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HEADQUARTERS
Defense Nuclear Agency
Washington, D.C. 20305

PREPARING AGENCY
University of Cincinnati College of Medicine
Cincinnati General Hospital
Cincinnati, Ohio 45229

Contract No. DASA-01-69-C-0131

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FOREWORD

This report was prepared by the following members of the University of Cincinnati College of Medicine:

Eugene L. Saenger, M. D.
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The research was supported by the Medical Directorate, *Defense Nuclear Agency, Washington, D. C. under Contract No. DASA-01-69-C-0131. The Project Officer for the contract is Dr. Robert Loind.

These studies were performed in conformation with the "recommendations guiding doctors in clinical research" as stated in the Declaration of Helsinki of the World Medical Association (1964) and have been approved by the Committee on Human Research of the University of Cincinnati College of Medicine.

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences, National Research Council.

*Formerly Defense Atomic Support Agency (DASA).

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INTRODUCTION

The University of Cincinnati studies in radiation effect in man continue as a carefully integrated effort to maximize clinical, psychiatric, therapeutic, biochemical, and theoretical approaches to whole and partial therapeutic irradiation as given for palliation of certain selected cancers. The methods of applying radiation have remained essentially the same since the inception of these studies. Previous DNA reports give details of earlier work (1).

The nature of the specific projects undertaken in our laboratories reflects the consideration of many of our faculty and the thoughts and problems of the other DNA laboratories and contractors as determined by the DNA conferences organized over the past several years by Col E. J. Huycke. Valuable interchange of ideas have been stimulated by visitors from Department of Defense laboratories who give our staff a more practical insight into military problems than we might otherwise have. Such contacts have resulted in cooperative studies with AFRRI and mutually profitable discussions with personnel at Air Force Laboratories at Wright Patterson AFB and Kirtland AFB.

A few of the newer developments in our research will be discussed briefly in this introduction as they have important implications in regard to our ongoing goals. Many of the new directions in our investigations stem from concurrent advances in cytogenetics, organ transplantation, bio-chemical aspects of molecular biology, and clinical aspects of cancer therapy.

A renewed interest is manifested in chromosome aberrations as being eventually an index of "effective radiation dose," particularly since almost all exposures encountered in nontherapeutic circumstances will have varying degrees of nonuniformity of dose rate and dose distribution. In spite of current technical complexity, these changes seem to be potentially one of the most useful indicators both of recent exposure and long term effects. Within the next year we shall have a new 4 Mev. linear accelerator available for these studies and the impact of a somewhat higher dose rate can then be evaluated.

In association with these studies are those which attempt to analyze hematological changes on the basis of "active" bone marrow. This work stems from efforts of other investigators to analyze the effects of inhomogenous radiation using a model based on survival studies of mouse bone-marrow stem cells. Our work is investigating the use of a human marrow system. Eventually we hope to relate this approach to a well standardized method of analyzing chromosome aberrations at various times following exposure.

As an outgrowth of our needs to afford maximum protection to patients receiving doses in the LD_{50} range, some new technical advances have been developed in bone marrow transfusion in patients. It has been possible to demonstrate in an increasing number of subjects that the actual removal of marrow and infusion are safe. We have also shown repeatedly with allografts that such infusions decrease significantly the white cell nadir. Other investigators have utilized homografts in human beings primarily in the treatment of leukemia and lymphomata with varying degrees of success (mostly temporary). The use of antilymphocyte globulin has been of value in some cases. Although not a primary goal of our studies, these attempts should be investigated further since the availability of successful therapy against radiation doses of 1 to 2 LD_{50} equivalents would have enormous implications.

In regard to biochemical studies, a number of interesting developments have occurred. Several years ago while investigating urinary excretion of deoxycytidine following irradiation, our attention was drawn to the problem of combined injury. Since the Cincinnati General Hospital and Shrine Burn Center attract many severe cases of thermal injury, urines were obtained from some of these patients. As mentioned in previous reports, the use of deoxycytidine as an indicator of radiation injury in humans proved unsatisfactory at the levels tested due to the presence of deoxycytidine deaminase in the blood. Yet in severly burned individuals deoxycytidinuria occurs late (in 2 to 4 weeks) and in the several patients studied the levels seemed directly related to the extent and depth of the burn. Radiation induced deoxycytidinuria when found occurs within 2 to 3 days and then disappears. Additional studies may suggest this test as a way of differentiating relative contributions of these two modalities of injury.

Also deoxycytidinuria may be of value in providing an independent index of the severity of the thermal injury as expressed by the total organism in contradistinction to the more obvious local effects.

The more detailed analysis of ultraviolet absorbing compounds in irradiated human beings was studied in only two cases in cooperation with Oak Ridge National Laboratory. A great many known and unknown compounds,were excreted postirradiation, many of which are known metabolites of purine and pyrimidine bases. These very preliminary results suggest a rich field of investigation directed to the biochemical lesions of the human being and have important implications of diagnostic, prognostic, and therapeutic interest.

Also the increase in serum amylase has been documented in patients with total and upper body radiation and was not observed in lower body radiation (with a single exception). This approach along with that of UV absorbing compounds suggests that biochemical or enzymatic "biopsy" may be developed as a practical way to evaluate radiation effects.

In regard to psychiatric changes the steady increase in the number of patients available for study and the improvement in selection in respect to severity of illness and level of education have proven helpful. A new paired association test has been added and appears to be more useful than previous ones in the analysis of these patients. In addition, some additional data on the occurence and control of nausea are presented.

HEMATOLOGICAL STUDIES

HUMAN CHROMOSOME ABERRATIONS AS A RADIATION DOSIMETER

Over three decades of in vitro studies on the abnormalities in human chromosome morphology caused by various modalities of ionizing irradiation (1 to 10) have not established unequivocal dose-response curves applicable to human subjects receiving equivalent (whole-body) irradiation. Yet the participants at a recent international IAEA-WHO conference on biological radiation dosimeters agreed that, at this time, analysis of radiation-induced chromosome aberrations was the most reliable method available (11). Our group has analyzed chromosome aberration of lymphocytes of six patients receiving whole-body irradiation up to 200 rads.

Methods.

Patients.

Five of the six patients had adenocarcinoma of the colon metastatic to the liver. Two (091, 107) were bedridden because of pathologic femoral fractures but were otherwise healthy except for mild anemia (hematocrit over 30 percent). One of these (107) had previous therapeutic irradiation (4, 000 rads to right femur and ilium). The other three (095, 096, 098) were ambulatory with normal hematologic values. All were clinically stable in that they maintained their weight and could perform activities of daily living. The sixth patient (105) had a Ewing's tumor of the eleventh thoracic vertebrae irradiated with 4, 600 rads to the upper thoracic and lower lumbar vertebrae. He remains entirely well over 1 year since the diagnosis was made.

All patients gave informed consent after the possible risks of irradiation were explained.

Total-Body Irradiation Dosimetry.

The radiation is delivered by a Cobalt 60 Teletherapy unit under the following exposure conditions.

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The radiation beam is directed horizontally at a wall 338 cm. away with the patient midline at 282 cm. from the source. The beam area for the 50 percent isodose curve at the patient midline distance is a square approximately 72 cm. by 72 cm. The patient is placed in a sitting position with legs raised and head tilted slightly forward. The irradiation is given by delivering half the specified exposure laterally through one side of the patient. The patient is then turned, and the other half exposure is delivered laterally through the other side.

The variation of air exposure with distance from the source was determined with a Victoreen 25 R chamber. The results indicated no departure from the inverse square law relationship for distances used in the study. Therefore, no correction was required for a possible dose contribution to the patient due to backscatter from the wall.

Preliminary measurements were made in a masonite phantom using dosimeters placed on lateral surfaces and at the midline of the head, trunk, and knee portions of the phantom. If the midline doses to the trunk, head, and knees are compared, the maximum variation in these doses is about 16 percent.

The exposure to the patient was determined as follows. The percentage depth dose at different depths for a 400 cm.^{2} field area and a source-skin distance of 80 cm. is given by H. E. Johns, "The Physics of Radiology," Charles C. Thomas, Springfield, Illinois, 1966, pp. 309-351. The depth dose at the greater source-skin distances used for the patients was found by multiplying the depth doses at 80 cm. by the "F" factor postulated by Mayneord and Lamerton (Brit. J. Radiol. 14: 255, 1941).

$$F = \frac{(D_d)_{f_2}}{(D_d)_{f_1}} = \left[\frac{f_2}{f_1} \times \frac{f_1 + d}{f_2 + d} \right]^2$$

Where: f_1 and f_2 are source-skin distances.
d is the depth.

By using the corrected depth dose at the patient midline (one-half lateral dimension of the trunk) and a conversion factor of 0.97 rads per roentgen for cobalt gamma radiation, the surface dose and midline air exposure required to give a desired midline absorbed dose in rads was calculated.

A direct comparison of the calculated and measured (phantom) doses was made for one patient who had the same lateral trunk dimensions as the phantom. The relative depth dose for each lateral exposure to this patient and the phantom compare quite well with the calculated doses. The combined dose of the two radiation fields shows a good homogeneous dose distribution through this patient. The maximum variation in lateral dose distribution was +13 percent for one patient having a lateral trunk dimension of 36 cm.

Doses of 100 and 200 rads midline irradiation were employed with a dose rate of 3 to 5 rads per minute.

Chromosome Studies.

Blood was obtained for chromosome analysis immediately after irradiation by the following protocol adapted from Moorehead (12).

PROTOCOL FOR CHROMOSOME CULTURE

1. A 10 ml. syringe is moistened with 0.1 ml. heparin (1 ml. = 5,000 U.S.P. units containing 1 percent benzyl alcohol) and 10 ml. venous blood is aspirated under sterile conditions.

2. Blood is then transferred to a sterile screw-cap tube. It is mixed gently and the red cells are allowed to sediment at room temperature for 1 hour.

3. 0.5 to 1.0 ml. leukocyte rich plasma is added to 4 sterile, disposable flasks containing 4 to 5 ml. of the following media: 80 percent Minimum Essential Media, * 15 percent Fetal Bovine Serum, 2.4 percent phytohemagglutinin, 1.2 percent l-glutamine, and 1 percent penicillin and streptomycin.

4. The culture is incubated at 37° C. for 46 to 50 hours at ambient pO_2. No CO_2 is added to the system.

5. Then 0.1 ml. 0.0004 percent colchicine in Hank's** balanced salt solution is added to each culture which is returned to incubator for 1 to 2 hours.

*Gibco, Grand Island Biological Co., Grand Island, New York.

**Difco Pharmaceuticals, Detroit, Michigan.

6. The flasks are agitated and contents emptied into a serologic tube which is centrifuged for 2 minutes in an Adams Sero-Fuge.

7. Then the supernatant is aspirated and discarded. The cells are washed with 37° C. 0.7 percent sodium citrate and placed in a 37° C. water bath for 4 to 7 minutes, then centrifuged as in Step 6.

