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LOS ALAMOS SCIENTIFIC LABORATORY
of the
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ANNUAL REPORT, BIOMEDICAL RESEARCH GROUP
HEALTH DIVISION
1953

HEALTH AND SAFETY

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ANNUAL REPORT OF THE BIOMEDICAL RESEARCH GROUP OF THE HEALTH DIVISION
1953

Wright H. Langham, Group Leader
John B. Storer, Alternate Group Leader (on Military Leave)

1. General Comments on Group Activities

This report covers the activities of the Biomedical Research Group (Group H-4) of the Health Division during the period of January 1 to December 31, 1953.

With minor exceptions the form is essentially that used in the previous annual reports. General comments are made on group and section activities including comments on what was considered the outstanding activities of the year. A detailed account of the past year's research is given in the form of project abstracts under the various section headings. No attempt is made to specify the degree of completeness of each project except by indicating the results obtained and by giving a list of published or distributed documents issued by each section. An additional section has been given which lists the proposed research program of the Biomedical Research Group for 1954.

1.1 Outstanding Activities During 1953

1.1.1 Further Studies of Effects of Massive, Rapid Doses of Gamma Radiation on Monkeys

During 1952 a preliminary study was made of the effects of massive doses of gamma radiation on monkeys. This project was carried

out jointly with Randolph Field School of Aviation Medicine and consisted of exposing eight monkeys to doses of 1000 - 30,000 r of gamma rays from multi-kilocurie sources of Ba¹⁴⁰-La¹⁴⁰. These results were reported in LA-1558, December 1953 (Confidential).

During 1953 the studies were repeated using refined techniques, additional criteria of effects and 120 monkeys. The studies included median survival time, physiological, biochemical and pathological effects, and observations of incapacitation and performance defect from massive, rapid doses of gamma radiation. No specific details of results are given here. They will be reported in a joint classified report from Group H-4, LASL, and the School of Aviation Medicine.

1.1.2 Radiation Hazards to Personnel Within an Atomic Cloud

At the 1953 Nevada bomb tests, studies were made of the relative radiation hazard from external gamma radiation and from inhaled fission products resulting from early passage through the mushroom head of an atomic cloud. The results will be distributed as report number WT-743, 1954 (Secret).

1.1.3 Liquid Scintillators and Their Application to Large Scale Detectors and Low Level Counting

In response to the need for extensive fundamental knowledge regarding the principles and properties of scintillators, a research program into the chemistry and characteristics of liquid solution scintillators has been pursued.

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Various oxazoles, 1,3,4-oxadiazoles and oxazole quaternary salts have been synthesized. Thirty-eight of these are now reported as new compounds.

Emission spectra of 3 g/l toluene solutions of these solutes have been run with radium excitation. Energy transfer between solutes has been noted by this method with toluene solutions of two solutes. Fine absorption spectra in cyclohexane solution and photon mean free path data have been obtained.

Solvents have been studied as to their light absorption and chemical properties of peculiar interest in scintillation work.

Relative photomultiplier anode current and pulse height methods of testing have been applied to liquid solution scintillators yielding information directly applicable to counting problems.

Applications of these solutions in H^3 and C^{14} counting of biological interest, natural C^{14} counting and to the fabrication of large volume gamma detectors have been pursued as a part of the program.

1.1.4 Relative Biological Effectiveness of Neutrons and Other Radiations

Approximately four years ago a program was initiated to study, develop and apply a number of mammalian biological test systems to the determination of the relative biological effectiveness of radiations of different types and different energies. Using the various biological test systems the effectiveness of various radiations of interest to the Los Alamos Laboratory are being determined relative to the effect produced by

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a standard radiation, 250 KVP X ray. The test systems so far applied or studied are weight decrease of the spleen and thymus of the CF_1 female mouse, 30-day lethality in mice and rats, incidence of lens opacities in mice, testicular atrophy in mice, Fe^{59} uptake of the red blood cells of rats, decrease in gut weight in rats and mice, life span of rats and mice, median survival times of mice, rats, and monkeys and quite recently the susceptibility of CF_1 mice to AK leukemia.

At present the radiations studied using one or more of the above test systems are thermal neutrons, 4.9 Mev gamma radiation from the homogeneous reactor, fission neutrons from the Godiva assembly, 14 Mev neutrons from the DT reaction in the Cockcroft-Walton accelerator, 250 KVP X rays, Co^{60} and Ba^{140} - La^{140} gamma rays delivered at different dose rates and beta rays of tritium. Since the RBE of a specific radiation is equal to the effectiveness in rem divided by energy imparted to the biological system expressed in rep, actual values for physical dose to tissue are needed for further refinement of the RBE's. Physical dose measurements using tissue equivalent and graphite ionization chambers will constitute an important part of the 1954 research program.

1.1.5 Preliminary Studies Using Godiva

Godiva is a naked critical assembly of U^{235} which can be either operated at a constant power level over a relatively long period of time or made supercritical for a few microseconds. The radiation given off is predominantly fission neutrons. Some fission gamma rays are also given off but this is believed to constitute less than 10 per cent

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of the total radiation dose. The device may be unique for its range of dose rate. With the appropriate arrangement of biological materials it may be possible to subject them to neutron radiation over the dosage range of from ~ 50 rem/min to $\sim 10^7$ rem/min.

Preliminary studies of the effect of Godiva radiation on splenic and thymic weight loss of the mouse supported the feasibility of using the assembly for biological studies and indicated that $(1-2) \times 10^8$ fission neutrons was equivalent to one rem.

During 1954 it is anticipated that the use of Godiva for dose rate studies and for the determination of RBE of fission neutrons will be one of the major projects of the Biomedical Research Group.

1.1.6 Follow-Up Physical Examinations on Los Alamos Pu Exposure

Cases

In December of 1952 arrangements were made through the Division of Biology and Medicine for Dr. L. H. Hempelmann, of Rochester, to collaborate with the Health Division in obtaining physical examinations on 27 of the early workers at Los Alamos who had accumulated 0.1 to 1.3 μg of Pu. Dr. Hempelmann was successful in obtaining complete physical work-ups including X rays on 22 of the 27 persons. Most of these people had accumulated their body burden during 1944-1945, approximately seven years prior to the examination. No unusual findings which could be attributed to the small body burden of Pu were found. These examinations will be repeated again in 1955.

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1.2 Changes in Physical Facilities

1.2.1 Occupancy of the New Laboratory

In August of 1953, the new Biomedical Research Laboratory was turned over to the University of California and occupied immediately. The building was officially designated as the Health Research Laboratory (HRL). The facility houses the Health Division Property Section and stocks, the Industrial Hygiene Group (H-5), and the Biomedical Research Group (H-4). The total cost of the installation was approximately 2.2 million dollars. The structure contains laboratories, administrative offices, medical library, seminar room, animal quarters, work shops, photographic darkrooms, X-ray exposure room, cobalt source exposure room, a special counting room, stock and storage space, a lobby, and a repository for classified material. The first preliminary plans for HRL were drawn in January 1947.

1.2.2 Equipment for Animal Quarters

During 1953 the Biomedical Research Group obtained new animal cages and racks. New equipment was ordered throughout with the exception of rabbit cages and racks. New cages for mice, rats, guinea pigs, and dogs or monkeys are of galvanize dip, sheet metal, and wire mesh construction.

1.2.3 Other Equipment

In keeping with an agreement between the Health Division and the Los Alamos Medical Center, a new 250 KVP General Electric X-ray machine was purchased by the hospital and transferred to Group H-4 as a replacement

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for the machine purchased by the Health Division and installed a year ago in the Los Alamos hospital. The new X-ray unit was not yet installed in HRL on December 31, 1953.

Facilities were provided in HRL for the installation of a cobalt⁶⁰ source. At the end of 1953 arrangements had not been made for procurement of the source. It is anticipated that the procurement and installation of a large cobalt source will be one of the major equipment items during 1954.

1.3 Changes in Organization and Personnel

The principal organizational change in Group H-4 during 1953 was the establishment of a Biophysics Section with Dr. E. C. Anderson designated as Section Leader.

During 1953 the following persons terminated their connections with the Biomedical Research Group: L. E. Browning (Military), P. S. Harris (Military), Frances Ficus, L. E. Ellinwood, Marion Vier, and R. E. Carter (terminated from Military Leave status).

Dr. John Storer (Alternate Group Leader) went on Military Leave and Major Sam Rothermel was appointed Acting Section Leader in Dr. Storer's place. Mr. John Furchner went on leave to complete his Ph.D. degree at the ~~CONFIDENTIAL - SECURITY~~

During the year the following new employees were added to the Group: John Larkins, Lora Belle Hughes, John Spalding, Rita Smith, Helen Furchner, Catherine Aguilar, William Schweitzer, and Jeanne Nordberg.

1.4 Summer Employment Program

The summer employment program was curtailed during 1953 because of the anticipated move to HRL during the summer months. Mr. Tom Moore was the only summer employee. A much greater summer employment program is anticipated for 1954.

2. Progress Reports of the Various Sections

2.1 Biochemistry Section (Gordon Gould, Section Leader)

2.1.1 General Comments on Section Activities

The investigative activities of the Biochemical Section during 1953 may be grouped under the following general categories.

- a. The dynamics of steroid metabolism and its relationship to atherosclerosis.
- b. Biochemical effects of massive doses of radiation.
- c. Metabolism of radioactive materials of special interest to the Los Alamos Scientific Laboratory and effect of Ca-ethylenediamine-tetracetate on their excretion.
- d. Metabolism of C¹⁴-labeled drugs and other compounds of biological and medical interest.
- e. Effects of radiation on the cholinesterase levels of erythrocytes.
- f. Miscellaneous studies.

During 1953 R. G. Gould served as a member of the program committee of the American Society for the Study of Arteriosclerosis, served as a consultant to the U. S. Public Health Service, Cardiovascular Study Section, and refereed numerous papers for the J. Clin. Inv., J. Biol. Chem., Arch. Biochem. and Biophysics.

All members of the Biochemistry Section participated in the Further Studies of Effects of Massive, Rapid Doses of Gamma Radiation on

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Monkeys and in the Study of the Radiation Hazards to Personnel Within an Atomic Cloud (WT-743).

