was installed for studies of positron-emitting radiopharmaceuticals in patients. Novel compounds labeled with carbon-11 were synthesized for use in ECAT diagnostic studies.
- Epidemiology Program was initiated and the Center for Epidemiologic Research established.

1978

- Spontaneous colon adenocarcinomas were reported to occur in the cotton-top tamarin monkey and its potential as an animal model for human colon cancer realized.

1979

- Platelet activating factor (PAF), the most potent biologically-active compound known, was discovered by biochemists at the Medical Sciences Division.

1980

- Scope of Epidemiology Program was expanded to include all DOE and predecessor agencies nationwide (600,000 persons).
- REAC/TS was designated as a World Health Organization (WHO) collaboration center.

1983

- Studies of radiolabeled monoclonal antibodies for cancer diagnosis and therapy were initiated.

1986

- A novel metabolic pathway was discovered for the de novo synthesis of PAF.
- Plans for setting up the Center for Personnel Security Assurance, Research, and Analysis were initiated.

1987

- A dedicated IBM mainframe computer was installed for the Epidemiology Program.

1988

- A statistically significant increase in lung cancer was identified among Oak Ridge Y-12 plant workers.
- ORAU began collaboration on an international, multiple population study of nuclear workers.

1989

- Statistically significant increase in bladder cancer identified among former centrifuge process workers at the Oak Ridge K-25 plant.
- The program of Radiopharmaceutical Internal Dose Information Center, established at ORAU in 1971, was transferred to the Medical Sciences Division.

1990

- The 2,500th scientist participated in a REAC/TS course.
- REAC/TS responded to the 600th call for assistance in a radiation accident.

Three international conferences related to radiation accidents were conducted by REAC/TS in 1979, 1988, and 1990.

1028880

table of contents

scope of programs

Biochemistry 1

Nuclear Medicine 2

Cytogenetics 3

Microbiology 4

Radiation Emergency Assistance Center/Training Site 5

Radiopharmaceutical Internal Dose Information Center 6

Center for Epidemiologic Research 8

Center for Personnel Security Assurance, Research and Analysis 9

publications, abstracts, and invited lectures

Full-length Journal Articles in Peer-Reviewed Journals 11

Submitted Papers 13

Chapters, Review Articles, Government Reports, and Other Publications 14

Abstracts, Oral Presentations, and/or Posters 16

Invited Lectures at National and International Conferences 19

collaborative research 21

training programs 23

major awards, honors, and current appointments 24

patents pending 26

non-DOE research funding 26

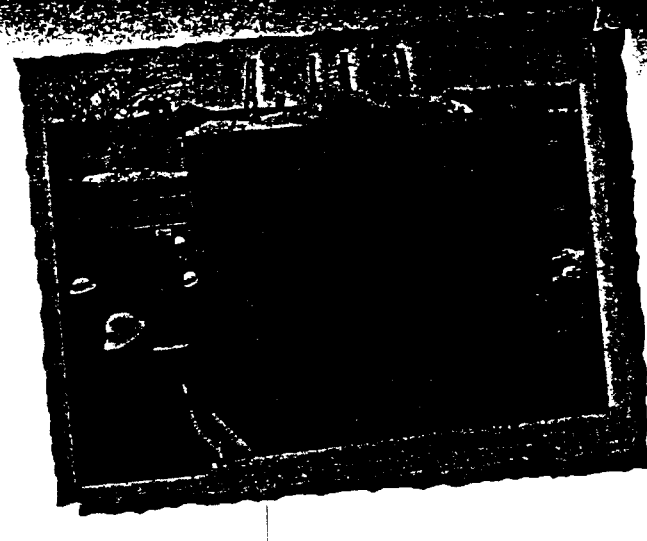
medical sciences division staff 27

consultants 29

administration and committees 29

member universities 30

1028881



The low-background whole-body counter being lowered into place at the Vance Rd. building. The counter is used to measure photon emissions from human subjects, down to a level where counting rates merge with the normal body background (1963). The Radiation Medicine Program was established in 1963.