8. The supernatant is again aspirated and discarded. Without disturbing the cell button, one adds 1 to 2 ml. Carnoy's fixative (3 parts Methanol: 1 part Glacial Acetic Acid) and allows the button to stand 30 minutes.

9. Then the cells are resuspended and again centrifuged as before.

10. Steps 8 and 9 may be repeated as needed, usually twice.

11. Next sufficient Carnoy's fixative is added to obtain an opalescent appearance, about 0.5 ml.

12. Slides are prepared by dropping 3 to 5 drops of cell suspension on clean slides which has been wet in cold distilled water, igniting them momentarily in an alcohol burner.

13. They are stained with 1:10 Giemsa stain for 14 minutes.

14. Next the slides are rinsed with acetone twice, acetone: xylol (1:1) once and then are placed in 100 percent xylol until ready for mounting.

15. The slides are mounted by adding 1 drop Permount*** and a coverslip.

Two hundred or more metaphases were counted except in patients whose lymphocytes grew poorly on culture. Metaphase plates were scanned at low power and a cell containing a ring or dicentric was recorded only if a fragment accompained it indicating the cell was in its first mitosis. Mitoses were also rejected if the spread was poor or the full complement of centromeres was obviously lacking. Two experienced chromosome technicians performed the analyses with frequent cross-checking of reproducibility between them.

Results.

Table I indicates the pre and postirradiation occurrence of ring and dicentric chromosomes in lymphocytes from patients receiving 100 to 200 rads whole-body irradiation.

***Fisher Scientific, Fairlawn, New Jersey.

TABLE I

INCIDENCE OF DICENTRIC AND RING CHROMOSOME ABNORMALITIES IN PATIENTS RECEIVING TOTAL-BODY RADIATION

Dose	Patient		Total cells counted	Dicentrics per cell	Rings per cell	Dicentrics + rings per cell
100 rads	096	Pre- R_X	200	0	0	0
		Immediately Post R_X	200	0.025	0.01	0.035
100 rads	105*	Pre- R_X	200	0.025	0.005	0.03
		Immediately Post R_X	200	0.105	0.01	0.115
200 rads	098	Pre- R_X	250	0.004	0	0.004
		Immediately Post R_X	200	0.03	0.01	0.04
200 rads	091+	Pre- R_X	1000	0.001	0	0.001
		Immediately Post R_X	250	0.044	0.02	0.064
200 rads	095	Pre- R_X	200	0	0	0
		Immediately Post R_X	200	0.03	0.015	0.045
200 rads	107*+	Pre- R_X	29	0.035	0	0.035
		Immediately Post R_X	16	0.375	0	0.375
		24 hours Post R_X	44	0.25	0	0.25

*Previous radiation
+Chemotherapy

We have graphed these data as the increment in rings and dicentrics over that patient's pretreatment count (Figure 1). Included for comparison are the data of Buckton et al. (13) who have obtained, with accurate dosimetry, similar data on 16 patients given whole-body doses (mid-plane trunk) of 17 to 50 rads at a dose rate of approximately 0.6 to 1.7 rads per minute from a 2 Mev. Van de Graaff generator. We believe our data and theirs are comparable since the relative biological effectiveness for dicentric formation from 2 Mev. X-rays is about 0.7 (14) and for cobalt-60 gamma rays the RBE is reported as 0.8 (15).

On the graph we have distinguished between two patients who received previous radiotherapy (105, 107, closed circles) and four who never had been irradiated therapeutically (091, 095, 096, 098, open circles). Chemotherapy (5-fluorouracil) had been given to one patient with (107) and one without (091) previous local radiotherapy but not within 4 months of the whole-body irradiation.

Because we have data on only six patients to date we have not attempted to fit the points to any curve, linear, quadratic, or powerfunction.

Discussion.

Because of the variation between mammalian species in dose-response curve for radiation-induced chromosome aberrations (16) extrapolation from animals to man is somewhat hazardous. The radiosensitivity of mammalian cells varies with the phase of cell cycle (17). The human lymphocyte has therefore a unique advantage as a chromosome dosimeter because it exists almost entirely in the G_1 (postmitosis, preDNA synthesis) phase of the cell cycle (18) where radiosensitivity is believed to be fairly uniform (19).

The ring and dicentric aberrations have been employed to estimate the effects of irradiation in the G_1 phase, for these abnormal chromosomes can only be formed in the G_1 phase of the cell cycle before the chromosome has visibly and functionably split into 2 chromatids. The ring and dicentric forms are more easily found than the more subtle translocation and inversion aberrations caused by irradiation. The sum of all visible aberrations apart from dicentrics come to 60 to 70 percent of the total dicentric yield (20).

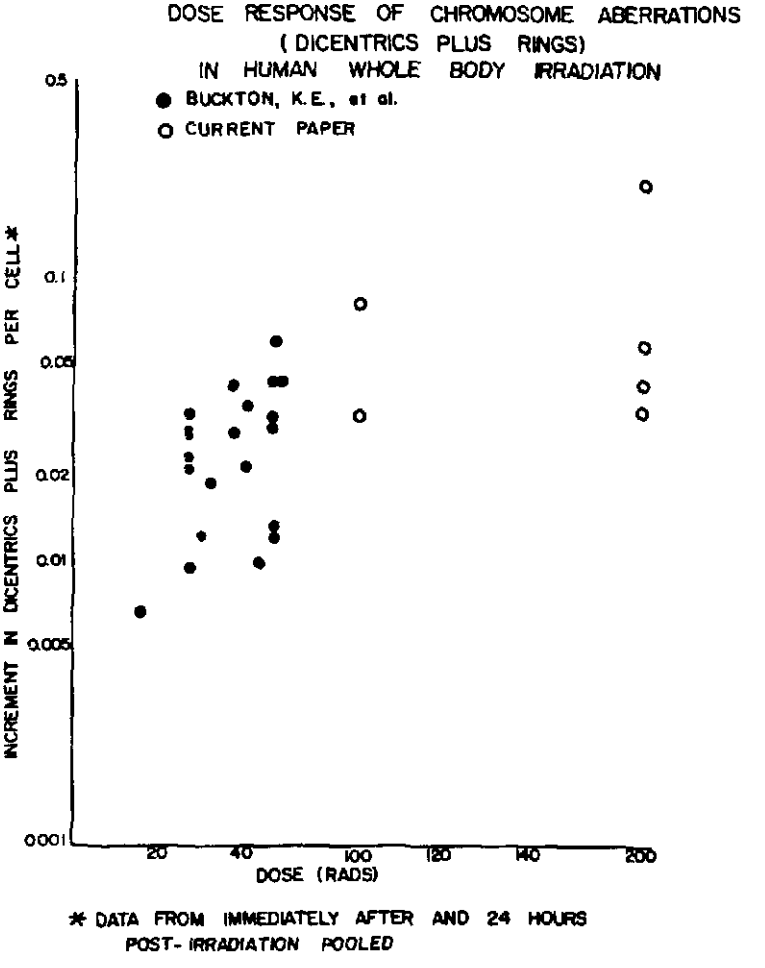

Figure 1. --Lymphocyte chromosome dicentric and ring aberrations as a radiation dosimeter: human in vivo data.

The data presented here, with that of Buckton et al. (13), provide, with accurate dosimetry, human data on whole-body irradiation to doses of 200 rads, at fairly comparable dose rates (1 to 5 rads per minute). From the fairly wide scatter observed the safest statement to make would seem to be that dose estimates made from this system is less predictable than similar observations made from in vitro systems (Figure 2).

An interesting discrepancy can be noted in the effect of irradiation on the chromosomes of two patients (105, 107) previously receiving conventional ^{60}Co radiotherapy. These two patients have aberration frequencies higher than those observed in the four other patients (091, 095, 096, 098) who had not been given therapeutic irradiation. Only one patient in each group (091, 107) had received 5-fluorouracil 4 or more months prior to whole-body irradiation so that prior damage from chemotherapy could not be implicated. One could hypothesize that previously irradiated blood may contain a population of lymphocytes carrying sub-microscopic damage or an increased susceptibility to breakage with further irradiation, perhaps through impairment of enzymes required for DNA repair. One of the two patients (107) has been represented by two points indicating aberrations found immediately after and 24 hours after irradiation. Buckton et al. (13) found a higher yield of aberrations at 24 hours than immediately after exposure only for the dose of 50 rads while at 24 hours our patient had a lower aberration rate than just postirradiation though a few mitoses could be found. Unfortunately patient 107 was lymphopenic prior to treatment and too few metaphases are available to be considered entirely reliable. Bender and Barcinski (8) note that if all of Buckton's samples are considered there is no significant difference between the aberration rate at the two times. Pooling the data from our patient 107 gives a ring plus dicentric frequency for 60 cells of 0.245 when corrected for preirradiation aberrations. Although the general supposition has been that in vivo and in vitro data are comparable the results herein suggest that there may be discrepancies between the systems. Employing 14 Mev. neutrons, chromosome aberration rates have been found to be higher in swine lymphocytes irradiated in vitro than in vivo (21). Since repair of chromosome damage appear

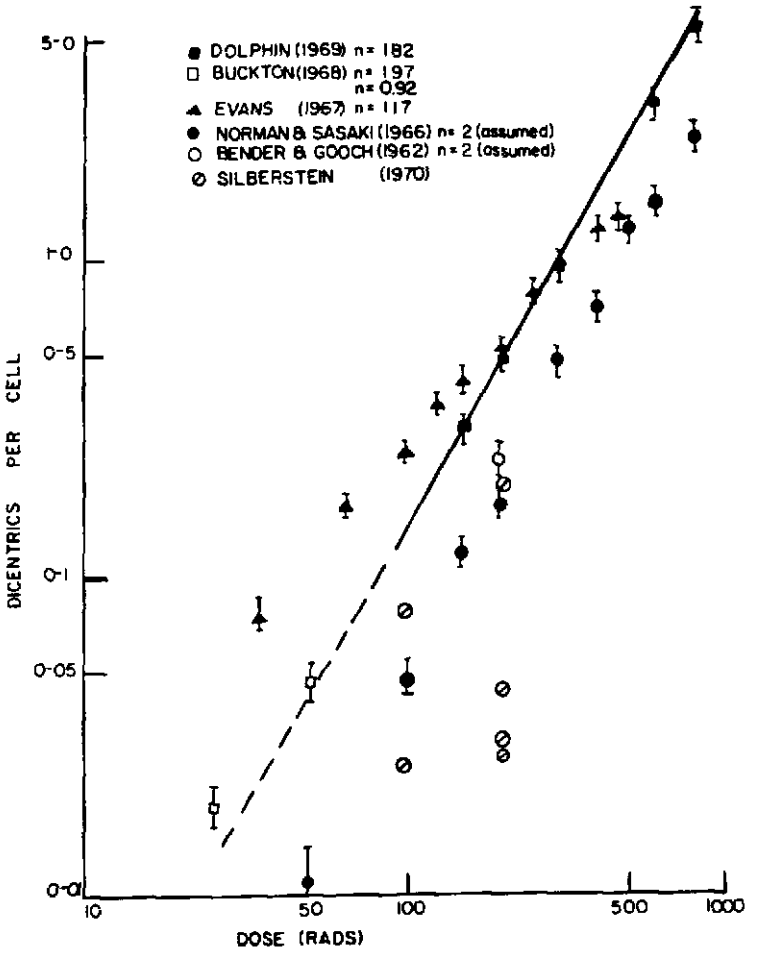

Figure 2. --Lymphocyte chromosome dicentrics as a radiation dosimeter: in vitro data plus in vivo data of this paper.

to occur rapidly in vivo and in vitro (22) the reduced in vivo yields may be due to elimination of cells in vivo.