2.1.2 Articles and Documents Originating in the Section

The following publications or documents were issued by the various staff members of the Biochemistry Section during 1953.

a. Cholesterol Metabolism. I. Effect of Dietary Cholesterol on the Synthesis of Cholesterol in Dog Tissue in vitro. J. Biol. Chem., 201 519 (1953). R. G. Gould, C. B. Taylor, J. S. Hagerman, I. Warner, and D. J. Campbell.

b. Liquid Scintillation Counting of Tritium Labeled Water and Organic Compounds, Science, 117 480 (1953). P. M. Hayes and R. G. Gould.

c. Isolation of a Crystalline Cholesteryl Ester from Normal Plasma, Federation Proc., 12 228 (1953). P. Keegan and R. G. Gould.

d. Factors Controlling Cholesterol Synthesis in the Body, Proc. of the Annual Meeting of the Council for High Blood Pressure Research, Amer. Heart Assoc., pp 3-19 (1953). R. G. Gould.

e. Acetate Metabolism and Blood Cholesterol Synthesis, J. Lab. and Clin. Med., 42 809 (1953). R. G. Gould, G. Okita, P. Keegan and G. V. LeRoy.

f. Use of Calcium Ethylenediaminetetraacetate in Case of Lead Intoxication, A.M.A. Archives of Industrial Hygiene and Occupational Medicine, 7 148 (1953). Harry Foreman, H. L. Hardy, T. L. Shipman, and E. L. Belknap.

g. Use of Calcium Ethylenediaminetetraacetate in Treating

Heavy Metal Poisoning, A.M.A. Archives of Industrial Hygiene and Occupational Medicine, 7 137 (1953). Harry Foreman.

h. The Metabolism of C¹⁴-Labeled Ethylenediaminetetraacetic Acid in the Rat, J. Biol. Chem., 203 1045 (1953). Harry Foreman, M. Vier, and M. Magee.

i. The Use of Chelating Agents for Accelerating Excretion of Radionuclides, J. Am. Pharm. Assoc., 42 629 (1953). Harry Foreman.

j. Role of Trace Elements in Animal Cells. A Tabulation in Handbook of Biological Data to be published by the National Research Council (in press). Harry Foreman.

k. The Metabolism of C¹⁴-Labeled Ethylenediaminetetraacetic Acid, J. Lab. and Clin. Med., (in press). Harry Foreman and T. Trujillo.

l. A Continuous Titration Method for Cholinesterase Determinations, AECU-2575 (1953). Jean Captain Sabine.

2.1.3 Progress of Specific Projects

a. Effect of Radiation on Metabolic Processes with Emphasis on Steroid Synthesis (Gould and Kossan)

Preliminary studies showed that 2400 r of whole body radiation in rats increased the rate of hepatic cholesterol synthesis more than threefold while showing no significant effect on extrahepatic cholesterol synthesis. Fatty acid synthesis was somewhat decreased in both liver and extrahepatic tissues. No studies on cholesterol synthesis have been reported from other laboratories; reports on fatty acid synthesis from the NRDL using liver slice methods rather than intact animals indicate the

opposite type of effect, probably due to difference in method. The only possible explanation for the cholesterol effect that can be suggested at present is that liver damage (e.g. anoxia) may increase hepatic cholesterol synthesis and even though the liver is considered to be extremely radio-resistant, this effect may represent a reaction to liver damage. These studies are being extended to other types of synthetic reactions and to other specific organs such as the intestine.

b. Effect of Radiation on the Central Nervous System (Gould, Kessan)

As a result of the failure to detect any changes in the permeability of the blood-brain barrier to blue dyes after massive doses of radiation in monkeys, an independent experiment was carried out on rats using I^{131} -labeled human albumin. Radiation dose was 50,000 r, the I^{131} albumin was injected immediately thereafter and the animals were sacrificed four hours later. No significant difference between control and irradiated rats was noted.

c. Coprogen as a Possible Protective Agent Against Radiation Damage (Foreman, Gould)

From literature reports, coprogen might be expected to have a protective effect and it was therefore tested in young rats but no evidence of protection was observed.

d. Cholesterol Metabolism in Humans (Gould, Kessan, Kobr, and LeRoy, Univ. of Chicago)

A series of experiments on 10 patients in the Argonne

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Cancer Research Hospital concerned with the metabolism of C^{14} -acetate was completed. An abstract has been published and a paper given; detailed papers will be written about March 1, 1954.

A method of determining both tritium and C^{14} on the same sample was worked out by appropriate adjustment of upper and lower discriminators in the liquid scintillation counter and used routinely in a number of these experiments. T-cholesterol was fed to each patient for a week before adrenalectomy and C^{14} -acetate injected one hour before removal of the adrenals. This made it possible to obtain much additional information from each patient.

e. Effect of Hypophysectomy on Cholesterol Syntheses (Keenan, Gould)

Two series of experiments on hypophysectomized rats have shown that a decrease in synthetic rate to about 1/5 normal occurs. One more experiment is planned before publication of these results.

f. Absorption of Tritium-labeled Sterols by the Rat (Kohr, Gould)

Eight different sterols have been labeled with tritium by exchange or by hydrogenation and their absorption in rats determined. Contrary to the statements in many textbooks, dihydrocholesterol and plant sterols were found to be absorbed. This material will be ready for publication after a few confirmatory studies have been completed.

g. Effect of Thyroid Status on Cholesterol Metabolism (Gould, Keenan, Kohr)

A number of hypothyroid rabbits and rats were studied by

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feeding T-labeled cholesterol and following the turnover rate of blood cholesterol. C^{14} -acetate was administered to the same animals and the relative rates of hepatic synthesis determined. It was found that hypothyroidism increases turnover time and decreases rate of synthesis but the change is not nearly as striking as in two patients with myxedema studied at the Argonne Cancer Research Hospital.

b. Mechanism of Control of Hepatic Cholesterol Synthesis by Dietary Cholesterol (Gould, Keegan and Lotz)

Additional studies on rats are being carried out to determine if it is the increase in liver esterified or liver free cholesterol fractions that is responsible for the sharp decrease in rate of synthesis observed after increase in dietary cholesterol.

1. Chromatographic Separation of Tissue Lipid Fractions (Keegan, Gould, Lotz)

A general method of separation of neutral fat from cholesterol esters has been worked out for plasma, liver and presumably any tissue. This method is being applied to the identification of fatty acids present in cholesterol esters.

1. Cholesterol Metabolism in Patients with Cardiovascular Disease (Gould, Kohn, Hauerer of Santa Fe, N. M.)

A series of normal humans and a series of cardiovascular patients in Santa Fe were fed standard doses of T-labeled cholesterol and the specific activity of plasma free, esterified and red cell cholesterol followed. Consistent results for the controls were obtained but additional

cardiovascular patients will need to be studied before it can be stated whether atherosclerosis alters the pattern of appearance of labeled dietary cholesterol in blood as reported by Biggs and Colman (UCRL).

k. Metabolism of C^{14} -Labeled Ca EDTA in Man (Foreman, Truillo)

Administration of C^{14} -Ca EDTA to normal human subjects showed that the material passes through the body essentially unchanged. It appears that the only interaction in the body is combination with certain di- and tetravalent metal ions and that any therapeutic or deleterious effects occur solely via this mechanism.

The material is excreted principally by the kidney. The turnover time from the blood is approximately one hour after intravenous administration and one and one-half hours after intramuscular injection. It quickly mixes with almost all of body water except that it does not pass into red cells and passes relatively slowly into the spinal fluid compartment. It is poorly absorbed from the gastrointestinal tract (a maximum of 5 per cent) and practically not at all through the skin.

l. Ca EDTA as Skin Decontaminant (Foreman, Truillo)

The findings, as indicated above, that Ca EDTA is not absorbed through the skin, suggested a reconsideration of the recommendation that it not be used as a skin decontaminant. A study has been started to determine if the agent can carry radioactive ions across the skin. $La^{140}NO_3$ was incorporated in a water soluble ointment paste and spread over the hand and forearm for one hour. The uptake of La^{140} in the body was measured by assaying the urine collected over a 24-hour period and by measuring the activity built up in the other arm by means of the "arm counter." The

procedure was repeated using the La^{140} EDTA and the results of the two compared. In a third series La^{140} was injected as the nitrate and the excretion and blood clearance measured before and after skin application of Ca EDTA in a water soluble ointment.

These studies are in progress and so far indicate that EDTA did not carry measurable amounts of La^{140} across the skin barrier.

Ba-La Metabolism (Foreman, Truillo)

The question of the significance of Ba^{140} - La^{140} activity in the urine of workers exposed to the material suggested an investigation of the correlation of urinary excretion with body burden. Preliminary to carrying out the study in humans, a study was done with rats, namely to develop techniques and to check the results of previous investigations. This study has been completed. The results verified previous findings and gave some additional information.

The body milks the La from an injected Ba-La mixture. The La is taken up principally in the liver, some by the other soft tissues and very little in the skeleton. The Ba goes principally to the skeleton, some to the soft tissues and none in the liver.

The body selectively excretes the Ba in both the urine and feces. Less than two per cent of La was excreted in four days.

A markedly simplified technique for assaying the Ba and La content in tissues and excreta without resorting to actual chemical separation was developed. The procedure involves the use of scintillation counting using a NaI crystal.

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The study in humans has been set up and is in process.

n. Metabolic Studies of C¹⁴ Isoniazid in Pyridoxine Deficient Rats (Boone, Magee, Turney)

A metabolic study on vitamin B₆ deficient rats with C¹⁴-labeled isoniazid was started because of the previously observed relationship between B₆ deficiency and isoniazid. A dose of 10 mg/kg of a mixture of cold and labeled isoniazid was injected prior to placing the animals in the metabolic cages. Carbon dioxide, feces, urine and tissues were analyzed in the usual manner by direct plating and wet oxidation. To date, 5 deficient animals and 4 controls were analysed at one hour, 6 deficient and 3 controls at six hours, and 2 deficient and 1 control at twenty-four hours. Very little difference was noted between the metabolism of the controls and

lower abdominal aorta, and intravenous injections into the jugular sinus. Tissues were analyzed by the Van Slyke wet oxidation method, precipitated as barium carbonate, plated and counted in a nuclear measurement proportional counter.

Tumor tissues showed a significantly higher specific activity of the drug when given intra-arterially. Muscle also showed a higher specific activity, and liver, spleen, thymus and lymph nodes showed a lower specific activity with intra-arterial injection.

The toxicity of nitrogen mustard by intra-arterial and intravenous injection was studied. Using 48 male Sprague-Dawley rats per route the LD_{50}^{30} of nitrogen mustard given intra-arterially was 1.25 mg/kg, given intravenously was greater than 1.5 mg/kg and less than 1.75 mg/kg.

Rats bearing the Walker tumor were injected intra-arterially and intravenously with 1.0 mg/kg HN_2 and sacrificed at six hours, twenty-four hours, three, seven, and fourteen days. Autopsies were done and sections turned over to histopathology for microscopic examination to find any variances in tissue damage when the drug was given by different routes.