scope of programs

biochemistry

Program Director: ***Fred Snyder***

The broad aim of this research is to determine the function of lipids as components of membranes and as cellular mediators in mammalian cells. Attention is also focused on a new group of phospholipid mediator analogs [an outgrowth of our platelet activating factor (PAF) studies] that possess selective antitumor properties with inhibitory activities towards the AIDS virus; the cytotoxic actions of these lipids are targeted against the surface membrane rather than towards the cell nucleus as are other conventional chemotherapeutic drugs. Bioactive lipid mediators, such as PAF and arachidonic acid metabolites, can provoke severe inflammatory reactions and other pathological consequences, including serious impairment of respiratory, hematopoietic, cardiovascular, and renal functions; however, these mediators also play a physiological role since they can trigger cellular signal transduction mechanisms involved in the control of cell growth, cellular differentiation, blood pressure control, and reproduction.

Specific questions being addressed are: (1) How do membrane lipids function as both structural components and precursors of potent biologically active messengers in cellular signal transduction phenomena? (2) How are biologically active glycerolipids metabolized and what are the regulatory mechanisms involved? (3) What is the role of PAF and arachidonate mediators and their structural analogs in cancer, hypersensitivity responses, and diseases associated with respiratory and immunocompetent reactions? (4) What is the mode of action of membrane-targeted PAF analogs that exhibit selective antitumor properties and the ability to inhibit the HIV-1 AIDS virus? and (5) How do membrane plasmalogens function as oxygen/free radical scavengers in protecting cells from radiation damage?

Major research accomplishments during FY 90 are:

- A highly specific arachidonoyl-specific phospholipase A_2 was shown to initiate the first step in PAF synthesis during inflammatory responses. This is the first evidence to indicate that only membrane polyenoic phospholipids are the preferred precursor source of PAF. These results also emphasize the close tie between the production of PAF and arachidonate mediators in inflammatory disease.
- Rate-limiting reaction steps in the production of PAF were identified.
- Results were obtained to indicate newly synthesized PAF is rapidly translocated from its site of synthesis (endoplasmic reticulum) to the plasma membrane, where it is sequestered in a compartment not readily accessible to extracellular acceptor molecules.
- Human leukemic cells, that possess no PAF receptors, were shown to develop specific functionally active PAF receptors when they are differentiated into a mature granulocytic form (i.e., neutrophils). These data suggest specific PAF receptors are not expressed in cancer cells and could explain the inability of cancer cells to respond to PAF stimuli.
- Collaborative work with scientists at the University of Ottawa and Queens College of the City University of New York led to the development of several new types of selective antitumor lipid analogs that are targeted towards the surface membranes of cells rather than the nucleus as are all other chemotherapeutic drugs. This work indicates that modifications of the polar head group of the cytotoxic lipids appear to enhance the antitumor activity of these unnatural lipid analogs.

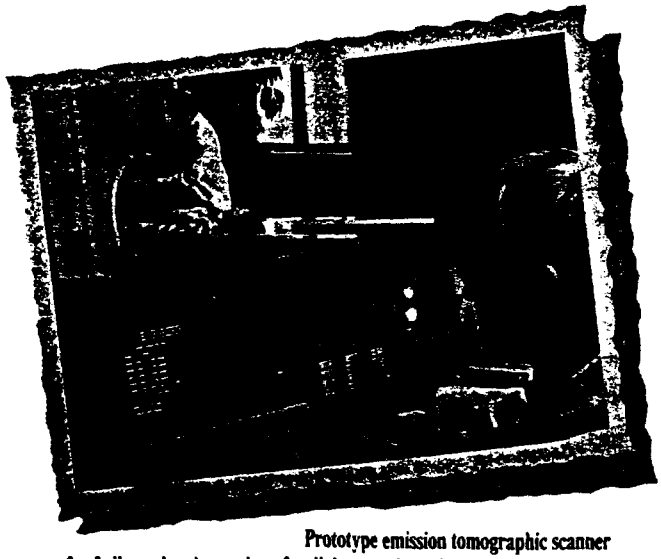
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- Subcellular experiments with a tritiated methoxy analog of antitumor phospholipids have documented their high affinity for the plasma membrane of tumor cells sensitive to their cytotoxic properties.