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HUMAN MARROW TRANSPLANTATION

Despite the exciting advances made in many areas of organ transplantation over the past few years (1) bone marrow transplantation remains an experimental procedure, rarely successful except in the few instances when the technique has been applied to immunologically deficient children (2, 3, 4), or a victim of radiation accident with an identical twin (5). Graft versus host disease (GVHD) caused by the donor marrow has been the limiting factor in successful marrow transplants to patients with leukemia or aplastic anemia (6), although recent reports suggest that anti-lymphocyte globulin may be of value in preventing the acute and subacute stages of GVHD (7).

The technique of marrow transplantation requires the infusion of an estimated minimum of 1.1 to 1.3 x 10^6 nucleated cells/kg or about 1 to 10 x 10^9 cells for an adult (8). To obtain such a volume of bone marrow, most commonly aspirated from the sternum and ilia of the patient, general anesthesia is required. The marrow has then either been filtered or given directly without filtration. The routes without filtration have been intravenously (2, 7, 9) or intraperitoneally

1 sterile red top Vacutainer tube (no anticoagulant), labeled with patient's name and number for the blood bank, plus a blood bank form even if blood for autotransfusion was obtained the previous week.

6 Kurnick needles

2 Conrad-Crosby needles for marrow biopsy.

10 20-gauge needles

3 Bierman needles

\+ 200 cc. heparin solution containing 50 units heparin per cc. This is made by putting one cc. of preservative-free heparin, 10, 000 units per cc., in 199 cc. of normal saline. An extra vial of this heparin is brought to the operating room.

= TC-199 culture medium, 100 cc. buffered with sodium bicarbonate to pH 7. 4 and added to a Fenwal pack.

150 12 cc. sterile plastic (polypropylene) syringes.

\# Sigmamotor Pump Model TM-20-4 with tubing attachments.

4 2-way stopcock adapters

* Sterilized double filter system with two mesh filter openings of 297 and 149 micra diameter. This is made pyrogen-free at 300° F. for 3 hours in a dry oven, then autoclaved at 15 lbs. for 20 minutes.

o 2 Fenwal Blood Pack Units No. TA-10, 1000 cc.

1 thioglycolate culture tube

1 box sterile microscope slides

1 box cover slips

\+ Abbott Venopak adapter

Normal saline for injection, 1- 30 cc. vial

\+ 3 Abbott blood administration sets (No. 4636) with filter opening measuring 150 by 200 micra.

10 isopropyl alcohol sponges

1 hemostat

+Abbott Laboratories, North Chicago, Illinois (Panheprin) 60064

*Buckeye Supply Co., 7775 Montgomery Rd., Cincinnati, Ohio 45236

oFenwal Laboratories, Div. of Travenol Laboratories, Inc., Morton Grove, Ill. 60053

#Sigmamotor, Inc., 3 North Main Street, Middleport, N.Y. 14105

=Difco Laboratories, Detroit, Michigan 48201

containing 100 cc. TC 199 through a two-way stopcock adapter to which the plastic tubing of the Fenwal pack has been attached. The emptied syringe is left in place but the stopcock handle is turned each time so as to close off the Fenwal pack system. After each aspiration another assistant applies pressure to the area from which marrow has been obtained, to decrease hematoma formation. The areas to be aspirated will be posterior and anterior iliac crests and the sternum. After 500 cc. of marrow are collected, the bag is inverted 5 to 10 times and then a sample is obtained for nucleated cell count and differential.

Infusion.

For infusion we employ the Sigmamotor pump and infusion set, alcohol sponges, hemostat, 10 cc. syringe fitted with a 20-gauge needle, a sterile bottle of 30 cc. 0.9 percent saline for injection, scissors and the double filter system noted above. Three Abbott blood infusion sets are present for emergency use (cf. infra).

The marrow has been collected and stored in the blood bank at 4° C. for several hours while the patient receives whole-body irradiation. It is then taken to the patient's ward where the Fenwal pack holding the marrow is inverted several times to insure homogeneous distribution of cells and then fitted to the Sigmamotor infusion system (Figure 3). The Sigmamotor pump is turned on and the marrow passes through the double filter system downstream from the pump at a rate of 60 to 90 drops (4 to 6 cc.) per minute. The filtered marrow enters the patient through a needle inserted into the rubber connector of a standard intravenous system containing 0.9 percent saline. Aliquots of filtered marrow are collected immediately after the pump is turned on for the determination of cell viability, nucleated cell count and differential, as well as blood culture.

If there is evidence of malfunction of the filter system, indicated by bubbles appearing in the infusion tubing just after the marrow is filtered, the pump is turned off at once. Then the intravenous infusion set containing 0.9 percent saline, through the rubber connector of which the marrow has been given, is restarted. The Fenwal pack containing the marrow is then connected to a standard Abbott blood infusion set which is attached "piggyback" to the tubing

2 50 cc. syringes with 18 gauge needles

2 10 cc. syringes with 18 gauge needles

Formalin tube for Pathology Department study of marrow biopsy.

Scissors

Procedure.

The patient is premedicated with 0.6 milligrams atropine and 100 mg. Seconal intramuscularly, at 6 a.m. The patient goes to the operating room at approximately 7 a.m. where, after induction with Pentothal, endotracheal intubation is performed, and the patient is given general anesthesia with Halothane and placed in the prone position. After appropriate antiseptic preparation of the patient's iliac crest area, bone marrow aspiration may begin.

At this time, prior to aspiration, blood for peripheral white cell count and differential is obtained and 5 cc. of blood are sent to the blood bank for typing and crossmatching of 2 units of whole blood. As an alternative 500 cc. of blood from the patient may be obtained 1 week before the transplant and reinfused during the marrow aspirations.

Bone marrow aspirations are performed using the Bierman needles for the sternum, and Kurnick needles for the anterior and posterior iliac crests. The Vim-Silverman needle has bent too easily in our experience to be of value in repeated marrow aspirations. No more than 3 cc. are obtained with the needle bevel in one position. The bevel is rotated through four quadrants for each aspiration. From the posterior iliac crest we have found that up to 20 cc. may be obtained from one site before marrow cellularity diminishes. Aspiration is performed with a syringe which has been filled with 1 cc. of heparin solution containing 50 units per cc. An Abbott Venopak adapter connects a sterile bottle containing 200 cc. of heparin solution, 50 units per cc., to a two-way stopcock adapter in which each syringe is placed to fill it with 1 cc. of heparin. This stopcock is also turned so that the heparin solution is a closed system between aspirations.

The syringe containing aspirated marrow is inverted several times to distribute the heparin and handed to an assistant who injects it into the Fenwal pack

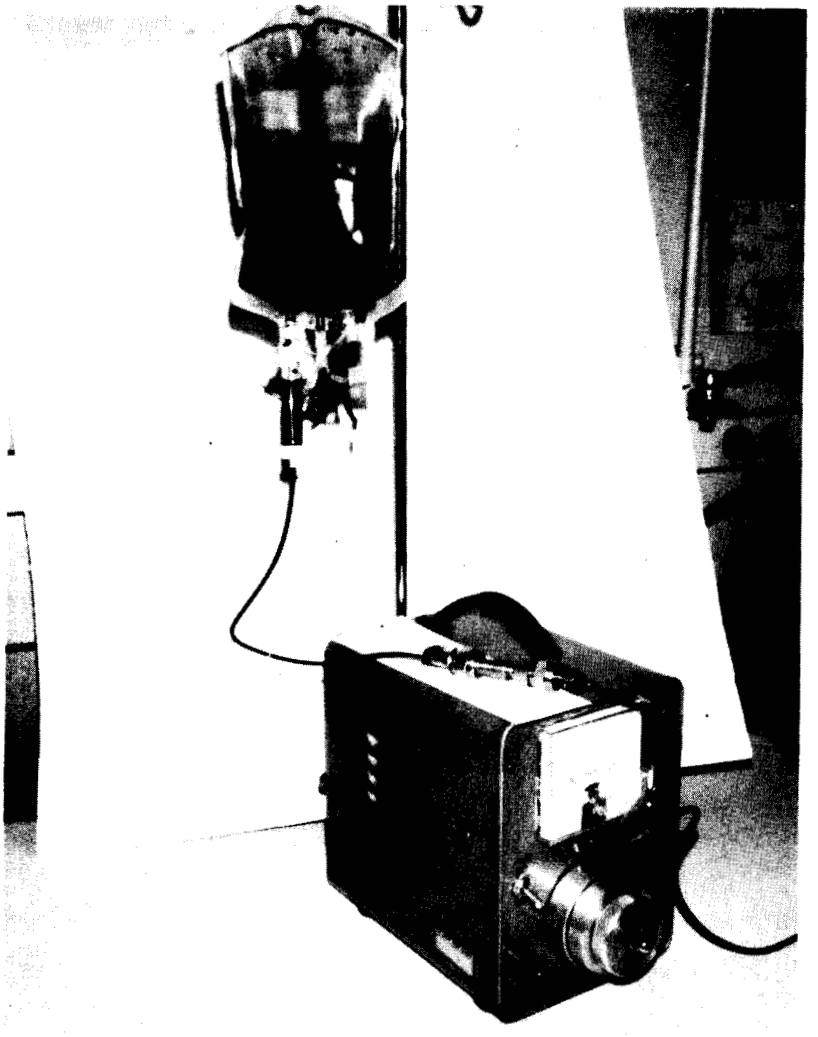

Figure 3. --Bone marrow infusion pack, double filter and pump.

containing the 0.9 percent saline solution and the infusion begun again. After the infusion bag has been emptied but marrow remains in the tubing 30 cc. sterile 0.9 percent saline is injected into the tubing proximal to the filter set and thus into the patient.

The marrow infusion is regularly accompanied by chills and fever unless 10 grains of aspirin are given orally or rectally during the procedure. Blood cultures are routinely obtained and have always been sterile.

To calculate the actual number of marrow cells administered, we count a filtered specimen using Kurnick's technique (13), correct appropriately for dilution and obtain the nucleated cell count per single hemocytometer square by counting eight squares and dividing the total by eight. Then we multiply this number by (10, to get count per mm.^{3}) x (40, dilution factor in counting) x (1000, number of mm.^{3} in 1 cm.^{3}) x (fraction of viable cells) x (500 volume of marrow) x (1.4, the dilution correction from 100 cc. TC 199 and 100 cc. of heparin in the Fenwal pack) x (fraction of marrow made up of erythroid and myeloid precursors, including stab cells). This simplifies to (cell count per hemocytometer square) x (2.8) x (10^8) x (fraction of viable cells) x (fraction of marrow cells). Cell viability is tested by the Trypan Blue exclusion technique (14).