B. Metabolism of C^{14} -Caffeine (Visc. Mages)

A metabolic study of caffeine labeled in the 1 and 7 positions was made. Preliminary work showed that tissues and urine could be counted as direct plates of water homogenates without loss of activity when dried in air without application of heat.

This study shows that both $1-C^{14}$ and $7-C^{14}$ caffeines are

excreted rapidly in the urine when injected into the jugular sinus of Sprague-Dawley rats. When the animals were sacrificed at six hours, about 40 per cent of the injected doses was recovered in the excreted urine. GI tract and feces contained about 10 per cent. Liver, muscle and bone marrow followed in that order. When sacrificed at twenty-four hours up to 75 per cent of the injected dose had been excreted in that order. Per cent remaining in the body was negligible. The liver was highest with about 1.5 per cent, then muscle and bone marrow.

Less than 1 per cent was excreted in the respiratory air even at twenty-four hours. The per cent concentration in tissues appeared to be the same with the 1-C¹⁴ and 7-C¹⁴ compounds.

Paper partition chromatography shows five or six metabolites in addition to some unchanged caffeine. These metabolites appear to have the same RF factor with 1-C¹⁴ or 7-C¹⁴ caffeine.

Attempt to separate the metabolites of the urine for chemical identification by use of columnar chromatography has been started and will be continued.

g. Effects of Total Body Radiation on the Cholinesterase Levels of the Erythrocytes in Mice (Sabina, Miller)

Following a single dose of 300 r (X ray, 250 KVP, 15 MA) the red cell cholinesterase values rose to a peak (144 per cent of the control value), then fell sharply to values around 60 per cent of the controls and then returned to normal levels in about sixteen days. The fourth day high value is also found following the injection of plutonium, as was reported last year.

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Mice have been subjected to varying doses of X rays from 50 r to 525 r, and the cholinesterase values in the red cells obtained on the fourth day post-radiation. Increased values (5 to 45 per cent of the controls) have been found at all dose levels. So far there is no apparent correlation between dose and magnitude of effect. Individual variations are so great that a very large number of animals would be required to detect a mathematical relationship if such exists. The more profitable way to extend this work is probably to use larger animals so that changes can be detected in individuals.

The fourth day high values with subsequent rapid fall are difficult to interpret at the present stage, but have several interesting aspects. The rise presumably reflects a transient reaction of the bone marrow, a release of young cells in numbers too small to give a reticulocytosis. The sudden drop is unprecedented in any conditions so far observed. In human clinical conditions the "recovery" curve is a gradual return to the normal level. What happens to these young cells in the irradiated mice is not known at present. Another question to be answered is whether the observed value, referred to the normal level for the individual, either on the fourth day or on the twelfth, when a much wider spread of data is found than at other times, has prognostic value. The investigation should also be extended to doses lower than 50 r, since the phenomenon of the fourth day rise might conceivably be useful in evaluating the dose received by the bone marrow in an individual on any one occasion.

The fact that any change can be demonstrated in the peripheral red cell picture with doses of 300 r and less is in itself of

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considerable interest.

r. Blood Lactate and Pyruvate in Monkeys Subjected to Large Doses of Gamma Radiation (Sabine, Turner, Miller)

Blood lactate and pyruvate determinations were made on monkeys following 1000 - 30,000 r of gamma radiation. Regardless of radiation dose no highly significant changes were found in either constituent immediately or two hours after exposure.

2.2 Radiobiology Section (S. Rothman, Acting Section Leader)

2.2.1 General Comments on Section Activities

The activities of the Radiobiology Section during 1953 were directed along the following general lines:

- a. Observations of long term effects of single and multiple doses of X and gamma rays and neutrons.
- b. Studies of the acute effects of massive, rapid doses of X rays, gamma rays and neutrons.
- c. Determination of the RBE of radiations of different types and different energies using a variety of biological test systems.
- d. Studies of the mechanism of biological action of various types of radiations.
- e. Investigation into prophylaxis and therapy of radiation damage.
- f. Metabolic studies in mammals and bacteria using C¹⁴-labeled compounds.
- g. Toxicity of various materials of specific interest to the

Los Alamos Scientific Laboratory.

h. Participation in group projects on effects of massive doses of gamma radiation on monkeys and studies of the radiation hazards to personnel within an atomic cloud.

The Section lost many valuable persons during 1953, some, fortunately, only temporarily. John Storer left for two year's service with the Army Medical Corps. L. E. Ellinwood left to enter Medical School. John Furchner took a year's leave of absence to complete training for his Ph.D. degree. Major Payne Harris, upon completion of his tour of duty at Los Alamos, was transferred to Sandia Base. Lt. Col. L. E. Browning was transferred to Washington, D. C., where he assumed duties as Surgeon of the Armed Forces Special Weapons Project.

Mr. William Schweitzer joined the Section during the year. Mr. Tom Moore worked temporarily in the Group as a summer employee.

2.2.2 Articles and Documents Originating in the Section

The following reports, written in whole or in part by members of the Radiobiology Section, were issued or in manuscript by the end of 1953:

- a. Comparative Biological Effects of U^{233} and U^{238} (Normal Uranium), Renal Function Studies, LA-1333 (1953). W. J. Eversole, LASL Consultant.
- b. Studies on the Mechanism of Action of Thermal Neutrons in Producing Biological Effects. I. Lack of Effect of Hypoxia and Glutathione in Altering Mortality in Mice, LA-1527 (1953). J. B. Storer, L. E. Ellinwood,

and W. H. Langham.

c. The Relative Biological Effectiveness of Tritium in Depressing Iron Uptake in Rats, LA-1544 (1953). J. E. Furchner, J. B. Storer.

d. Additivity of Thermal Neutrons and X Rays in their Acute Lethal Action on Mice, LA-1502 (1953). J. B. Storer, P. S. Harris.

e. Effect of Pituitary Extracts on Radiation-induced Testicular Atrophy in Rats, Proc. Soc. Exp. Biol. and Med., 83 259 (1953). J. B. Storer, P. Sanders.

f. The Relationship of Pyridoxine and Its Derivatives to the Mechanism of Action of Isoniasid, Proc. Soc. Exp. Biol. and Med., 84 292 (1953). I. U. Boone, K. T. Woodward.

g. The Toxicology of Lithium Compounds (in manuscript). L. E. Ellinwood.

h. The RBE of Tritium Beta Radiation Using the Spleen-Thymus Weight Decrease of CF-1 Female Mice (in manuscript). F. C. V. Worman.

2.2.3 Progress of Specific Projects

a. Effects of Radioactive Particles in the Respiratory Tract of the Rat (Boone, Turner with Anderson, Worman, Farringa and Larkins)

A long term study to determine the local effects of radioactive particles in the lung of the rat has been undertaken. Small spherical pellets containing varying amounts of beta and alpha activity are to be implanted in the lungs of rats. The rats will be observed extensively for signs of lung damage. At present, the surgical techniques and physical problems are being worked out. Small gold pellets are being imbedded into

the lungs without the aid of thoracotomy and then located in the lungs by chest X rays. Post-mortem lung X rays with pathological sections of the beaded areas are being studied. CMR-4 has kindly offered to work on the difficult problem of incorporating the radioactivity into the gold pellets as an alloy.

b. Studies of the Relative Effects of Neutrons, Gamma Rays, and X Rays on Cataract and Tumor Incidence and on Longevity of Mice.
(Rothermel, Strang, Sanders, Harris and Storer)

Mice were irradiated in 1951 and have been followed over the ensuing period. The work has been described in the two previous reports. Most of the original 1200 mice have, by now, died. One publication has been prepared on the results at the end of 30 weeks, and a second is being prepared on the results after one year. A final statistical analysis is also being made.

In general, it may be stated that insofar as lethality is concerned, there appears to be no significant difference in the RBE of thermal column exposure as compared with X rays whether 30 days, 30 weeks, or 1 year is used as the time for making the comparison. This value has been found to be 1.7. Likewise, there has been little change in the RBE of thermal neutrons in producing lens opacities, the corrected value remaining about 6.

c. Cataractogenic Effect of Thermal Neutrons and 250 KVP X Rays when Administered in Fractional Doses. (Rothermel, Strang, Sanders, Schweitzer and Storer)

This project was begun in 1952 and was described in the

previous annual report. Doses of X rays and thermal neutrons were given to groups of mice in single acute exposures and in fractions of from four to twelve exposures at two-week intervals. The primary goal was to compare the additivity of multiple neutron exposures with the additivity of multiple X-ray exposures, especially in their cataractogenic effect.

A preliminary analysis of the data to date indicates that the chronic neutron exposures have been far more effective in producing lens opacities than chronic X-ray exposures, i.e., there has been less recovery after each partial dose of neutrons. Lethality and tumor producing effects appear roughly comparable. The over-all tumor incidence, however, is too low to make positive statements.

A one-year report is being prepared.

d. The Effects of Massive, Rapid Doses of X Rays Given Selectively to the Rat Brain. (Woodward, Rothermel and Worman)

Reports in the literature, based upon a limited number of animals, indicate that the general features of the syndrome produced by whole body irradiation with doses in excess of 10,000 r can be duplicated by irradiation of the head alone. The general features of the illness and the median survival times were reported as the same. On the other hand, another report in the literature indicates that the same features can be produced by irradiating the rump alone. An evaluation of these conflicting reports was undertaken. The extensive series of animals given massive, rapid, whole body gamma irradiation in this laboratory served as excellent basis for comparison.

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Using a 50 KVP X-ray machine with a beryllium window, it was possible to obtain a dose rate of about 4000 r/min through the rat skull to the brain. The rest of the body was shielded and received no radiation. Doses of from 2500 to 100,000 r were given to the brain. Above 10,000 r, it was found that survival time did indeed parallel survival time after whole body irradiation. It appears, therefore, that the limiting factor in survival is the central nervous system, and that animals receiving such doses to the whole body die a central nervous system death. Physical signs of the animals given selective brain irradiation likewise paralleled those from whole body irradiation insofar as neuromuscular signs are concerned (e.g., in-co-ordination, irritability, and above 20,000 - 30,000 r, convulsions); however, other body signs, such as the diarrhea so prominent after whole body irradiation, were lacking. In a few preliminary studies of animals given spot radiation to other sites, such as the rump, neuromuscular signs were absent, and diarrhea pronounced. Furthermore, the survival times of animals receiving massive radiation to the rump, though far less than the three and one-half days reported as the intestinal death time, were not so short as those given the same doses to the brain.

g. Median Survival Times of Mice Following Large Doses of X Rays and Thermal Neutrons. (Rothermel and Woodward)

This study, mentioned in last year's report, has been extended in order to compare the effects of massive doses of X rays and of neutrons. The rem value used in calculating neutron doses was that obtained from 30-day lethality studies.