- Preliminary experiments indicate that total body irradiation causes significant differences in the enzyme activities responsible for catalyzing the PAF cycle in spleen tissue. Such an imbalance in lipid metabolism could be of key importance in the cellular responses associated with radiation injury in skin and hematopoietic tissue.

- Ongoing collaborative studies with the Regional Neonatal Center at the University of Tennessee Medical Center support the concept that PAF is an important cellular mediator to consider in the pathogenesis of neonatal lung injury and bronchopulmonary dysplasia in premature babies.

Future research initiatives particularly relevant to Department of Energy (DOE) interests concern the involvement of lipid mediators and membrane lipids in burn injuries and altered functions of hematopoietic cells induced by radiation and in inflammatory, respiratory, and immune responses resulting from accidental or environmental exposure to energy-related oxicants. In addition, projected studies of anti-PAF and anti-eicosanoid receptor antagonists and/or enzyme inhibitors will explore their potential as drugs for treating radiation injuries and toxicant-induced inflammatory disorders. Long-range plans will incorporate molecular biology approaches to investigate the regulatory controls and active catalytic sites of key enzymes that catalyze rate-limiting steps in the formation of PAF and related ether-linked glycerolipid membrane components.



Prototype emission tomographic scanner for 3-dimensional scanning of radioisotopes in patients and experimental animal models (1977).

nuclear medicine

Acting Director: ***Lee C. Washburn***

The major objectives of the nuclear medicine program are to develop new radiopharmaceutical agents and to carry out phase I and phase II testing of such materials in subsequent clinical studies. The work involves identification of medical needs, design and synthesis of potential radiopharmaceutical agents for diagnosis and therapy, tests of these substances in animals for effectiveness and toxicity, and efficacy trials in humans, according to U.S. Food and Drug Administration (FDA) guidelines.

Some recent accomplishments of the nuclear medicine program include:

- A Competing Continuation application for the National Cancer Institute (NCI) grant, "Radiolabeled Monoclonal Antibodies for Cancer Therapy," which is now in its sixth year of support, was submitted.

- Studies with ^{90}Y-labeled CO17-1A, a monoclonal antibody with specificity for colorectal and pancreatic carcinomas, have advanced to the point that an Investigational New Drug (IND) application for phase I/II clinical trials of this agent for treatment of patients with disseminated colorectal carcinoma has been submitted to the FDA. The planned clinical study is a three-way collaboration involving the Medical Sciences Division of Oak Ridge Associated Universities (ORAU), the Thompson Cancer Survival Center (Knoxville, Tennessee), and the Wistar Institute (Philadelphia, Pennsylvania).

- Of a series of bifunctional chelate methods studied for radiolabeling CO17-1A with ^{90}Y, a technique involving site-specific conjugation of the macrocyclic ligand, (p-aminobenzyl)DOTA-3A, which was synthesized by O. A. Gansow and M. W. Brechbiel of the NCI (Bethesda), yielded the greatest tumor specificity and in vivo stability.

- A preliminary experiment in collaboration with T. J. MacVittie and M. L. Patchen of the Armed Forces Radiobiology Research Institute (Bethesda) showed the potential of granulocyte colony-stimulating factor (G-CSF) as an adjunct to experimental radioimmunotherapy with ^{90}Y-labeled CO17-1A for stimulating hemopoiesis and therefore, regenerating radiation-damaged bone marrow.

- A study carried out in collaboration with B. W. Wessels of George Washington University Medical Center (Washington) investigated implantation of miniature thermoluminescent dosimeters (mini-TLDs) in

1028883

tumor-bearing nude mice as a means of measuring the radiation absorbed dose to tumor resulting from administration of ^{90}Y-CO17-1A. Dose estimates obtained in this manner were similar to those calculated from integrated tumor time-activity levels.

- Spontaneous colorectal carcinomas in cotton-top tamarins (small South American primates) were successfully imaged using monoclonal antibody WGHS 22-2 radiolabeled with both ^{123}I and ^{111}In, thus further enhancing the usefulness of this animal model.

- Two sessions of a practical mini-course, "Radiolabeling Monoclonal Antibodies," provided training to a total of 16 participants.