Results.

Eight patients at the Cincinnati General Hospital have received marrow transplants in the last 2 years employing this technique. Table II indicates the number of nucleated cells given to each, and the calculated number of marrow cells received. These were a group of ambulatory patients with metastatic cancer who received 200 rads (midline dose) of whole-body irradiation from a ^{60}Co source at a dose rate of 4 to 5 rads per minute. One exception was the allotransplant donor whose sister had drug-induced marrow aplasia.

In one method for determining the "true" number of marrow cells contained in the transplant the total number of peripheral blood leukocytes in an equivalent volume is subtracted from the total cell count of the volume of marrow aspirated. Another approach employed in this laboratory has been to determine the percent

TABLE II

NUMBER OF CELLS ASPIRATED IN 500 CC. MARROW BY CURRENT TECHNIQUE

Donor	Type of Transplant	Total Cells Given x 10^9	Marrow Cells x 10^9 (% Nucleated Cells in Parenthesis)	
			Calculated by Subtraction of Peripheral Leukocyte Count	Calculated as % of Leukocyte Precursors Observed In Marrow
087	Isograft	8.76	4.90 (56%)	---
090	Autograft	11.0	4.60 (42%)	
091	Autograft	11.95	4.90 (42%)	---
095	Autograft	3.07	0.76 (25%)	---
098	Autograft	10.77	7.42 (68%)	9.15 (85%)
099	Autograft	7.84	4.49 (57%)	5.32 (68%)
107*	Autograft	4.96	2.21 (45%)	3.19 (64%)
E. R.	Allograft	23.50	16.40 (70%)	12.69 (54%)
Mean		10.23	5.78 (57%)	7.59 (74%)
Mean of 10 other recent marrow aspirations exceeding 250 cc. (From Table IV)		11.51		

*522 cc. aspirated

of the marrow aspirate made up of erythrocyte, granulocyte precursors, and megakaryocytes (excluding all leukocyte forms seen in normal peripheral blood except "stab" cells), multiplying this percentage by the absolute number of cells aspirated. This latter technique clearly underestimates marrow aspirated since bone marrow contains a large number of polymorphonuclear granulocyte reserve estimated at between three to four (15) to 25 to 30 (16) times that of the total blood granulocyte pool as well as a vital component of lymphocytes (17). In fact the average number of mature marrow polymorphonuclear cells is 22 percent, and marrow lymphocytes 10 percent (18), suggesting very little dilution by peripheral blood (Table II). Yet this technique gave a larger number of marrow cells than that obtained by subtraction of the peripheral blood leukocyte count three out of four times (Table II).

There is no statistically significant difference between the mean number of cells infused by our technique and other transplants summarized in Table IV. Table III indicates the protective effect of the marrow in four patients given 200 rads midline whole-body irradiation immediately after marrow aspiration ($0.02 < p < 0.05$). These patients and seven control patients not receiving transplants were all ambulatory individuals with metastatic carcinoma and stable hematologic values, the only abnormality being mild anemia with hematocrits always exceeding 30 percent. All patients listed in Table III selected for marrow donation had normal granulocyte reserves assessed with etiocholanolone (19), a normal marrow scan (with technetium-99m sulfur colloid) as well as a marrow aspirate histologically within normal limits.

In Table IV are listed the volumes aspirated, absolute number of cells infused and the nucleated cell concentration both of our series and from several other recent articles on human marrow transplantation (11, 20-23). The mean nucleated cell counts for aspirates exceeding 250 cc. is 20160 ± 10667 and for volumes under 250 cc. 57700 ± 26161, a statistically significant difference.

Table V confirms our impression ($p<0.01$) that the larger the volume of marrow aspirated and the greater resultant contamination with peripheral blood. The table further indicates that our technique gives counts comparable to those previously described.

TABLE III

RESULTS OF RECENT SUCCESSFUL ISO- AND AUTO-TRANSPLANTS OF MARROW FOLLOWING 200 RADS MIDLINE WHOLE-BODY ^{60}CO IRRADIATION

Patient	Day of Leukocyte Nadir	Leukocyte Count at Nadir	Nucleated Cell Count Infused Per Kilogram Body Weight
087	26	2100	1.6×10^8
091	25	4100	2.3×10^8
095	33	2700	0.6×10^8
098	28	2200	1.7×10^8
Mean	28	2780 ± 920	1.6×10^8
Mean of Controls*	24	850 ± 380	---

*7 patients receiving 200 rads whole-body radiation without transplantation of marrow.

TABLE IV

ASPIRATED VOLUMES, VIABLE CELLS, AND NUCLEATED CELLS IN 29 PATIENTS IN CINCINNATI SERIES AND FROM LITERATURE

Volume Aspirated (cu. cm.)	Total Viable Nucleated Cells Aspirated x 10^9	Nucleated Cell Count per cu. mm. of Aspirate	Reference
1590	17.14	10,800	20
995	10.7	10,800	20
765	28.09	36,780	11
696	23.11	33,200	11
526	12.73	24,200	21
522	4.96	9,500	This Report
500	8.76	17,500	"
500	11.0	22,000	"
500	11.95	23,900	"
500	3.07	6,100	"
500	10.77	21,500	"
500	7.84	15,700	"
500	23.5	47,000	"
338	2.28	6,800	22
292	4.905	16,800	22
275	6.50	23,700	10
263	5.40	20,500	21
260	4.20	16,100	20
236	18.97	80,400	11
150	9.0	60,000	23
120	5.0	41,700	23
100	3.0	30,000	12
93	9.1	97,800	11
84	3.44	40,900	22
80	6.0	75,000	23
70	6.7	95,700	23
58	2.94	50,700	22
48	1.0	20,800	3
24	1.0	41,700	4

TABLE V

NUCLEATED CELL CONCENTRATION RELATED TO VOLUME OF MARROW ASPIRATED

Summary from Table IV of 29 Aspirated Marrow Volumes	Mean Marrow Aspirate Volume	Mean Concentration of Nucleated Cells per cu. mm.
All over 250 cc.	528 cc.	*20, 200
This series (all volumes over 250 cc.)	503 cc.	20, 400
Other ten aspirates in Table IV exceeding 250 cc.	600 cc.	19, 970
All under 250 cc.	97 cc.	*57, 700
All aspirates in Table IV	382 cc.	34, 400

*Difference significant, $p < 0.01$

Discussion.

We believe that the technique described for bone marrow transplantation provides several advantages over previously employed methods.

1. Little special equipment is required which is not readily available from local medical supply companies except for the filter system noted above. On two occasions when the filtration system has become clogged with marrow spicules and fat globules the plastic bag containing the remaining marrow could be switched immediately to an Abbott Blood Administration set with Nylon Filter (List No. 4636). This is a rectangular filter, each hole measuring 150 micra (0.005 inches) by 200 micra (0.007 inches) (24).

2. There is no loss of time in processing individual syringes of marrow since the syringes are simply passed to a technician for immediate insertion into the storage bag. The storage bag has been prefilled with 50 units of heparin by a technician just prior to use and the bag contains 100 cc. TC-199 culture medium buffered to pH 7.4.

3. Maintenance of sterility is guaranteed by the closed system with no re-utilization of the disposable aspiration syringe or transfer of marrow to multiple beakers and re-aspiration for final re-insertion into the storage bag (11).

4. Since our technique involves rotating the aspirating syringe through 270°, the tubing required for a totally closed circuit marrow aspiration system (three-way stopcock connecting aspirating needle, tubing to culture medium and tubing to storage bag (23)) would become twisted and therefore has not been employed. Furthermore, the use of a three-way stopcock requires the operator to draw up his own TC-199-heparin mixture prior to marrow aspiration which is time consuming, while in our method a technician pre-fills each syringe.

5. The marrow infusion is intravenous rather than intramedullary or intraperitoneal. Reinjecting marrow into the medullary cavity has no advantage over the intravenous route (8). The intraperitoneal route (3) has been employed to decrease the risk of fat and bone embolization to the lung but reduces the effective number of cells by a factor estimated at 20 to 100 (2). Filtration has been

successful in preventing the gross hemoglobinuria which may occasionally occur following direct marrow infusion (25, 26). Friedman has observed clumps of marrow up to 3 mm. in length when a filter was not used (25). The recipient of our marrow allotransplant was desperately ill at the time of infusion and died 2 hours later of overwhelming Gram negative sepsis, giving us the unique opportunity to look for marrow emboli in the pulmonary circulation. Post mortem examination of her lungs revealed no marrow cells in the lungs, and no fat or bone spicules, despite the fact that she received a marrow transplant containing 23.5×10^9 nucleated cells, one of the largest reported to date. Marrow cells were identified primarily in liver, spleen, and adrenal sinusoids and bone marrow in this patient. Each of our patients has been monitored electrocardiographically, radiologically, by several serum enzymes (SGOT, LDH, CPK) and in two cases by lung scan before and immediately after transplantation, without any changes. Data obtained in this laboratory indicate no effect of filtration on cell counts or viability (25, 27).

6. Our aspirates were, in 6 of 8 cases, from patients with metastatic malignant disease, some of whom had had previous radiotherapy. Table V shows the cellularity of marrow aspirates by our technique was not significantly different from that of other methods, yet was obtained with less handling and in a closed system.

7: Using general anesthesia for a period of 90 to 120 minutes, multiple puncture sites have been employed around both upper ilia where the majority of our marrow is obtained, as well as from the sternum. Not only are the ilia safely and easily aspirated but they contain as much as 40 percent of adult marrow (29). We believe that the more separate sites aspirated the less opportunity there should be for peripheral blood to contaminate the marrow. Employing only one site per ilium, as in Wilson's technique (28), it would seem probable that as the marrow architecture would be progressively ruptured at the one needle insertion site and peripheral blood would appear in the aspirate in increasing amounts; however, the nucleated cell counts listed in his article seem adequate.

Until the human hematopoietic stem cell can be identified with certainty it is impossible to know to what extent marrow is diluted with peripheral blood. The

most common method of approaching this problem has been to assume that the marrow volume administered has the same peripheral blood leukocyte count as a sample obtained from venous or capillary blood and therefore to subtract the total number of peripheral blood cells in the volume (obtained by multiplying peripheral blood count per cu. mm. by the volume of marrow administered) from the total nucleated cell count in the marrow. We also tried to approximate the number of marrow cells infused by obtaining a differential cell count on filtered marrow excluding all polymorphonuclear neutrophils, eosinophils and basophils, lymphocytes, and monocytes to obtain a percentage we arbitrarily called "precursor cells" (Table II). This percent of "precursor cells" obviously excludes a population of lymphocytes and polymorphs which are known to be part of the marrow and not peripheral blood, thus underestimating the true number of marrow cells infused. Yet it exceeds the marrow cell count calculated by peripheral blood subtraction in three of four cases, by 44 percent, 23 percent, and 18 percent. Our data from Table II (patient 098) further suggest that up to 85 percent of the cells obtained with our technique may be marrow cells.