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Doses of from 5000 to 20,000 rem were given. No significant differences were noted between mice receiving X rays and those receiving neutrons in this dosage range. Survival times were similar. Likewise, the effects of these exposures, given at dose rates of less than 100 r/min, do not differ significantly from the effects of massive gamma radiation given at dose rates of 1000 to 6000 r/min.

f. The Relative Biological Effectiveness of Thermal Neutrons in Producing Lethality in Rats. (Furchner, Ellinwood, Sanders and Storer)

Since the RBE of any type of radiation has significance only in connection with the biological system used, and since several systems have yielded different RBE's for thermal neutrons (e.g., mouse lens opacities vs mouse 30-day lethality), the studies on the RBE of neutrons have been extended to new systems. It is hoped that such RBE findings may be an approach to the mechanism and differences in mechanism by which various types of radiation produce their effects.

In this study an RBE on rat lethality was desired for comparison with the RBE obtained on depression of Fe^{59} uptake by the red blood cells. The two test systems gave RBE values of ~ 1.6 and ~ 1.1 , respectively.

g. The Relative Biological Effectiveness of Thermal Neutrons in Producing Intestinal Weight Loss in the Rat. (Ellinwood, Schweitzer, Sanders and Storer)

When mice are given comparable doses of thermal neutrons and X rays based on 30-day lethality an interesting difference in survival times is noted at the shorter time periods. Mice given thermal

neutrons in the dose range of 500 - 1000 rem show an earlier wave of death than mice given corresponding doses of X rays. Thermal neutrons produce a three and one-half day mortality peak at a lower level by about 200 - 300 rem than do X rays. Reports in the literature indicate that the three to four day death is a gut death. Therefore, it may be that neutrons have a higher RBE on the gut than they do on body systems responsible for a ten to fourteen day death which occurs with doses of less than 500 rem.

Rats were exposed to doses of thermal neutrons and X rays which were found to be equivalent in producing 30-day lethality. On successive days after irradiation, the intestines were removed from pylorus to ileocecal juncture, washed, and weighed. Preliminary results indicate that there may be greater weight loss after neutrons than after X rays.

h. The Relative Biological Effectiveness of Thermal Neutrons in Producing Testicular Atrophy in Mice. (Storer, Ellinwood, Furchner and Sanders)

This study, begun in 1952, was completed in the past year. When atrophy of the testis of the CF_1 mouse was used as the biological test system $\sim 8 \times 10^9$ thermal n/cm^2 produced an effect equivalent to 1 r or 250 KVP X radiation. Depending on various assumptions regarding depth of the testes with respect to the region of peak neutron collision, density calculation of the RBE of thermal neutrons gave values of 1.2 - 2.5 with 1.3 being the more likely figure.

i. The Relative Biological Effectiveness of Thermal Neutrons

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in Depressing Antibody Production in the Rat. (Woodward and Sanders)

Another system being considered for an RBE determination is that responsible for antibody production. Curves have been worked out for antibody depression after X rays. Doses of between 100 and 400 r depress the formation of antibodies in increasing degree if the antigen is given after the radiation. The production of hemolysins to sheep cells has been studied extensively in the rat.

A procedure involving the use of sheep cells in the rat is being developed preparatory to a comparison of the effects of neutrons and X rays.

j. Determination of RBE with the Use of AKm Leukemia in CF₁ Mice. (Boone, Turney, Rothermel and Woodward)

CF₁ mice are being inoculated with an AKm mouse strain of leukemia after exposure to varying doses of X rays and neutrons in an attempt to determine an RBE with the per cent leukemia takes as an end point. A preliminary study with X rays indicates 100 per cent takes following 400 r, about 90 per cent following 300 r, about 60 per cent following 200 r, and about 30 per cent following 100 r. There is a normal take in control CF₁ mice of about 20 per cent. Preparations are under way for a combined X ray and neutron study.

k. RBE of Various Radiations on Microorganisms. (Boone, Worman and Turney)

Several attempts were made to determine the RBE of various ionizing radiations using uptake of C¹⁴-nicotinic acid by microorganisms

as the biological test system. Several microorganisms were tried but many technical difficulties with exposure techniques were encountered. Difficulty in repeating the gamma ray doses with existing Co⁶⁰ and Ra sources has been the greatest problem. Some attempts to use tritium and determine the RBE of tritium beta particles have been made, but the work is temporarily in abeyance until the cobalt source room is made available so that conditions will be more constant.

1. The Relative Biological Effectiveness of 14 Mev Neutrons in Producing Lethality in Mice. (Ellinwood, Schweitzer, Woodward, Storer)

Deuteron bombardment of a tritium target in the Cockcroft-Walton accelerator is being used as a source of a pure spectrum of 14 Mev neutrons.

As an initial project to approximate the rem value of 14 Mev neutrons, an LD₅₀³⁰ study was made in 1953. The lethality data spread badly, however, for both the neutrons and the 250 KVP X-ray controls. Although the results were subject to a large statistical error, $\sim 1.3 \times 10^8$ 14 Mev neutrons/cm² was equivalent to 1 r of 250 KVP X ray. Plans are to refine the exposure techniques and repeat the experiment in 1954.

2. The Production of Lens Opacities in Mice with 14 Mev Neutrons. (Boone, Rothermel and Strang)

The mice remaining from the 14 Mev Cockcroft-Walton neutron exposures are being examined periodically for cataract incidence. Up to the present time no statement can be made regarding the relative effects of 14 Mev neutrons and 250 KVP X rays for producing lens opacities in mice.

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a. The Relative Biological Effectiveness of 14 Mev Neutrons in Producing Spleen-Thymus Weight Loss in Mice. (Ellinwood, Harris, Schweitzer and Sanders)

Atrophy of the spleen and thymus of the mouse was used as the biological test system to determine the RBE of 14 Mev neutrons compared to 250 KVP X ray. The results showed that 1×10^8 n/cm² was equivalent in effect to 1 r of X radiation. On the basis of rep calculated from theoretical single collision curves the RBE of 14 Mev neutrons was 1.6. Therefore, the RBE of the 7 Mev recoil protons must be between 1 and 2 for the biological response observed.

a. The Relative Effects of Energy Equivalent Injections of Pu and Ra in Depressing Fe⁵⁹ Uptake by Red Blood Cells of Rats. (Furchner and Lotz)

Rats were injected with equivalent amounts of Pu and Ra (on an energy basis) as a part of an extensive study on the effects of varying types of radiation upon bone marrow function as measured by Fe⁵⁹ uptake by red blood cells.

On the basis of results obtained it was not possible to assign values for the RBE of the alpha particles of Pu and Ra. The inhomogeneous distribution of these elements in the body made valid comparisons with total body radiations impossible. Pu was roughly 20 times as effective (on the energy basis) as Ra in depressing bone marrow function probably because Pu was deposited in the endosteum and the periosteum while Ra was deposited in the spatite structure of the bone. The results

dc, however, give the first experimental evaluation of the relative effectiveness of the two modes of deposition with regard to radiation of the bone marrow.

p. The Sulfhydryl Content of the Lens Following Cataractogenic Doses of Radiation. (Rothermel and Strang)

This work, undertaken last year, has been completed. The sulfhydryl content of lenses of rats which had been given cataractogenic doses of radiation were followed for 16 weeks after irradiation (short of opacity development). Some determinations were made immediately after irradiation and others at intervals during the ensuing weeks.

No decrease in the sulfhydryl content of the lens as a function of time after irradiation could be demonstrated. It appears unlikely that the biological effects of radiation can be explained by any immediate, detectable, over-all biochemical change in the tissue irradiated. Similar results to those reported here have recently appeared in the open literature.

q. Studies on the Mechanism of Action of Thermal Neutrons in Producing Biological Effects. The Effect of Prophylactic Agents on Survival. (Storer, Ellinwood and Sanders)

Preliminary experiments, mentioned in last year's report indicated that certain prophylactic agents protected against X rays but not against neutrons. These studies have been repeated. Mice pretreated with p-aminopropiophenone (PAPP) and glutathione were partially protected against X and gamma rays but not against thermal neutrons.

It thus appears that the effect of the high specific ionization resulting from neutron irradiation is independent of the concentration of molecular oxygen in the animal since the effect is immune from interference by added sulfhydryl groups. This work has been reported in LA-1527 (1953).

r. Prophylactic Value of Various Bone Marrow Stimulants Against X Radiation. (Ellinwood, Sanders, Schweitzer and Storer)

Naphthalene and pyrogallol were administered to mice to induce myelopoietic and erythropoietic hyperplasia before irradiation. No protective effect could be demonstrated and the study was dropped.

s. Studies on the Therapy of Radiation Illness. (Storer, Ellinwood and Sanders)

The reported beneficial effects of splenic implants upon radiation illness was used as a point of departure. Thymuses from Ak mice were implanted into irradiated Ak mice. No beneficial effect could be demonstrated despite the beneficial effect of spleens in this strain. Thymuses, lymph nodes, and spleens were also transplanted from white Swiss mice into irradiated white Swiss mice. There were no beneficial effects from any of these implants - including the spleens.

This work strengthens the belief that therapy by spleen implants is effective only in certain highly inbred strains.

t. The Effect of Dicumerol in the Treatment of Radiation Illness. (Ellinwood, Schweitzer and Storer)

Dicumerol has been shown to prevent clinical radiation pneumonitis when X rays have been delivered to the thorax. The possibility

reverse the inhibition in such organisms as Lactobacillus plantarum, Saccharomyces carlsbergensis and two strains of E. coli. No reversal of isoniazid inhibition with vitamin B₆ or its derivatives however could be obtained in any of the Mycobacteria tested. It seems more likely that if isoniazid acts as an antagonist of pyridoxine or its derivatives in Mycobacteria it is not a simple substrate competition or uptake phenomenon. Preliminary results indicate that the uptake of C¹⁴-labeled isoniazid by Mycobacteria is very small and as yet no conclusion can be drawn as to the mode of action of isoniazid in inhibiting their growth.

x. Isolation of C¹⁴-Labeled DPN and TPN from Lactobacillus Following Incorporation of C¹⁴-Nicotinic Acid and Its Amide into the Media. (Boone, Turner and Woodward)

While in the process of studying the uptake of C¹⁴-labeled nicotinic acid and its amide by Lactobacillus plantarum (arabinosus) (17-D AATCC No. 8014) and several other organisms, it was found that 50 per cent of the labeled material could not be removed from the cells with saline washes but was removed with pH 4 buffer. The material was chromatographed and identified to be diphosphopyridine nucleotide (DPN) and triphosphopyridine nucleotide (TPN). These compounds could be eluted from the filter paper and with the use of Hemophilus influenzae the quantity and specific activity of the materials could be determined. The conversion of the nicotinic acid to DPN occurred within an hour even when added to the bacteria suspended in physiologic saline. This method has promise as a means of preparing commercial amounts of DPN.