Future efforts by the nuclear medicine program will continue to focus on the development and application of radiolabeled monoclonal antibodies. Clinical trials of ^{90}Y-labeled CO17-1A will begin as soon as an IND exemption is granted by the FDA. The problem of dose-limiting radiation-induced myelotoxicity from administration of therapeutic dosages of radiolabeled monoclonal antibodies will be addressed in two ways: (1) continued improvement in bifunctional chelate methodology in order to produce ^{90}Y-labeled monoclonal antibodies with optimal in vivo stabilities ' tumor specificities and (2) development of adjunctive hemopoietic .ulation protocols for reversing bone marrow damage. Research in collaboration with J. Roche and C. D. Teates of the University of Virginia Medical Center (Charlottesville) will seek to develop a radiolabeled monoclonal antibody with thymus specificity to aid in distinguishing thymic from nonthymic tissues during surgical thymectomies.

cytogenetics

Program Director: L. Gayle Littlefield

Chromosome aberrations are sensitive and readily observable biomarkers of DNA damage resulting from exposures to radiation and clastogenic chemicals. The cytogenetics program conducts research to develop and improve various techniques for quantifying chromosome damage and applies cytogenetic endpoints as biological "dosimeters" in persons having real or suspected accidental exposures. Long-term follow-up studies are conducted in selected cohorts of patients having radiation ›sures to gain information on the correlation between survival of cells ...n chromosome damage and the subsequent risk of late effects. We also continue studies using human cells irradiated in the presence of radioprotective chemicals or inhibitors of DNA repair to learn how DNA damage may be ameliorated or potentiated by chemical modifiers of radiation action. Assays in animals as human surrogates are useful as experimental approaches for answering specific questions regarding cytogenetic and cellular toxicity resulting from exposure to genotoxic agents.

Highlights of selected research accomplishments are cited below:

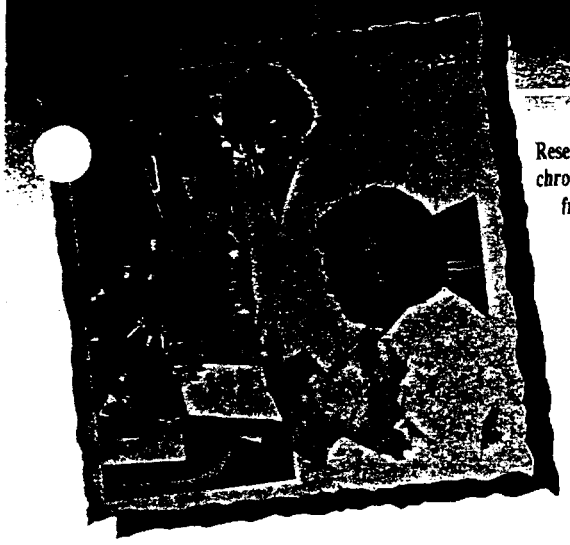
- Our laboratory has now studied chromosome aberrations in cultured lymphocytes of over 1,000 persons. Some 200 evaluations have been performed for biological dose estimation, and in parallel "cytogenetic epidemiology" studies we have evaluated several hundred persons who received partial-body radiation during various medical procedures up to 50 years ago. Our findings in populations exposed many years ago suggest that "stable" types of chromosome aberrations in lymphocytes may provide a "retrospective" biological index of the relative degree of cytogenetic injury sustained by lymphocyte stem cells that survived the radiation exposure. Efforts are underway to determine whether the cytogenetic findings in these irradiated populations can be correlated with the excess relative risk of acquiring various types of malignancies.

- "Unstable" types of chromosome aberrations and radiation-induced cell killing are strongly correlated, because many types of chromosome aberrations are lethal when the cell attempts to divide. Our recent studies demonstrated that, when human lymphocytes are exposed to low doses of X rays, about 50% of the cells with chromosome aberrations die during the first cell division after radiation exposure. When cells are exposed to higher doses of radiation, those with extensive chromosome damage are preferentially eliminated and upwards of 85% of the damaged cells do not survive a single cell division.