If our filter system clogs, which has happened twice, when large clumps of fat and bone settle to the bottom of the storage pack, transfer to a filtered gravity system (Abbott Venopak adapter) for marrow infusion has been performed without difficulty. Experiments performed in this laboratory indicate that at the same flow rate the Sigmamotor pump exerts over twice the pressure as the standard Abbott blood infusion set held as much as 4 feet above the patient. Since one hopes to press marrow cells out of the spicule-supported sinusidal network where they develop (30), we feel that this increase in pressure is of great value.

Each syringe employed is filled with 1 cc. of solution containing 50 units of preservative-free heparin. Presence of a preservative might diminish cell viability even over a few hours storage time (31). With approximately 100 syringes per procedure, there are 5, 000 units of heparin in the marrow storage bag. In our early experiences we found that a total of 4, 000 units in the mixture of 700 cc. (marrow 500 cc., heparin plus culture medium-200cc.) permitted small clots to form in the bag. 10, 000 units of heparin in this volume infused over 50 minutes led in one patient to significant bleeding in the marrow puncture sites.

Our compromise of about 5,000 units of heparin per infusion (from 50 units per syringe) has been quite successful.

The nucleated cell survival before and after filtration has averaged 97 percent, indicating the relatively atraumatic effect of both aspiration and infusion on the marrow obtained by our technique.

The amount of marrow removed in the four procedures summarized in Table III averaged 1.6×10^8 nucleated cells per kg. body weight or 1.5 percent of the estimated total-marrow cellularity in man (which is about 10.9×10^9 marrow cells per kg.) (8), so that the donor is always left with a large residue of marrow. The marrow scans with technetium 99m sulfur colloid of a marrow donor (E.R.) before and 5 days after the aspiration procedure shows no alteration (Figure 4) but the scan resolution is no better than 9 mm. Human marrow cellularity has been estimated at 430,000 cells per cu. mm. (8) but the aspirate with the highest nucleated cell count per cu. mm. in Table IV was only 97,800 (11), with an overall average for the 29 aspirates in Table IV of 34,400 per cu. mm. The calculated maximum contribution of peripheral blood to the aspirate depending on the method employed as noted in Table II may range as low as 15 percent. Thus, either our current technique would appear to be quite inefficient in obtaining available cells, or the marrow cellularity may be much lower than 430,000 cells per cu. mm. Marrow biopsies after our multiple aspirations may help answer this question.

The timing sequence of cytopenia in our series of transplants differed from a few of those previously reported where marrow given to one group of patients permitted their more rapid recovery from radiation-induced leukopenia (23). In our patients the day of the nadir of the leukopenia did not differ significantly from that of patients receiving the same dose of irradiation without a transplant.

The only side effects of marrow infusion are shaking chills, with fever as high as 103° orally occurring 30 to 60 minutes after the beginning of infusion. The fever and chills are preventable with aspirin, 640 mg. p.o. or p.r. given prior to the infusion. Since our marrow infusion samples and patient blood cultures have always been sterile, we hypothesize that some trauma to leukocytes

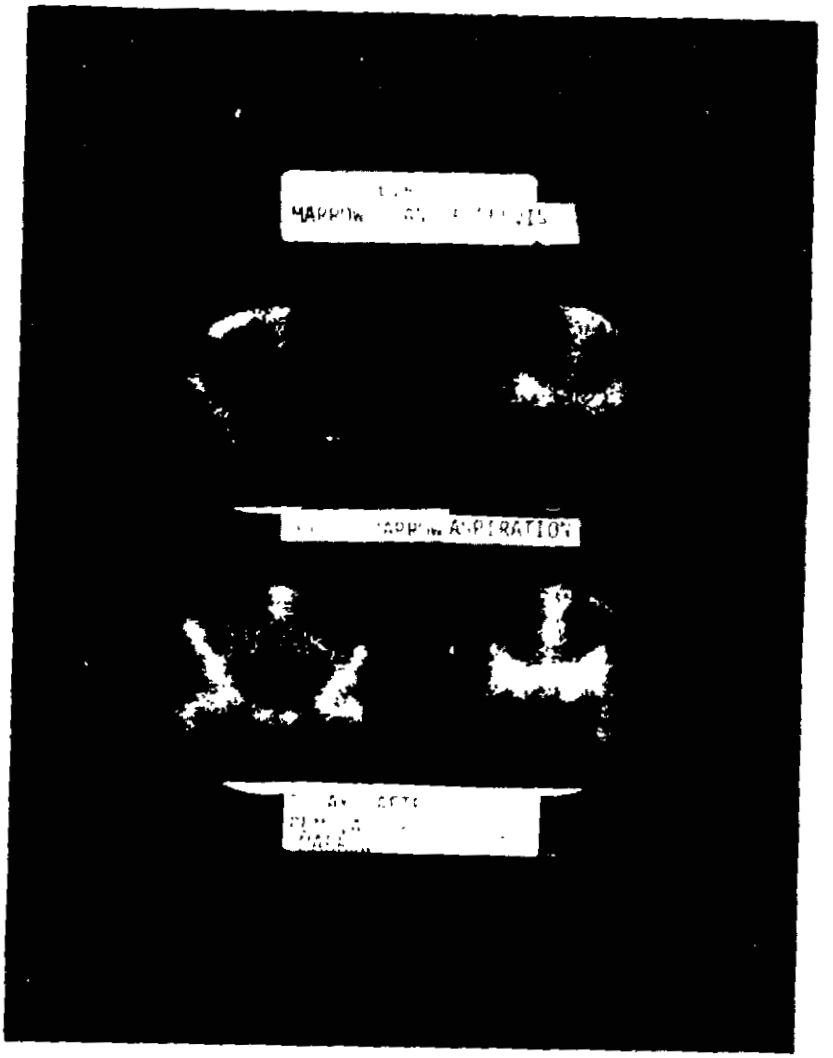

Figure 4. --Technetium-99m sulfur colloid bone marrow scans of marrow donor before and 5 days following aspiration of 500 cc. marrow.

in our technique releases sufficient endogenous pyrogen to have a clinical effect.

Of the eight transplants reported in Table II, four, noted in Table III, were successful. Of the remaining four patients, the allograft recipient was dying of Gram negative sepsis at the time of infusion. The second death (090) was anesthesia-related, four days after the procedure. Two patients, (099 and 107) were, in retrospect, poor candidates for autotransplantation. Although the transplants did not prevent radiation-induced leukopenia, infection was not the cause of death in either patient. Both had had widespread radiotherapy which had affected the reticuloendothelial framework necessary for stem cell development (32, 33), and preliminary cell aspirates in allegedly unirradiated areas did appear hypocellular. Fractionated radiotherapy exceeding approximately 3,000 rads over 2 to 4 weeks appears to prevent marrow regeneration permanently (34, 35). In fact 5 of 10 marrow isotransplants summarized by Harvey et al. (20) failed, and this group achieved success only on the third try. One of our two failures, (107), appeared to possess normal granulocyte reserves only because we were given an incorrectly high body weight on which to base our etiocholanolone dosage, falsely elevating her marrow granulocyte reserves. From this unfortunate experience we now insist that a donor candidate have a normal iliac marrow aspirate, a normal bone marrow scan (using technetium-99m sulfur colloid*), and normal granulocyte reserves measured with etiocholanolone (15, 19).

Between marrow aspiration and infusion we have kept our infusion bag at 4° C, while our patients have been irradiated, although Pegg et al. have found that fat may solidify at this temperature and interfere with infusion (36). One of our patients whose autotransplant failed (107) also demonstrated this phenomenon. Mathe has recommended keeping marrow at 37° for 2 hours to lessen graft versus host disease (37). Other investigators have found that maintenance of erythropoietic activity of mouse marrow cells declined to 80 percent of initial activity when the cells were kept in culture 6 hours at 37 percent with no deterioration at 4° (38). We will decide on the appropriate short-term (hours) storage

*Iron-52 would be preferable but it is not available to us because it is a cyclotron product with a very short half-life.

temperature for human marrow on the basis of our own cell survival experiments.

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ACTIVE BONE MARROW DOSE RELATED TO HEMATOLOGICAL CHANGES IN WHOLE-BODY AND PARTIAL-BODY EXPOSURES

We have been interested in finding an approach to allow the prediction of the hematological changes to be expected following the uniform and nonuniform exposures used in this program. A quantitative approach to evaluating the effects of nonuniform exposure has been proposed by Bond and Robinson (1, 2). In applying this approach to our specific project, one has to know the distribution of bone marrow (assumed to parallel that of stem cells) and the radiation dose distribution throughout the bone marrow. The detailed distribution of active bone marrow for standard man at age 40 adapted from the paper by Atkinson (3), is shown in Table VI. A tissue equivalent phantom (Rando) containing a human skeleton and simulated lung cavities was used to experimentally determine the active bone marrow dose under simulated whole-body and partial-body (upper, lower, and complete trunk) bilateral cobalt-60 exposure conditions. Capsules filled with lithium fluoride (LiF) were placed in bone cavities so demonstrated by radiographs of each section. The xiphoid served as the boundary of the field for upper and lower body exposures. The doses for each section were averaged and multiplied by total grams of active bone marrow in that section. The active bone marrow integral doses for upper body, lower body, and complete trunk are 48 percent, 61 percent, and 75 percent, respectively, of that determined for whole-body exposures (Table VII).

Using the radiation dose distribution to active bone marrow, we then proceeded to calculate the weighted stem cell survival (following the approach of Bond and Robinson) for the various exposure conditions. For mortality in the LD_{50} range, the normalized mouse bone marrow stem cell survival curve shown in Figure 5 was used. This curve is based on the work of McCulloch and Till (4). An example ot the procedure as applied to the pelvic region for whole-body and lower-body exposure is shown in Table VIII. The sum of the products of the fraction of total marrow irradiated (from Table VI) and relative stem cell survival for that radiation dose yields the weighted relative stem cell survival. The

TABLE VI

MARROW DISTRIBUTION OF THE AVERAGE MALE ADULT

SITE	MARROW WEIGHT g	FRACTION RED MARROW (Age 40)	RED MARROW WEIGHT (Age 40) g	% TOTAL RED MARROW
Head	250.9	0.75	188.2	14.2
Upper Limb Girdle	150.6	0.77	115.9	8.8
Sternum	50.0	0.65	32.5	2.4
Ribs	265.7	0.354	94.0	7.1
Vertebrae				
Cervical	64.5	0.75	48.3	3.7
Thoracic	263.9	0.75	198.0	15.0
Lumbar	203.1	0.75	152.3	11.5
Sacrum	226.6	0.75	170.0	12.9
Lower Limb Girdle	431.5	0.75	323.6	24.4