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v. Adsorption of C^{14} -Nicotinic Acid and Related Compounds by Lactobacillus. (Woodward, Turner and Boone)

The metabolism of C^{14} -nicotinic acid by Lactobacillus has been shown to be a cell surface phenomenon. Both growing and saline suspended cells adsorb C^{14} -nicotinic acid and reach cell surface saturation at a quantity 20-fold in excess of the requirement for maximum growth of the organism.

The adsorbed C^{14} -nicotinic acid is incorporated as DPN and TPM. No other labeled metabolic products or intermediates were isolated in the cellular desorbate or in the spent growth medium. The localization of coenzyme on the cell surface of a microorganism is in contrast to storage in the mitochondria of liver and kidney cells. Adsorbed coenzyme is transmitted to daughter cells with fission of the parent, as evidenced by the reproduction of saturated cells for several generations in nicotinic acid free medium.

Less than 0.1 per cent of C^{14} -nicotinic acid or amide is degraded to C^{14} -carbon dioxide. All known fractions account for 60 - 75 per cent of the total C^{14} activity.

Structural isomers of nicotinic acid also labeled with C^{14} are not adsorbed by Lactobacillus and suggest that the meta carboxyl position is an essential orientation of nicotinic acid and its amide for adsorption and assimilation.

2. The Uptake of C^{14} -Labeled Isoniazid by Mycobacterium. (Boone and Turner)

Various strains and species of Mycobacterium are being

used to measure the uptake of C^{14} -isoniazid. Strains which are resistant and nonresistant to the drug are being used to measure the uptake in media where concentrations of known or suspected antimetabolites or inhibitors of isoniazid are also present, such as pyridoxine and its derivatives, sodium pyruvate, and biotin. No conclusive results have been obtained to date.

aa. The Relationship of Pyridoxine to Isoniazid as an Antimetabolite in the Rat. (Boone and Turkey)

An extensive study was undertaken to determine what relationship, if any, exists between pyridoxine and isoniazid in animals. Eighty mature rats were divided into four groups of 20 animals each. These groups were fed a basal diet deficient in vitamin B_6 supplemented by intraperitoneal injection as follows: 1) Control (basal diet supplemented by B_6), 2) basal diet supplemented by desoxypyridoxine, 3) basal diet plus 50 mgm/kilo isoniazid, and 4) basal diet plus B_6 , plus 120 mgm/kilo isoniazid daily. All animals were pair-fed and injected with the supplement daily. All animals were observed for the effect of the various regimens on weight loss and signs of ill health, effect on tryptophane metabolism as measured by xanthurenic acid output, tissue pathology and metabolism of C^{14} -isoniazid.

The results indicate that animals that were deficient in pyridoxine and received only minimal doses of isoniazid had greater weight loss than any other group. These animals developed severe convulsive seizures earlier than those in Group 4. These seizures could be reversed

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or prevented with adequate doses of pyridoxine. The xanthurenic acid output with forced tryptophan does not appear to be increased by isoniazid.

2.3 Radiopathology Section (C. C. Lushbaugh, Section Leader)

2.3.1 General Comments on Section Activities

The activities of the Pathology Section can be summarized as follows:

- a. Research on cytological effects of radiation upon individual cells and tissues with particular reference to their ability to divide and reproduce themselves.
- b. Research on the nature of the damage produced in skin by beta irradiation with particular emphasis on the chemical changes produced by massive, rapid doses of ionizing radiation.
- c. The provision of service to other sections and groups requiring pathological interpretation and preparation of experimentally and clinically obtained materials.

The Section was fortunate in obtaining the services of John Spalding, Ph.D., Lora Belle Hughes and Rita Smith.

2.3.2 Articles and Documents Originating in the Section

- a. Experimental Acute Radiodermatitis Following Beta Irradiation. I. Its Pathogenesis and Repair, Cancer, 6 671-677 (1953). C. C. Lushbaugh, J. B. Storer and D. B. Hale.
- b. Experimental Acute Radiodermatitis Following Beta Irradiation. II. The Inhibition of Fibroplasia, Cancer, 6 678-682 (1953).

C. C. Lushbaugh and J. B. Storer.

c. Experimental Acute Radiodermatitis Following Beta Irradiation. III. The Changes in Water, Fat and Protein Content, Cancer, 6 683-685 (1953). C. C. Lushbaugh and D. B. Hale.

d. Experimental Acute Radiodermatitis Following Beta Irradiation. IV. Changes in Respiration and Glycolysis, Cancer, 6 686-689 (1953). C. C. Lushbaugh and D. B. Hale.

e. Experimental Acute Radiodermatitis Following Beta Irradiation. V. Histopathological Study of the Mode of Action of Therapy with Aloe vera, Cancer, 6 690-698 (1953). C. C. Lushbaugh and D. B. Hale.

f. The Effect of Rapid, Massive Doses of Gamma Radiation on the Behavior of Subhuman Primates. LA-1558 (Confidential). W. H. Langham, S. J. Kaplan, J. E. Pickering, C. C. Lushbaugh, W. Haymaker, J. B. Storer and P. S. Harris.

g. Comparative Study of Experimentally Produced Beta Lesions and Skin Lesions in Utah Range Sheep. Intra-Commission Project Report used as an Appendix to the final report of the Atomic Energy Commission on the 1953 sheep losses in Nevada. C. C. Lushbaugh, J. P. Spalding and D. B. Hale.

2.3.3 Progress of Specific Projects

a. Gross and Microscopic Pathology of Dermal Lesions of Nevada Range Sheep as Compared with Those Produced Experimentally with a Strontium⁹⁰-Yttrium⁹⁰ Application. (Lushbaugh, Spalding and Hale)

These studies are being continued in sheep in order to

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extend the observation of the excellent efficiency of the wool in protecting the skin from strong beta irradiation. Since 3 cm of wool appears to protect from exposure to 100,000 rep, it seems that fallout contamination of livestock during the 1953 Nevada test series should not be a concern of sheep raisers. Gross and microscopic pathology of the lesions of Nevada sheep indicated that they were not the same type as those produced by $\text{Sr}^{90}\text{-Y}^{90}$ beta radiation.

b. Pathological Effects of Rapidly Administered Large Amounts of Radiation. (Lushbaugh, Wallnitz and Hale)

1) Pathologic Studies of Morphologic Effects. -- The histopathology of monkeys exposed to large amounts of radiation is being studied and compared with that of similarly exposed rodents. Special stains are being used which selectively stain endocrinologically active cells in order to determine whether previously observed changes in the pituitary glands of monkeys under these conditions also occur in rats.

2) Cytological Studies on the Effect of Rapid, Massive Doses of Radiation on Mitosis. -- The previously reported occurrence of large numbers of mitoses in the livers and kidneys of heavily irradiated rats has again led to an interest in studying the amount of radiation necessary to prevent mitosis in the regenerating livers of rats. Using the normal mitotic processes of the jejunal crypts the coagulative effects of such large amounts of radiation are being investigated.

c. Endocrinologic Study of the Functional Capacity of the Irradiated Pituitary Gland. (Spalding and Wallnitz)

Paralleling the study of the morphologic changes of the

pituitary gland after irradiation is a study of the amount of radiation necessary to suppress pituitary function as determined by hormonal interrelationships of ovulation in the rat.

d. Additional Biological Test Systems for Determining the Relative Biological Effectiveness of Various Kinds of Radiation Available at Los Alamos. (Spalding, Lushbaugh, Harris, Hale and Hughes)

Because the RBE of various radiations may be dependent on the biological test system used it is desirable to develop as many test systems as possible and to apply them to the assortment of radiations available at Los Alamos for comparison of our results with those of others. Therefore, systems used by other investigators are being developed for use in this laboratory.

1) The Broad Bean Root as a Test System for Studying Biological Effectiveness. — This system was chosen for study and use because of the success obtained with it in the classical experiments of L. H. Gray. The problems of culture of these beans have now been overcome and the beans are growing well under easily reproducible conditions obtainable the year round in Los Alamos.

2) The Growth and Luminescence of Achromobacter fischeri as a Test System for Studying Biological Effectiveness. — This system, developed by Hoyt Whipple and others, is particularly adaptable to conditions at Los Alamos where many of the radiation devices emit their radiation in small parts. The growth and emission of light of these bacteria are being studied by the use of electronic equipment peculiar to this

laboratory. The attempt is being made to standardize the response of these organisms to radiation so that the chemical damage in the cycle responsible for the production of light can be studied.

g. The Pathological Effects of Isoniazid Compared with Those of Desoxypyridoxine. (Lushbaugh and Boone)

In conjunction with another Section, the histological effects of desoxypyridoxine competition with pyridoxine are being studied in order to aid in the determination of the mechanism of action of isoniazid. The interest of the Pathology Section in this problem stems from the reported mitotic arresting properties of these substances for lymphopoietic tissues.

h. Studies of Dermal Beta Ray Injuries in Sheep and Other Animal Species. (Lushbaugh, Spalding and Hale)

Studies which are now in progress are concerned with:

- 1) The Precise Amount of Radiation Required to Produce Permanent Damage to the Wool Follicle of Range Sheep.
- 2) The Mechanisms Involved in Epilation and Regrowth of Hair in Colored Mice Injured with Beta Rays.
- 3) The Effect of Previous Epilating Doses of Radiation Upon the Effects of Subsequent Radiation.
- 4) The Effect of Local Radiation Upon the ATP Content of Skin.

The minute amounts of this important substance present in the skin has led to the utilization of the method of ATP analysis based upon the emission of light by extracts of firefly lanterns when ATP is added.

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This method has now been standardized and tested but its experimental use awaits procurement of additional firefly lanterns.

g. The Effect of Spleen Shielding as A Means of Increasing Resistance to Radiation. (Lushbaugh, Hughes, Hale, Wallnitz and Spalding)

These studies are being continued as three closely related projects:

- 1) The Effect on Serum and Tissue Phosphatases.
- 2) The Effect on the Resistance of Partum and Post-Partum Rats.
- 3) The Effect on the Resistance of the Walker Rat Tumor.

h. Application of Histochemical Techniques to Radionathology. (Wallnitz, Hale, Smith and Lushbaugh).

These techniques are being used in order to determine morphologically where enzymatic alterations have occurred in irradiated tissues after whole or partial body irradiation.