- Scientists in the cytogenetics program were recently awarded four-year support by the National Institutes of Health to continue and expand basic research using dimethyl sulfoxide (DMSO) as a model radioprotective chemical in studies aimed at elucidating mechanisms involved in radiation-induced cellular injury. Studies are currently underway to quantify the amount of chromosome damage induced by radiochemical mechanisms in human cells exposed to differing doses of 14-MeV neutrons.

- We have continued to evaluate the genotoxicity of selected chemicals in animal models. Particular emphasis has been placed on testing compounds by the micronucleus assay. While this system

1028884



Researchers discuss a chromosomal pattern from cells isolated from a radiation accident victim (1978). MSD's Cytogenetics Program was established in 1963.

affords faster and theoretically more accurate evaluation of the clastogenic action of chemicals, adequate comparisons to the results of other assays have not been made. Detailed testing of the clastogenicity of compounds as simultaneously measured by the micronucleus, chromosome aberration, and sister chromatid exchange assays is currently underway.

- A potential for improvement for the micronucleus assay by separating nucleated cells from samples prior to scoring was thoroughly evaluated. Use of the system did not alter the outcome of tests, neither did the procedure increase the ease or speed of scoring sufficiently to warrant the additional time required for the separation process.

In collaboration with scientists at the Radiation Epidemiology Branch at NCI, we will continue studies of chromosome aberrations induced in patients who received radiotherapy using 14-MeV neutrons produced by the cyclotron at M. D. Anderson Hospital. Comparisons of findings in these patients with those obtained in persons receiving orthovoltage X-radiation for similar types of tumors will allow us to derive estimates of the relative effectiveness of the two qualities of radiation in inducing chromosome damage in human cells. We will also continue studies using human lymphocytes and DMSO as a model OH radical scavenger to estimate the amount of neutron-induced chromosome damage that results from OH radical attack for neutrons having a range of energies encompassing those of concern to DOE and other regulatory bodies involved in setting standards for human exposures.

Several "chromosome-specific DNA probes" (i.e., small pieces of human or mammalian DNA that selectively bind or "hybridize" to specific genes, chromosomes, or chromosomal regions) have been recently developed by scientists at Los Alamos, Lawrence Livermore, and other national laboratories or universities. Such probes may be labeled with fluorescent dyes so that their locations on chromosomes may be readily observed in preparations of human metaphases. Scientists in the cytogenetics program will continue to expand several ongoing collaborative projects using various DNA probes as tools for quantifying levels of chromosome damage and in investigations of cellular or molecular mechanisms involved in cell injury.

microbiology

Program Director: ***Howard I. Adler***

The emphasis of this program is currently being shifted from a study of the mechanisms involved in determining the oxygen sensitivity of bacteria to a study of the role played by anaerobic organisms in waste remediation. In addition, we have initiated experiments designed to isolate and characterize anaerobic microorganisms that are effective in degrading some of the more highly polychlorinated biphenyl (PCB) compounds. In recent years evidence has been presented by other groups that naturally-occurring anaerobes can remove chlorine molecules from highly chlorinated compounds. This makes these PCBs amenable to further breakdown by aerobic bacteria. Although several strains of anaerobes have this ability, they all carry out the dechlorinations only very slowly. Because our group has developed some novel methods for isolating and cultivating anaerobes, we are attempting to find efficient PCB-degrading organisms not revealed by the techniques being used in other laboratories. Our methods involve the use of specific enzymes for producing anaerobic conditions. This approach contrasts with the frequently used nonspecific chemical reducing agents. Because it is known that some anaerobes are inactivated by chemical reducing agents, we anticipate that our methods will reveal organisms not usually isolated from the complex mixtures normally encountered in soils and sediments. The PCB degrading ability of newly isolated organisms is being evaluated in collaboration with a group of investigators in the Chemical Technology Division of the Oak Ridge National Laboratory.

During FY90 our studies on the mechanism of oxygen sensitivity have made significant progress. Major accomplishments are:

- Established that the oxygen-sensitivity of an *Escherichia coli* mutant is due to a genetic alteration in a 3 to 1 kilobase fragment of DNA. This fragment also includes the hemB locus that codes for the enzyme 5-amino levulinate dehydratase.
- Sonicates of the oxygen-sensitive mutant can convert one or more

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