TABLE VII

TOTAL GRAM-RADS TO THE ACTIVE MARROW OF A "STANDARD MAN"
AGE 40

SKELETAL ANATOMY	WHOLE BODY (g-rads)	PARTIAL BODY (g-rads)		
		Upper	Lower	Trunk
Head				
Cranium	44, 508	41, 590	1, 185	1, 787
Mandible	4, 248	4, 254	141	329
Upper Limb Girdle				
Humeri, head and neck	6, 012	5, 407	485	4, 789
Scapulae	11, 705	11, 573	1, 384	8, 686
Clavicles	3, 767	4, 128	193	890
Sternum	5, 896	6, 360	620	4, 753
Ribs (1-12 pair)	18, 585	11, 999	12, 288	18, 203
Vertebrae				
Cervical	9, 892	10, 113	426	1, 586
Thoracic	38, 176	29, 315	22, 827	38, 744
Lumbar	31, 615	2, 572	30, 781	32, 300
Sacrum	33, 652	1, 308	32, 241	32, 751
Lower Limb Girdle				
2 Os Coxae	55, 278	1, 985	53, 972	54, 027
2 Femoral head and neck	10, 197	314	10, 212	6, 174
	273, 531	130, 918	166, 755	205, 019

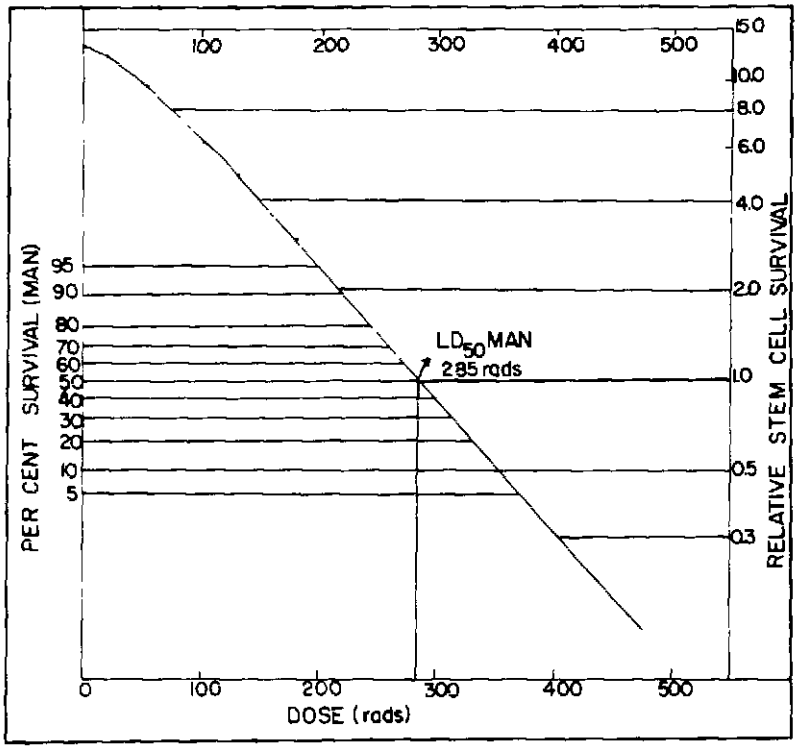

Figure 5. --Dose-survival curve for man exposed uniformly to penetrating X- or gamma-radiation and relative survival rate of bone marrow stem cells; the survival rate of stem cells is normalized to the number surviving at the LD_{50} dose for man, uniform total-body exposure.

TABLE VIII

RELATIVE STEM CELL SURVIVAL (WEIGHTED)

300 R Midline Air Exposure

Body Section	Active Marrow Weight g	Fraction Total Active Marrow (from Table VI)	WHOLE BODY			LOWER BODY		
			Dose rad	Relative Stem Cell Survival (RSCS) (from Figure 5)	Weighted RSCS	Dose rad	Relative Stem Cell Survival (RSCS)	Weighted RSCS
Pelvic Region								
Sacrum	170.0	.129	198	2.40	.309	190	2.60	334
R & L Os Coxae	272.0	206	203	2.30	474	198	2.42	499
R & L Femurs	51.6	039	198	2.45	096	198	2.42	095
					.879			.928

calculations, also extended to other midline air exposures, are shown in Figure 6. Thus, for any of the given nonuniform exposures, one can determine the dose of uniform whole-body irradiation that would result in the same mortality rate. The corresponding "doses" thus derived for uniform whole-body exposures can be thought of as being "dose equivalent".

In extending this approach to the circulating fractions of the peripheral blood elements at the nadir point, the un-normalized mouse bone marrow stem cell survival curve (D_0 = 95 rads) was utilized as well as the survival curve for human hematopoietic cells (D_0 = 137 rads). Senn and McCulloch (5) have recently shown that the sensitivity of human bone marrow (by colony-forming ability in culture technique) to irradiation is of the same order as that predicted on the basis of experiments in mice. The survival curve they obtained for a class of human hematopoietic cells has a D_0 of 137 rads and an extrapolation number of 1.0. It is assumed in this extension of their approach that the circulating fraction for a given blood element at the nadir is equal to the surviving fraction of marrow stem cells for the given exposure. The validity of this extension was tested by comparing the predicted and measured nadir circulating fractions of white blood cells and platelets for several groups of patients. Peripheral blood counts of the patients were obtained prior to irradiation and were followed for as long as practical following exposure. The data reported here were obtained from patients shown to have normal blood counts prior to exposure. The patients were grouped as to type of exposure and the midline dose received. Table IX shows the comparison for three groups of patients who received whole-body exposures to achieve 100, 150, and 200 rads midline-absorbed doses respectively; and two groups of patients who received lower-body exposures to achieve 200 and 300 rads midline-absorbed doses, respectively. The assumptions given above as well as the application of the mouse stem survival curve to man appear to yield fair agreement between the proposed model and the average clinical findings. Because of the wide range observed in the clinical findings, however, additional clinical data are obviously needed. Also more work is needed on the specific dose-effect curve for human stem cells. If the value for D_0 or extrapolation number for man in vivo is markedly different from those used in the calculation,

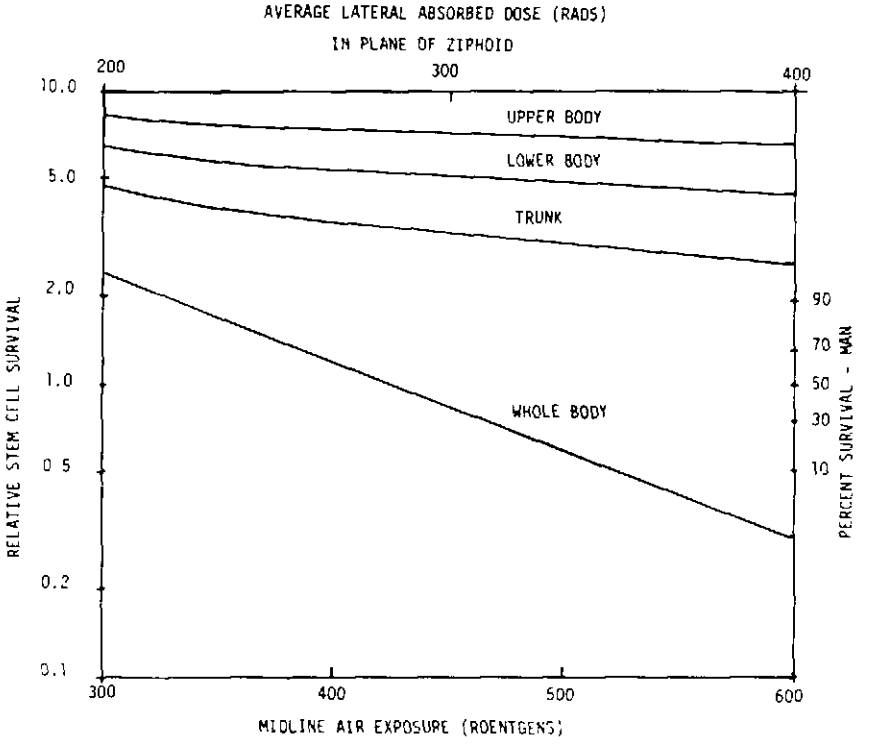

Figure 6. --Weighted relative stem cell survival (and associated percent survival for man) and dose expressed either as midline air exposure in roentgens or as average lateral absorbed dose in rads in the plane of the xiphoid.

TABLE IX

MEASURED AND PREDICTED NADIR CIRCULATING FRACTIONS OF BLOOD ELEMENTS FOR PATIENT EXPOSURE CONDITIONS

Exposure Conditions	Average Lateral Absorbed Dose Rads	Number of Patients	Nadir Fraction		
			Measured Average Range	Predicted D_O = 95 rads	Predicted D_O = 137 rads
WHITE BLOOD CELLS					
Whole Body	107	6	.30 (.14-.51)	.43	.45
Whole Body	160	4	.14 (.07-.19)	.26	30
Whole Body	214	4	.17 (.06-.24)	.15	21
Lower Body	210	5	.58 (.47-.64)	.45	.47
Lower Body	321	4	60 (.60-.95)	37	.38
PLATELETS					
Whole Body	107	6	.47 (.13-.74)	.43	.45
Whole Body	160	3	.14 (.06-.24)	.26	.30
Whole Body	214	4	.18 (.15-.22)	.15	.21
Lower Body	210	5	.78 (.44-1.0)	.45	.47
Lower Body	321	4	.78 (.49-1.0)	.37	.38

the model as applied to our phantom measurements would have to be altered. In addition, patient data presently being obtained on lower body and complete trunk exposures will allow extension of the model to these modes of nonuniform exposures.

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BIOCHEMICAL STUDIES

Deoxycytidinuria in Burn Patients - Possible Relation to Combined Injury.

Our previous studies of deoxycytidinuria (1) indicated radiation-induced urinary excretion of deoxycytidine (CdR) is not as sensitive a biological indicator of radiation dose in man as in a rat and is not a dosimeter specific to radiation injury. Several burned patients also showed abnormally high urinary CdR excretion. In order to obtain further information concerning deoxycytidinuria in combined injury, CdR contents in 24-hour urine on a number of patients with burns of varying extent and degree and patients with severe trauma were determined. Significant increases in urinary CdR excretion were noted in patients with burned surfaces greater than 50 percent; 18.5 μg./day of CdR were found in the patient with 10 percent third degree and 42 percent second degree burns, 25.4 μg./day in the patient with 72 percent second degree burn, and 326.7 μg day in the patient with 4 percent second degree and 87.5 percent third degree burns (Table X) as compared to the normal value of 6.68 $\pm$ 3.67 μg./day determined previously (2). The amount of CdR excretion seems to be related not only to the severity but also to the extent of the burn. The maximum excretion was usually found 2 to 4 weeks after the burn and the elevated CdR excretion still could be observed more than a month after the accident. It is of interest to note that radiation-induced deoxycytidinuria usually disappears 2 to 3 days after irradiation. The reason for the delayed and prolonged deoxycytidinuria caused by the burn is not known at present but it is possible that bacterial infection of burned wounds might be partially responsible for the prolonged elevation of CdR in urine. CdR in urines of three patients with severe trauma was measured up to 3 days after the accident. Daily urinary excretion of CdR was found to be normal in all patients (Table X). In the course of this study, it was found that about 30 percent of the persons of Chinese or Japanese ancestry tested gave urinary CdR excretion greater than 20 μg/day as compared to 6.68 $\pm$ 3.67 μg/day for occidental population ranging from 6 to 60 years of age (Table XI). This increased CdR excretion seems to be a familial characteristic.