2.4 Biophysics Section (E. C. Anderson, Section Leader)

2.4.1 General Comments on Section Activities

The Biophysics Section was not formally organized until December 1953. There was, therefore, no organized section program during the year. However, the activities of the various staff members transferred to the section are grouped under the section heading as though the section had been in existence during the year.

The various members of the Biomedical Research Group transferred to the new section were E. C. Anderson (Leader), J. D. Ferrings,

F. N. Hayes, F. C. V. Worman, Robert Schuch, Betty Rogers, Louise Larkins, John Larkins and Kenneth Kohr.

During the year Robert Schuch transferred to J-Division, F. N. Hayes was loaned for a time to J-Division, and John Larkins transferred into the Biophysics Section from CMR-7.

During 1953 the activities of the section were grouped into the following general categories:

- a. Continuation of the Neutrino Program.
- b. Studies of the Fundamental Principles of Liquid Solution Scintillators.
- c. Construction and Study of Large Volume Liquid Scintillators.
- d. Dosimetry Measurements and Calculations for Other Sections of the Group.
- e. Participation in the Group Projects for Godiva Radiation Studies, High Dose Effects on Monkeys, and Studies of the Inhalation Hazards to Personnel within an Atomic Cloud.

2.4.2 Articles and Documents Originating in the Section

- a. Determination of Total Body Radiation Using Liquid Scintillation Detectors, *Nature*, 172 521 (1953). F. Reines, R. L. Schuch, C. L. Cowan, Jr., F. B. Harrison, E. C. Anderson and F. N. Hayes.
- b. The Production and Distribution of Natural Radio Carbon, *Ann. Rev. Nuclear Science*, 2 63 (1953). E. C. Anderson.
- c. Copenhagen Natural Radiocarbon Measurements, *Science*, 118

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6 (1953). E. C. Anderson, H. Levi, and H. Tauber.

d. Incidence of Abnormal Lymphocytes in the Peripheral Blood of Rabbits During and After Chronic X Radiation, LA-1494 (1953).

F. C. V. Worman and R. E. Carter.

e. Further Study of Hematological Changes in Humans Chronically Exposed to Low Level Gamma Radiation, LA-1440 (1953). R. E. Carter and F. C. V. Worman.

f. Critical Sedimentation and Viscosity Studies on Calf Thymus Sodium Desoxyribonucleate, J. Colloid Science, Aug. 1953.

V. L. Koenig and J. D. Perrings.

g. Large Liquid Scintillation Detectors, Phys. Rev., 90 493 (1953). C. L. Cowan, Jr., F. Reines, F. B. Harrison, E. C. Anderson and F. N. Hayes.

h. Liquid Scintillation Counting of Natural C^{14} , Phys. Rev., 92 512 (1953). F. N. Hayes, D. L. Williams and Betty Rogers.

2.4.3 Progress of Specific Projects

a. The Neutrino Program. (Anderson, Hayes, Schuch and Rogers)

Participation in the neutrino experiment at Hanford occupied the first three months of 1953. The results of this experiment have been reported in several papers in the Physical Review. From the point of view of H-4, the principal result of the participation was the experience gained in the design and operation of large volume scintillation detectors.

All scintillators used in the program were designed and

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prepared at Los Alamos by Group H-4 and were then transported to Hanford where operations such as filling and emptying the detector and testing the solutions were performed.

b. Synthesis and Study of Organic Scintillators. (Hayes, Rogers, Sanders and Kohn)

New scintillation solutes in the 1,3,4-oxadiazole series have been prepared and tested.

A very detailed study of liquid scintillation solvents has been completed. Relative pulse height and current measurements as pure solvents and as mixtures with toluene have been run with the use of 2,5-diphenyloxazole as a standard solute.

The relative merits of plastic and liquid scintillators have been studied with a variety of sources, noting both source strength and particle energy effects.

c. Construction and Study of Large Volume Scintillation Detectors for Biological Use. (Anderson, Hayes, Schuch, J. Larkin, Fennings and Rogers)

1) The Whole Body Human Counter. -- Preliminary measurements of the gamma emission of the human body were obtained using a modification of the neutrino detector. These results are extremely impressive in that they showed it to be possible to measure *in vivo* the natural potassium content of humans and indicated a sensitivity to Ra of less than 1/10 of the maximum permissible level. These results are published in Nature, 172 521 (1953). Confirmation of the value of this detector is given by the work of Spiers at Leeds and Sievert at Stockholm. They have constructed elaborate ion

chamber installations capable of essentially the same sensitivity. However, two to four hours are required by them to make a measurement which can be made in our counters in 10 minutes. The agreement between our K-40 measurements and Sievert's are striking. We found seven subjects to show activities ranging from 79 to 158×10^{-10} g Ra equivalent with an average of 123. Sievert's values for 12 subjects ranged from 78 to 168 with an average of 114. Sievert has shown a striking linear increase of whole body activity with age, which seems to be in good agreement with the hypothesis that this rise is due to Ra ingested from drinking water supplies. His results suggest that our counter would be of great use in studying the very important problem of the natural Ra level in humans. Of equal or greater importance is the fact that the results of Spiers indicate that the apparatus can be expected to detect in vivo Sr^{90} - Y^{90} at somewhat less than the maximum permissible level.

2) Whole Body Counter for Dogs. — A scintillation detector similar but smaller than the human counter mentioned above was built for the University of Utah for measurement of total Ra and RaTh in the intact dog. The counter, called K-9, is in successful operation and will be described in a forthcoming paper in Nucleonics. It is planned to design and build a somewhat similar detector to measure Sr^{90} through its Bremsstrahlung.

3) The 2.5 Liter Detector (Arm Counter). — A smaller detector than either of the ones mentioned above has been designed, built and tested. It has a 2.5 liter capacity, uses six photomultiplier tubes and is shielded with mercury. Its purpose and use is low level detection

of gamma photons from grossly amorphous samples with volume less than 2.5 liters. Its specific application is counting the human forearm, mice, rats, guinea pigs, rabbits and 24-hour urine samples or other materials of biological interest which can be accommodated in the 12 in. deep by 4 in. diameter cylindrical sample section. The detector portion surrounds all but the opening of the sample section thereby allowing high geometrical efficiency. A thickness of 4.5 in. of liquid scintillator viewed by six photomultipliers with associated electronics and surrounded by mercury shielding makes up the complete detector.

This counter will be used in biological problems and will serve as a small testing model for the human counter yet to be built.

d. Small Scintillation Detectors for Measurement for Beta Radiation. (Anderson and Perrings)

Some work has continued on small detectors for measurements of C^{14} and tritium. The consultant services of Prof. J. R. Arnold of the University of Chicago have been valuable in this connection. Measurements of natural radiocarbon have been made and the stability of the counter considerably improved. Tritium measurements are being made for a W-2 cross-section problem. (Richard Olcott, W-2).

An installation for counting single photon pulses using a Swiss photomultiplier tube was constructed for the Radiopathology Section for adenosine triphosphate assay by the firefly method.

e. Neutron Dosimetry Studies. (Anderson, J. Larkins and Perrings)

One of the most important problems concerning the

biological aspects of the atomic energy program is the determination of physical dose (in rep) delivered to tissue by neutrons. During 1953 a program was started to fabricate, calibrate and use tissue equivalent ionization chambers. Chambers of graphite and tissue equivalent plastic have been constructed and are now in process of assembly. They will be applied to specific problems of determination of RBE for the various neutron sources available at the Los Alamos Scientific Laboratory. It is anticipated that continuance of this project will constitute one of the major efforts of the Biophysics Section during 1954.

Foil measurements have already been made using Codiva and the Cockcroft-Walton accelerator. These measurements will be continued and correlated with tissue equivalent ion chamber measurements and biological effectiveness studies.

f. Ultracentrifuge Studies of Heavy Molecules (Koenig and Perrin)

During the past year sedimentation and viscosity studies were conducted on calf thymus sodium desoxyribonucleate, type III pneumococcus desoxyribonucleic acid and type III pneumococcus somatic and capsular polysaccharides. These results have been submitted for publication and the projects have been discontinued.

g. J-Division Program. (Havas, Schuch, Rogers and Kehr)

Since November 1953, some members of the Biophysics Section have transferred their efforts completely, or in part, to problems of interest to the bomb test division. These efforts evolve around

the application of liquid scintillator techniques to certain diagnostic measurements of atomic detonations.

2.5 Organic Chemistry Section (A. R. Rossi, Section Leader)

2.5.1 General Comments on Section Activities

During 1953 the general activities of the Organic Chemistry Section were as follows:

- a. Synthesis of Labeled Compounds.
- b. Services to other Groups and Divisions of the Los Alamos Scientific Laboratory in the Form of Preparation and Purification of Organic Compounds, Micro-Analysis of Special Materials and Preparation of Scintillator Solutions.
- c. Preparation of Manuscript of a Book to be Published by Interscience Publishers Entitled "Organic Syntheses with Isotopes."
- d. Preparation of Special Scintillator Solutes and Solvents.

The Organic Chemistry Section lost some time moving into the new building.

The isotopic labeling of vitamin B₆ has proved a very difficult job. About 50 per cent of the time of two persons was expended following various syntheses that had promise of leading to the C¹⁴-labeling of this material. Success with the project has not been achieved but additional leads have been developed.

2.5.2 Articles and Documents Originating in the Section

The following documents or articles were prepared by the members of the Organic Chemistry Section:

- a. A Contribution to the Total Synthesis of Alizarin.
Synthesis of 1,2-Dihydroxy-9, 10-Anthraquinone-9-C¹⁴, Org. Chem., 18
489 (1953). D. Lloyd Williams.
- b. Preparation of a Standard Sample of Benzoic-C¹⁴ Acid,
LADC-1385. D. Lloyd Williams.

2.5.3 Progress of Specific Projects

a. Organocadmium Compounds for Scintillation Counting of Neutrons. (Williams and Hayes)

The preparation and study of dimethylcadmium and diphenylcadmium as agents for scintillation detection of neutrons was continued from 1952.

The organocadmium compounds, dimethylcadmium and diphenylcadmium were prepared via the Grignard reaction. Dimethylcadmium, which appeared the more promising of the two for scintillation work, was purified by distillation under a dry nitrogen atmosphere. These compounds are very sensitive to moisture and oxygen and are, in addition, quite toxic.