TABLE X

URINARY EXCRETION OF DEOXYCYTIDINE BY PATIENTS WITH BURNS AND TRAUMA

PATIENTS		Time of Urine Collection (days after accident)	Urinary CdR (μg/day)
Sex/Age	Diagnosis		
1. M/24	4%-2°; 87.5%-3°	10	19.0
		16	326.7
		30	155.5
2. M/16	42%-2°; 29%-3°: total body except head and feet	36	57.3
		240	28.0
3. F/12	50%-2°; 5%-3°: legs, arms, chest, neck, face	26	32.6
		240	5.7
4. M/57	75%-2°	1	4.4
		7	25.4
5. M/5	15%-2°; 35%-3°: body, legs	60	15.6
6. F/24	42%-2°; 10%-3°: body	24	18.5
		38	11.4
7. M/5	40%-2° and 3°	1	10.5
		7	7.0
8. F/4	20%-2°; 9%-3°: body	11	11.6
9. F/6	9%-2°; 17%-3°: body	4	9.2
10. M/42	23%-2°	1	12.7
11. M/62	9%-2°; 9%-3°: legs	14	10.0
12. M/32	10%-2°: hand	2	3.2
13. M/19	Brain stem contusion, paraplegia (auto accident)	1	3.9
14. M/23	Multiple trauma (motor-cycle accident)	1	9.4
		3	8.0
15. F/33	Multiple trauma (motor-cycle accident)	2	4.2

TABLE XI

URINARY EXCRETION OF DEOXYCYTIDINE BY ORIENTALS

SUBJECT	SEX	AGE	CdR EXCRETION	COMMENT
			µg/24 hrs.	
I.C.	M	35	77.5 ± 10.9*	
T.C.	M	39	41.0 ± 10.2*	Brother of I.C.
H.C.	F	3	24.1 ± 4.3*	Daughter of I.C.
A.C.	F	2	19.4	Daughter of I.C.
S.K.	F	30	5.7	Sister of I.C.
K.C.	F	60	2.9 ± 0.4*	Mother of I.C.
M.C.	F	30	8.1 ± 0.5	Wife of I.C.
R.H.	M	35	14.3	
D.L.	M	26	33.4	
M.H.	M	25	10.2	
K.T.	M	33	40.9	
T.T.	M	4	12.8	Son of K.T.
M.H.	M	30	12.0	
H.S.	M	29	6.0	

*Average values of 3 to 6 samples.

Other Ultraviolet-Absorbing Compounds in Urine.

Recently, the so-called high-resolution analytical techniques, which are capable of separating and quantifying many of the individual constituents of a physiological sample automatically, have been developed (3). One of these analytical techniques is the automated high resolution anion exchange column chromatography for analysis of ultraviolet-absorbing compounds in urine. Urinary constituents are extremely complex. More than 1,000 urinary molecular constituents of low molecular weight were reported to have possible pathologic significance or to be drugs and their metabolites (4). It is impracticable to isolate and quantify each urinary constituent of metabolic importance in search for a biological indicator of radiation dose. It was thought, therefore, that the automated high resolution anion exchange column chromatography could be used to quantitatively compare ultraviolet-absorbing compounds in urine specimens obtained before and after radiation therapy. Although it is still not possible to identify all compounds separated by the column, the number of urinary constituents of interest to us should be greatly reduced and their identification should be accomplished without much difficulty.

One ml. aliquots from 24-hour urine samples obtained from cancer patients before and after irradiation were applied on the automated high resolution anion exchange column and their UV chromatograms were compared quantitatively. * Results obtained from two patients are summarized in Table XII, Figure 7, Table XIII, and Table XIV. The first patient studied was a 14-year-old male with Ewing's Sarcoma treated with 100 rads to the whole body (Case No. 105). Chromatograms of his pre and postirradiation urine samples showed no significant change with the exception of some of the compounds listed in Table XII. 5-acetylamino-6-amino-3-methyluracil, 7-methylxanthine, 3-methylxanthine, and 1-methylxanthine are known to be highly diet dependent and are of no use as a biological dosimeter. Uracil shows 1.83-fold increase, hypoxanthine 3.88-fold increase and xanthine 1.48-fold increase after irradiation. These compounds are known to be metabolic end-products of pyrimidine and purine bases.

*This work is being carried out jointly with Dr. Charles D. Scott and his associates at the Oak Ridge National Laboratory.

TABLE XII

ULTRAVIOLET-ABSORBING COMPOUNDS IN URINE OF A CANCER PATIENT RECEIVING 100R WHOLE-BODY IRRADIATION (CASE NO. 105)

COMPOUNDS	EXCRETION RATE (mg/Kg-Day)			POST / PRE
	PRE-IRRADIATION	POST-IRRADIATION	AVERAGE NORMAL VALUES	
Creatinine	13.90	10.5	10.20	0.76
Pseudouridine	1.730	1.75	1.46	1.01
Uracil	0.055	0.101	0.066	1.83
5-Acetylamino-6-amino-3-methyluracil	0.065	0.190	0.128	2.92
N'-Methyl-2-pyridone-5-carboxamide	0.246	0.235	0.200	0.96
1-Methylinosine	0.233	0.118	0.147	0.51
7-Methylxanthine	1.900	0.795	1.130	0.42
Hypoxanthine	0.164	0.636	0.399	3.88
Xanthine	0.132	0.195	0.136	1.48
3-Methylxanthine	0.074	0.048	0.051	0.65
Urocanic acid	0.005	0.007	0.006	1.33
1-Methylxanthine	0.043	0.147	0.080	3.44

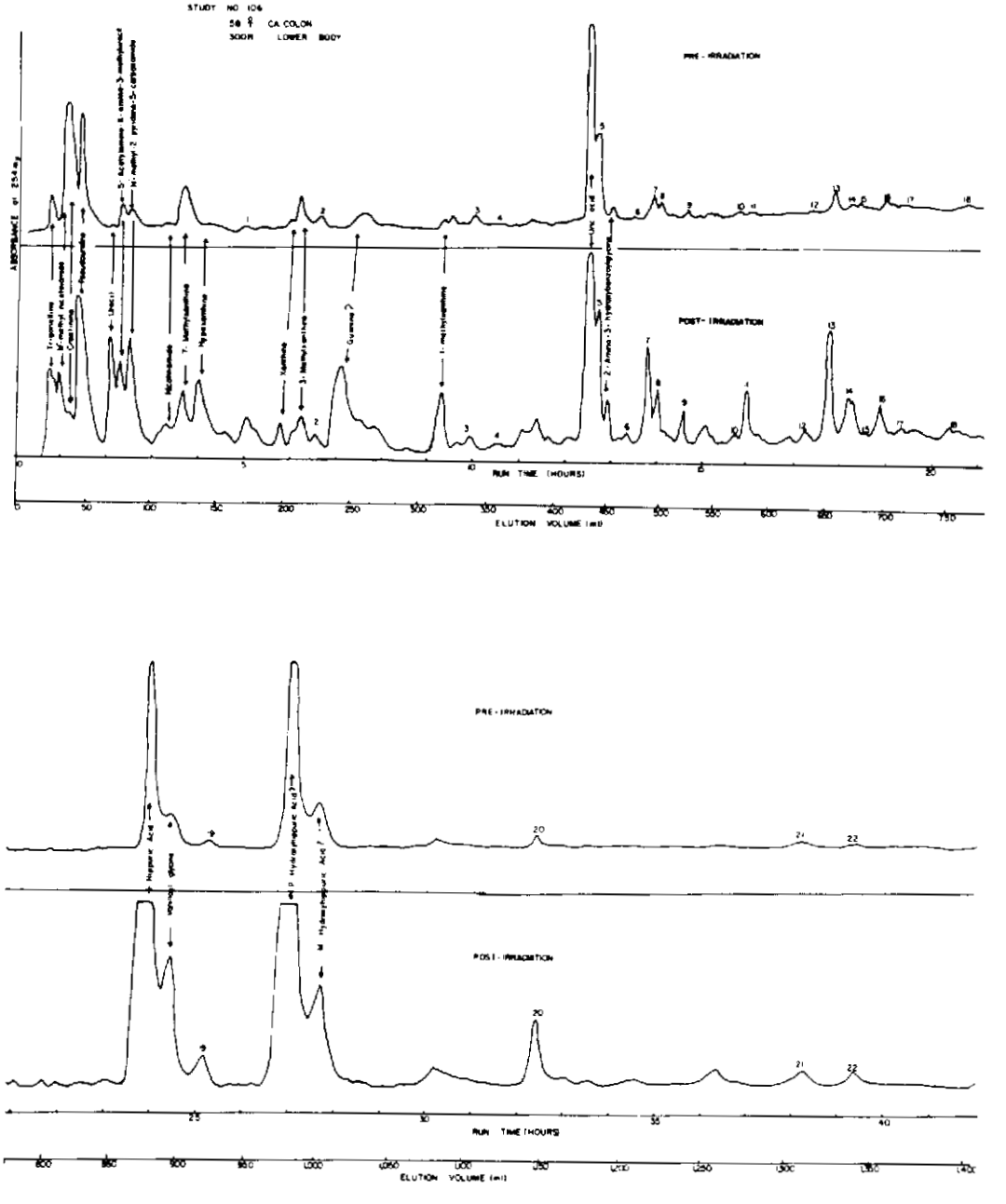

Figure 7. --Pre and postirradiation urine chromatograms from high-resolution UV analyzers (Case No. 106).