Solutions of dimethylcadmium in dry toluene were prepared for evaluation in the liquid scintillator phase of the neutrino detection program. Tests made with the pulse height discriminator apparatus indicated this class of compounds exhibited serious quenching properties and were of no value to the neutron detection program.

b. Preparation, Purification and Drying of Cadmium Propionate for Neutron Studies. (Williams, Murray and Ronsio)

Because of its favorable neutron capture cross-section

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very pure, dry cadmium propionate was desired for the neutrino program and for neutron detection studies. Cadmium propionate was prepared from cadmium hydroxide and propionic acid. Since it was suspected that the instability of the solutions was due to hydrolysis of the salt, a sample of the air dried material was tested for water of crystallization. It was found that the two moles of water formed in the reaction were present as water of crystallization. This was completely removed upon drying the salt for one and one-half hours under vacuum at the temperature of boiling alcohol. Solutions of the dry cadmium propionate in the methanol-toluene scintillator medium were prepared for testing. These solutions remained clear for more than one month.

Six kg of cadmium propionate was prepared commercially for the neutrino program. This material was tested for water content upon arrival and was found to contain 2.9 per cent moisture, which made drying, prior to use, necessary. This material was successfully converted to the anhydrous form without decomposition.

c. Preparation of Carbon¹⁴ Scintillation Standards. (Williams)

Two samples of benzoic-C¹⁴ acid were prepared for use as internal standards in determining the efficiency of liquid scintillation C¹⁴ detection systems. The first of these was prepared from a Bureau of Standards C¹⁴ Na₂CO₃ solution. This standard was of doubtful value because of the rather wide limits of error involved in the sodium carbonate standardization.

A second standard was prepared as follows: C¹⁴-carbon

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dioxide was prepared from a sample of C^{14} -barium carbonate and was divided into two approximately equal fractions. One of these was converted to benzoic- C^{14} acid via the Grignard reaction; the other fraction was analyzed on the mass spectograph by R. J. Kandel of GM-2. A sample of the benzoic- C^{14} acid was burned to carbon dioxide which was also analyzed by Dr. Kandel. Isotopic ratio correlation between the two samples was extremely good.

A liquid scintillation C^{14} detection system was standardized with the above benzoic- C^{14} acid for the determination of the natural radiocarbon content of contemporary p-cymene (from trees) by F. N. Hayes.

d. Synthesis of Methyl- C^{14} Alcohol. (Williams)

The reduction of carbon dioxide to methyl alcohol by lithium aluminum hydride reagent was investigated. Methyl alcohol theoretically would be a good medium for the scintillation counting of natural carbon samples. The method was carefully worked out and the only difficulty was in running a lithium aluminum hydride reduction on a large scale; e.g., 30 grams of methanol.

The method was used to prepare a 10 mc amount of methanol- C^{14} for use in the labeling of a hydantoin.

e. Synthesis of Pyridoxine- C^{14} . (Williams)

Labeling of pyridoxine via the known synthetic procedures would be quite undesirable from the point of view of isotopic yield. Therefore, an approach to the problem was taken whereby pyridoxine itself is converted to an intermediate which can be labeled via either the Grignard or the halogen-metal interconversion reaction. This is best

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accomplished by oxidizing either the 4- or 5-hydroxymethyl group to a carboxyl group. Replacement of the carboxyl carbon by an amine group, via either the Curtius, Hofmann or Schmidt reaction, which is in turn replaced by halogen in the Catterman reaction, would give the desired intermediate for the labeling step.

Actually, pyridoxine (2-methyl-3-hydroxy-4-hydroxymethyl-5-hydroxymethylpyridine) is difficult to work with because of the varied and reactive functional groups. Since 4-halopyridines are less stable than 5-halopyridines it was decided to make the 5-bromoderivative. Permanganate oxidation of the 3-methoxy derivative, prepared with diazomethane, gives a majority of the 5-carboxy compound mixed with the other possible products. A much better approach is the formation of a 3,4-ketal with acetone which blocks the 3,4-positions and allows nearly complete oxidation of the 5-hydroxymethyl group to carboxyl. The ketal block is stable only in neutral or basic media and the gamma-hydroxy acid configuration resulting from loss of the block is stabilized by formation of a gamma-lactone. The required hydrazid has been prepared from the lactone with hydrazine hydrate but application of the Curtius reaction conditions results in a reversion to the lactone.

Future work will be along the two immediately available avenues with the intermediates at hand. The barium salt of the 2-methyl-3,4-ketal-5-carboxypyridine will be subjected to the Schmidt reaction. In addition, the 5-amido derivative will be prepared from the lactone for use in the Hofmann reaction.

A third approach, which may be useful if all others fail, is a total synthesis patterned after the improved method now in use by Merck and Company.

This work will be continued.

f. Synthesis of Compounds for Liquid-Scintillator Studies.

(Williams)

The synthesis of a series of furan derivatives for testing as scintillators has been initiated. 2,5-Diphenylfuran was prepared via dibenzoyl ethane. An intermediate, beta-benzoylpropionic acid, useful in preparing other members of the furan series has been prepared.

g. Recovery and Purification of C¹⁴-Nitrogen Mustard. (Murray)

Badly decomposed C¹⁴-nitrogen mustard (8.45 mc) from the Oak Ridge stockpile was processed for recovery and purification.

The project was completed and 5.5 millicuries of pure compound was packaged and delivered to Oak Ridge effecting a saving of several hundred dollars.

h. Preparation of Special Solvents for Scintillator Program.

(Murray)

In order to evaluate the effects of hydrogen in the hydrocarbon solvents of the liquid-scintillator system a series of solvents was desired in which an increasing amount of hydrogen is substituted by fluorine. In order to fill the gaps left by the compounds unattainable commercially, a few fluorocarbons were synthesized.

Highly pure samples of cyclohexyl fluoride and 1,4-difluorobenzene were prepared. The preparation of 1,2,4-trifluorobenzene was unsuccessful because of instability of an intermediate compound.

1. Preparation of Synkayvite-C¹⁴. (Murray)

A synthesis was developed for the preparation of 1,4-naphthalenediol-2-methyl-C¹⁴-diphosphate tetrasodium salt. The investigation included a study of two methods for reducing 2-methyl-1,4-naphthoquinone and a number of methods for separating the Synkayvite from inorganic phosphate. Trial experiments on the 1.7 millimole scale gave yields of approximately 50 per cent based on the quinone. The potentiometric titration curve of the acid (equivalent weight, 81; calc., 83.5) proved to be quite dissimilar to that of phosphoric acid.

No isotopic synthesis has been made because of interest along similar lines by a group of investigators in England.

1. Investigation of Methods of Preparation of Beryllium Hydride. (Murray)

A sample of hitherto unobtainable pure beryllium hydride (free of ether and other contaminants) was desired. A literature study was made of known methods for preparing the hydrides of all elements associated with beryllium in the periodic table. A literature study was also made of the known reactions of ether and etherates with the view to dissociating the complex.

A section report was made recommending what appear to be the more profitable avenues of approach to the synthetic problem.

k. Synthesis of Halogen Substituted Pyridine Ring Compounds. (Murray)

The reaction of Bockemuller and Hoffmann* permits the preparation of alkyl and aryl halides in good yield through treatment of the silver salt of the appropriate carboxylic acid with halogen. Attempts were made to adapt the method to the hetero-nitrogen ring system by preparing 4-bromopyridine and 3-bromopyridine. The silver salts of both nicotinic and isonicotinic acids were extremely resistant to reaction with bromine, even under forcing conditions, and no recognizable product was formed.

l. Synthesis of C¹⁴-labeled 1-Hydrasinophthalasine. (Murray and Langham)

1-Hydrasinophthalasine exerts a profound effect in conjunction with other drugs in effectively reducing hypertension but its metabolism and mode of action is not understood. A labeled sample of the drug would obviously be of value in furthering its investigation. A study of the literature of phthalasines has turned up several promising methods for synthesizing the drug labeled in the diastine ring. This preparation may be attempted in the coming year.

m. Studies of the Synthesis of Pyridoxine-C¹⁴. (Murray)

The vitamin pyridoxine has been discovered to be an antagonist to the tuberculostatic drug isonicotinic acid hydrazid. To further an investigation of this phenomenon, which has been studied already with C¹⁴-labeled Nydrasid, the labeled compound is desired and

*W. Bockemuller and F. H. Hoffmann, Ann., 520 165 (1935).

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several syntheses have been explored. The several known total syntheses of the vitamin require far too numerous steps to be attractive in any carrier-free preparation of the ring labeled compound in the high specific activity requisite to such a study. Of the three substituted groups on the ring that possibly might be labeled late in the synthesis, position 2 is uniquely specific in that it must be methyl to retain the vitamin activity. For these reasons it was decided to attempt the preparation of methyl-labeled pyridoxine.

There are a half dozen known reactions in pyridine chemistry that result in alpha substitutions with an alkyl group, and at least four that produce halogen substitution. It might be hoped in the latter case that a methyl group could be introduced by a coupling reaction such as was employed in the labeling of vitamin K. The desired starting material could be made available by 1) degradation, and 2) synthesis:

1) An attempt was made to oxidize the pyridoxine substituents at 2,4, and 5 to carboxyl with permanganate, since the 2-carboxyl can be preferentially removed. The procedure was not successful, largely because of the small amount of pyridoxine available at the time and an unsatisfactory method of isolating the product. Both factors have been remedied and further trials are warranted.

A series of attempts was made to convert pyridoxine or the triacetate to the stilbasole, in the known manner of dimethylating alpha-picolines. No reaction was found to take place, although every known condition of stilbasole formation was tried.

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2) Effort was directed toward synthesizing the starting material by the oxidative degradation of a suitable isoquinoline derivative, 4-methoxy-5-amino-isoquinoline. This new compound was prepared in good yield by the following series: Isoquinoline, 4-methoxy-5-nitroisoquinoline (new), 4-methoxy-5-aminoisoquinoline (new). Permanganate oxidation of this compound under various conditions, however, never yielded more than minute amounts of 3-methoxycinchomeric acid, despite the fact that oxidation of 4-bromoisoquinoline gave better than 20 per cent yields of 3-bromocinchomeric acid. No method was found to convert this latter compound to the ether. 4-Methoxyisoquinoline proved to be not easily separable from its starting material 4-bromoisoquinoline.

Present effort is being directed toward a total synthesis of the starting material by a modification of the excellent new (1952) pyridoxine synthesis of A. Cohn and J. A. Silk. Ethylbenzylaminoacetate is to be condensed with ethyl alpha-formyl succinate and the ring closed by a Dieckmann cyclization. Treatment of this product with sulfuryl chloride followed by hydrogenolysis is expected to give 3-hydroxycinchomeric ester in good yield. This will be etherified by treatment with benzyltrimethylphenyl ammonium hydroxide with none of the usual complications of addition of the methylating agent to the nitrogen.

3. Proposed Program for 1954

It is not possible to give in detail all the specific projects that will be pursued by the Biomedical Research Group during 1954 because it is felt that the primary responsibility of the Group is to always gear its program, as the problems arise, to the acute needs of the Los Alamos Scientific Laboratory and the Division of Biology and Medicine of the AEC. These problems cannot always be predicted in advance because of the changing nature of the weapons development program. It is possible, however, to list the projects which the various sections are continuing from the 1953 program and those in which there is an obvious foreseeable interest on the part of the above agencies. These projects are listed by title under the section heading to compose a proposed program for each section.

3.1 Biochemistry Section (R. G. Gould, Section Leader)

- a. Effect of Radiation on Synthetic and Metabolic Patterns in Animals with Specific Reference to Lipids, Proteins and Steroids.
(Gould, Lots and Trujillo)
- b. Factors Controlling Cholesterol Metabolism. (Gould and Lots)
- c. Effect of Beta Radiation on the Steroid Composition of Skin.
(Gould, Lots and Lushbaugh)
- d. Effect of High Doses of Radiation on Absorption of HTO from the Gut. (Gould, Trujillo and Langham)
- e. Effect of High Doses of Radiation on Synthetic Functions of

the Gut Wall. (Gould and Trujillo)

f. Effect of Adrenal Function on Water Balance Using HTO. (Eversole and Gould)

g. Comparative Study of the Effects of Radiation on Enzymatic Synthetic Processes in the Intact Animal and on Tissue Slices Following in vitro and in vivo Radiation. (Gould)

h. Significance of Red Cell Cholinesterase Values on the Fourth Day After Irradiation to Radiation Dose and Effect. (Sabine and Miller)

i. Prognostic Value of Red Cell Cholinesterase Levels on the Twelfth Day Post-Radiation. (Sabine and Miller)

j. Influence of the Reticulo-Endothelial System on the Post-Radiation Syndrome. (Sabine and Miller)

k. Effect of Chronic Low Level Radiation Exposure on the Cholinesterase Levels of Red Blood Cells. (Sabine and Miller)

l. Mechanism of Inactivation of Purified Cholinesterase by Radiation. (Sabine and Miller)

m. Studies of the Applications of Ethylenediaminetetraacetic Acid to Toxicology and Metabolism of Radioactive Isotopes.

1) The Effect of Delay in Treatment on the Ability of the Drug to Remove Internally-Deposited Pu.

2) The Effect of Parenterally Administered Drug on Pu Deposited in the Lung.

3) The Effect of the Drug on Pu Deposited in Wounds.

4) Use of the Drug as a Skin Decontaminant and Possible

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Inverse Absorption of the Radio Contaminant.

5) The Extent of Injury to the Kidney Tubules Brought on by the Administration of Large Doses of the Drug under Various Physiological Conditions.

n. Screening Experiments with a Variety of Agents to Attempt to Hasten Excretion of Internally-Deposited Sr^{90} .

o. A Quantitative Investigation of the Comparative Roles Played by the Gall Bladder and the Lower Bowel on the Intestinal Excretion of Parenterally Administered Sr^{90} .

p. Studies of the Mode of Pu Binding by Bone.

q. Studies with the So-called 2.5 Liter Scintillation Counter (Arm Counter) to Measure:

- 1) Turnover of K^{42} and Na^{24} in Irradiated Rats.
- 2) Turnover of K^{42} and Na^{24} in Normal and Hypertensive

Individuals.

3) Cardiac Output.

4) Iron Turnover in Anemia and Polycystemia.

5) Circulation Curves in Peripheral Vascular Disorders.

r. Metabolism of C^{14} -Caffeine Administered to the Rat. (Magee, Ott and Langham)

3.2 Radiobiology Section (S. Rothermel, Section Leader)

a. RBE of 14 Mev Neutrons as Measured by Fe^{59} Uptake in Red Blood Cells of Rats. (Schweitzer, Rothermel and Sanders)

b. RBE of Fission Spectrum Neutrons from Godiva as Measured by

k. Determination of the RBE of Neutrons Using Susceptibility of CF₁ Strain and Possibly Other Strains of Mice to AKa Leukemia. (Boone and Turney)

l. Continuation of the Particle Size Radiation Studies in the Respiratory Tract of the Rat, Concentrating on the Injection of Small Radioactive Particles into the Lungs. (Boone, Turney, Anderson et al.)

m. Completion of the Metabolic Studies of C¹⁴-Isoniazid in Pyridoxine Deficient Rats. (Boone, Turney and Magee)

n. A Continuation of Antimetabolic Studies of Isoniazid, Pyridoxine and Related Compounds in the Mycobacterium as Related to the C¹⁴-Isoniazid Uptake. (Boone and Turney)

o. Continuation of the Preliminary Studies of Mouse Cataract After Exposure to 14 Mev Neutrons. (Boone and Strang)

p. Determination of the RBE of Fission Fragments Using Neutron Exposure of Animals Injected with Pu²³⁸ and Pu²³⁹. (Harris and Langham)

q. RBE of Na²⁴ Beta Radiation in Depressing Fe⁵⁹ Uptake by Red Blood Cells of Rats and in Producing Splenic and Thymic Atrophy in Mice. (Schweitzer and Langham)

r. The Effect of Fission Neutrons from Godiva on the Incidence of Lens Opacities in Mice. (Boone, Rothermel and Strang)

3.3 Radiopathology Section (C. C. Lushbaugh, Section Leader)

a. Microscopic Studies of the Effect of Beta Radiation on the Wool Follicle. (Lushbaugh, Spalding and Wellnitz)

b. Pathological Effects of Massive, Rapid Doses of Gamma Radiation on Radio Resistant Tissues. (Lushbaugh, Wellnitz and Hale)

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- c. Endocrinologic Studies of the Functional Capacity of the Irradiated Pituitary Gland. (Spalding and Wellnitz)
- d. Application of the Effect of Radiation on the Rate of Growth of the Root of the Broad Bean to the Determination of RBE. (Spalding, Lushbaugh and Hale)
- e. The Effect of Irradiation on Growth and Luminescence of Achromobacter fischeri and Its Possible Application to the Determination of RBE. (Hughes and Lushbaugh)
- f. The Effect of Local Radiation Upon the ATP Content of Irradiated Skin. (Hughes and Lushbaugh)
- g. The Effect of Spleen Shielding as a Means of Increasing Resistance to Irradiation. (Lushbaugh, Hughes, Hale, Wellnitz and Spalding)
- h. Application of Histochemical Techniques to Radiopathology. (Wellnitz, Hale, Smith and Lushbaugh)
- i. Pathology Services to the Biomedical Research Group and to the Los Alamos Medical Center. (Lushbaugh, Wellnitz and Smith)
- j. Effect of Radiation on the Functional Capacity of the Reticulo-endothelium Using Colloidal $\text{CrP}^{32}\text{O}_4$. (Wellnitz, Lushbaugh and Langham)

3.4 Biophysics Section (E. C. Anderson, Section Leader)

- a. Development and Application of Tissue Equivalent Ion Chamber Techniques to Neutron Dosimetry. (Anderson, J. Larkins and Perrings)
- Suitable tissue equivalent and graphite ionization chambers are being fabricated and tested. These chambers will be applied to problems

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of neutron and gamma ray dosimetry. Special emphasis will be given to the study of neutron to gamma radiation dose ratios from the Los Alamos homogeneous reactor and the Godiva assembly. Physical measurements using these chambers will be correlated with biological response.

b. Studies of the Acute Tritium Hazard Problem. (Perrings, Anderson and Langham)

Studies will be conducted concerning the biological effects of massive exposures to tritium beta radiation.

c. Construction and Study of the Fundamental Properties of Large Volume Scintillation Detectors. (Larkins, Perrings, Hayes and Anderson)

The construction of a scintillation detector capable of measuring the total gamma emission of the intact human body is contemplated.

d. Synthesis and Study of the Characteristics of Organic Scintillation Solutes (Hayes, Rogers and members of the Organic Chemistry Section)

Studies will be made of the scintillation properties and characteristics of furans, benzoxazoles, oxazole quaternary salts including betaines, polyalkylated polyphenyls, heterocyclic polymers, and solutes into which have been incorporated elements of high Z number.

e. Spectroscopic Studies of Organic Scintillators. (Hayes, Rogers and Kohr)

Emission spectra will be run on various organic scintillators and excitation under the mercury arc will be compared with emission following various types of particle excitation. Secondary solute effects and emission spectra through long spectral paths as well as fine absorption

spectra will be studied.

f. Electronic Testing of Organic Scintillator Solutes. (Hayes and Rogers)

Studies will be made of the relative pulse height vs relative current output of organic scintillators during photoelectric excitation from photons of various energies.

g. Other Studies of the Properties of Organic Scintillators. (Hayes, Rogers and Kohr)

Absorption, adsorption and quenching problems in liquid scintillation counting will be investigated.

h. Application of Scintillation Detectors to Low Level Counting. (Anderson, Perrings and Larkins)

Work will continue on the application of scintillators to the detection of radium and mesothorium in the dogs used in the Utah experiment. Attempts will also be made to study the detection of Sr^{90} in animals by Bremsstrahlung.

i. Installation and Calibration of 250 KVP X-ray Machine. (Worman and L. Larkins)

A new GE Maxitron 250 KVP X-ray machine is being installed. This machine will be calibrated with both physical dosimeters and biological test systems. Much of the time of the above persons will be taken up providing radiation exposure services for other members of the Biomedical Research Group.

j. Procurement, Instrumentation and Calibration of Co^{60} Gamma Source. (Worman and L. Larkins)

It is contemplated that a 1000 curie Co^{60} source will be procured, instrumented and calibrated during 1954 for use in the Biomedical Research Group.

3.5 Organic Chemistry Section (W. E. Leachman, Section Leader)

- a. Continued Compilation of Book on "Organic Syntheses With Isotopes" for Interscience Publishers. (Murray and Williams)
- b. Syntheses and Study of Organic Scintillator Solutes and Solvents. (Williams, Murray, Ott in collaboration with the Biophysics Section)
- c. Labeling of Vitamin B_6 with C^{14} . (Williams and Murray)
- d. Labeling of Beta-Hydroxybutyric Acid with C^{14} . (Williams and Gould)
- e. Special Services to Other Groups and Divisions of the Los Alamos Scientific Laboratory. (Williams, Murray and Ott)
- f. Studies of Methods of Isolation of Metabolites of Labeled Compounds from Urine of Experimental Animals. (Ott and Magee)
- g. Preparation and Standardisation of C^{14} Standard Preparations. (Williams, Kandel of GMK-2, Grilly of CMR-9, and National Bureau of Standards)
- h. Isotopic Labeling of Other Compounds of Biological and Medical Interest to the Biomedical Research Group. (Williams, Murray, Ott, Kohr and Rogers)

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