TABLE XIII

ULTRAVIOLET-ABSORBING COMPOUNDS IN URINE OF A CANCER PATIENT RECEIVING 300 R LOWER-BODY IRRADIATION (CASE NO. 106)

COMPOUND	EXCRETION RATE (mg/Kg-Day)		POST
	PREIRRADIATION	POSTIRRADIATION	PRE
Trigonelline	0.46	0.36	0.8
Creatinine	7.84	0.95	0.1
Pseudouridine	0.70	1.91	2.7
Uracil	0.006	0.18	30.0
5-Acetylamino-6-amino-3-methyluracil	0.10	0.22	2.2
N'-Methyl-2-pyridone-5-carboxamide	0.058	0.22	3.8
7-Methylxanthine	0.28	0.32	1.1
Hypoxanthine	0.04	0.232	5.8
Xanthine	0.026	0.106	4.1
1-Methylxanthine	0.080	0.295	3.7
Uric acid	5.13	4.08	0.8
2-Amino-3-hydroxybenzoylglycine	0.37	1.78	4.8
Hippuric acid	4.32	6.23	1.4
Vanilloyl glycine	0.38	0.72	1.9
p-Hydroxyhippuric acid	1.18	1.39	1.2
m-Hydroxyhippuric acid	1.06	1.29	1.2

TABLE XIV

UNIDENTIFIED ULTRAVIOLET-ABSORBING COMPOUNDS IN URINE OF A CANCER PATIENT RECEIVING 300 R LOWER-BODY IRRADIATION (CASE NO. 106)

PEAK NO.	EXCRETION RATE (mg/Kg-Day)		POST
	PREIRRADIATION	POSTIRRADIATION	PRE
1	0.115	0.661	5.8
2	0.222	0.380	1.7
3	0.545	0.474	0.9
4	0.028	0.340	12.1
5	1.58	1.15	0.7
6	0.051	0.533	10.4
7	0.619	1.575	2.5
8	0.409	.823	2.0
9	0.219	0.271	1.2
10	0.097	0.117	1.2
11	0.125	0.599	4.8
12	0.055	0.226	4.1
13	0.958	2.271	2.4
14	0.363	0.850	2.3
15	0.399	0.154	0.4
16	0.498	0.593	1.2
17	0.399	0.133	0.3
18	0.042	0.296	7.0
19	0.292	0.914	3.1
20	0.400	1.244	3.1
21	0.076	0.919	12.0
22	0.040	0.800	20.0

The second patient was a 58-year-old female with carcinoma of the colon, who received 300 rads to her lower body (Case 106). Again, marked increases in the amounts of uracil (30-fold), hypoxanthine (5.8-fold), and xanthine (4.1-fold) in postirradiation urine was observed (Table XIII). The tryptophan metabolites, 2-amino-3-hydroxybenzoylglycine and N'-methyl-2-pyridone-5-carboxamide also seem to undergo significant increases in excretion rate, 4.8- and 3.8 increases, respectively. A 1.9-fold increase in vanilloyl glycine could be explained based on the patient's diet. A marked decrease in the postirradiation excretion rate of creatinine could not be explained at present. However, low creatinine excretion rate usually reflects incomplete 24-hour urine collection. If this is the case, the changes in excretion rate of the compounds mentioned above would be more distinctive than we thought.

In addition to the compounds listed in Table XIII, many unidentified ultraviolet-absorbing constituents were also found in the chromatograms (Figure 7). The excretion rate of these compounds were calculated from the area of each peak, assuming molecular weight of 100 and molar extinction coefficient of 1,000 for each unknown compound. Compounds corresponding to peaks 4, 6, 21, and 22 showed increased in excretion rate greater than 10-fold (Table XIV). Chemical and physical identification of these compounds will be attempted. If they are found to be not diet dependent, i.e., endogenous in origin, they might serve as a sensitive biological indicator of radiation dose. Enzymatic analysis of urinary xanthine and hypoxanthine are currently being carried out in our laboratory.

Amylase.

In our previous report (2), we demonstrated that amylase activity in serum and urine was elevated in cancer patients receiving radiation therapy to the upper or whole body. The maximum serum and urinary amylase levels were observed 16 to 24 hours after irradiation (Figure 8 and 9). Significant increases in serum amylase activity were observed in 8 out of 11 patients receiving whole-body irradiation (Table XV). The average increase for five patients receiving 200 rads was 13.9-fold whereas that for four patients receiving 100 rads was 4.8. The serum amylase was not elevated significantly in all patients receiving radiation to their lower bodies (excluding head) with the exception of Case No. 102 who

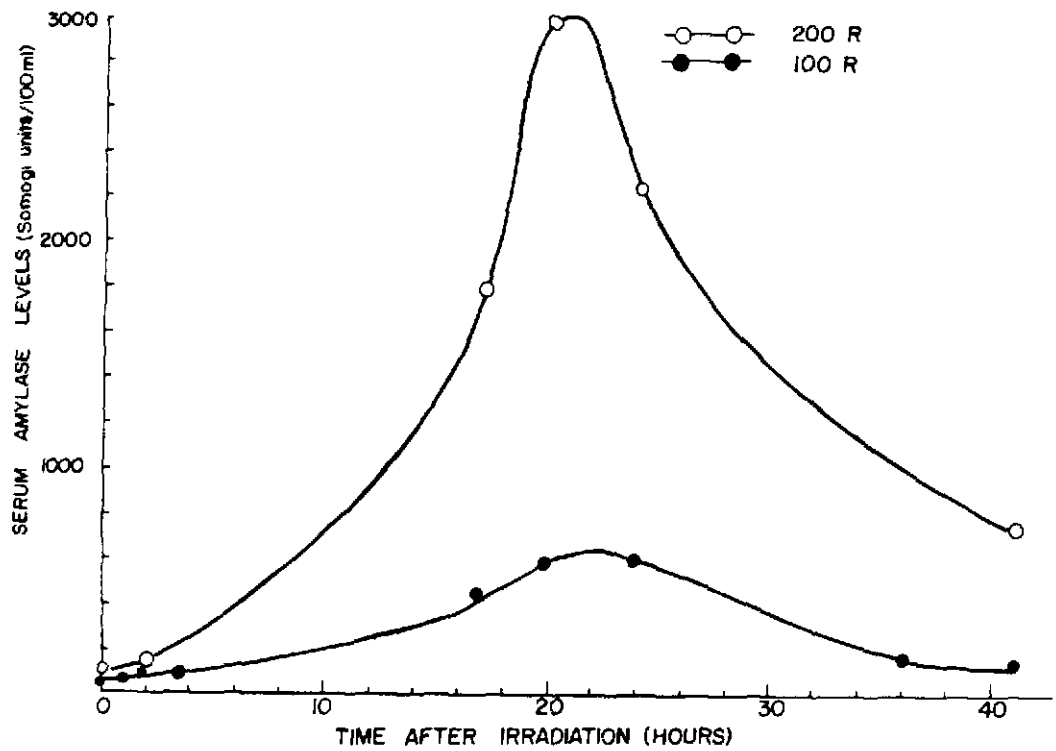

Figure 8. --Serum amylase response following whole-body irradiation.
Open circle: Case No. 87, 200 rads.
Closed circle: Case No. 96, 100 rads.

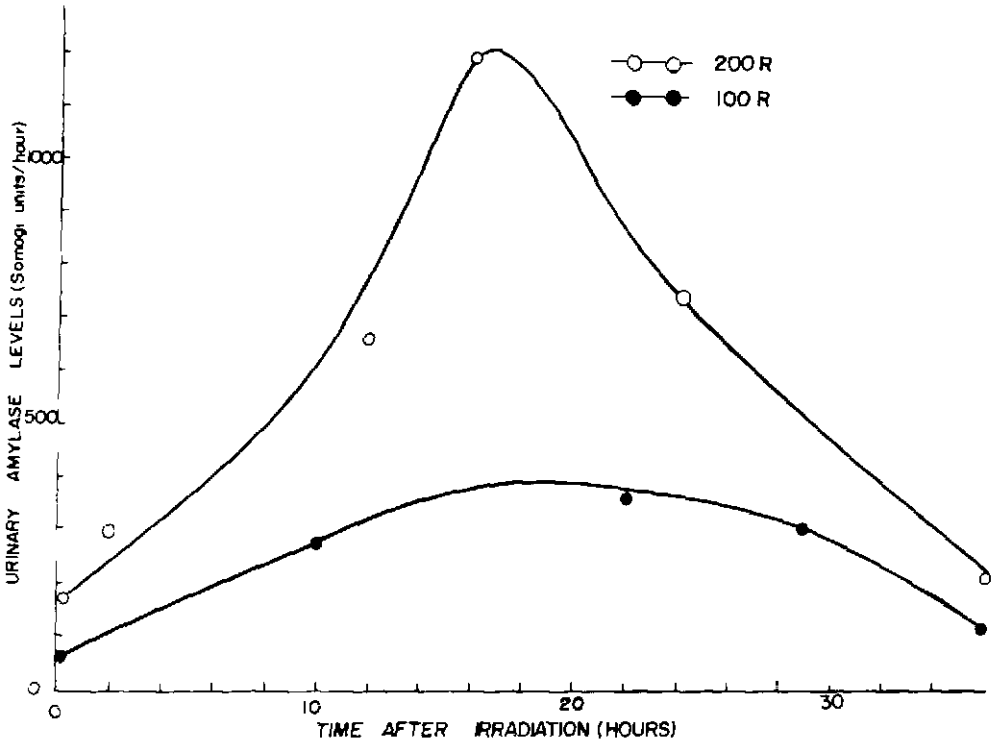

Figure 9.--Urinary amylase response following whole-body irradiation.
Open circle: Case No. 98, 200 rads.
Closed circle: Case No. 105, 100 rads.

TABLE XV

SERUM AMYLASE LEVELS IN CANCER PATIENTS
RECEIVING WHOLE-BODY IRRADIATION

10

PATIENTS				Amylase Levels (Somogi units/100 ml)		Post / Pre
Case No.	Sex/Age	Diagnosis	Dose* (Rads)	Preirradiation	Postirradiation+	
87.	F/11	Ewing's Sarcoma	200(197)	112	3,000	26.8
98.	F/45	Ca Colon	200(200)	126 ± 15	1,200	9.5
95.	F/66	Ca Colon	200(179)	150 ± 0	225	1.5
107.	F/58	Ca Colon	200(280)	59 ± 4	1,800	30.1
99.	M/45	Ca Rectum	200(205)	127 ± 11	180	1.4
90.	F/80	Ca Bladder	150(141)	112 ± 2.7	300	2.7
88.	F/54	Ca Lung	150(160)	300	360	1.2
86.	F/57	Ca Broncogenic	100(108)	87 ± 11	600	6.9
96.	M/42	Ca Colon	100(119)	160 ± 10	600	3.8
97.	M/65	Lymphoma	100(101)	168 ± 14	225	1.3
105.	M/13	Ewing's Sarcoma	100(---)	65 ± 3	534	8.2

*Doses are total midline doses and the values in parenthesis represent dose readings of dosimeters placed on the head.

+Maximum values after irradiation.

gave a 3.2-fold increase after receiving 300 rads (Table XVI). Both patients with 300 rads to their upper bodies (including heads) showed marked increases in the serum amylase activity, a 20-fold increase for Case No. 108 and a 17-fold increase for Case No. 110. Increases in urinary amylase activity ranging from 12.9- to 2.4-fold were observed in all patients receiving whole- or upper-body irradiation (Table XVII).

Since serum amylase level is increased only when the total or upper body is irradiated, the serum amylase produced after irradiation is probably originated from salivary glands. In order to confirm this, 3 μl of serum samples before and after irradiation were applied on agar gel slides and electrophoresis was carried out at 300 volts for 2 hours at room temperature. Human saliva and aqueous extract of human pancreas were used as standard. Amylase activities were revealed on starch agar slides by staining with iodine solution after amylase was imprinted on the starch agar slides and incubated at 30° C. for 1 hour (5). Pancreatic amylase was found to move faster toward the cathode than salivary amylase (Figure 10). Three μl of preirradiation serum failed to give an amylase spot on the slide whereas those of postirradiation serum gave an amylase spot which migrated together with the amylase in human saliva, suggesting that the increase in serum amylase level is caused by radiation effect on salivary glands.

Amylase activity in urine of rats did not respond to 200 rads whole-body irradiation. Serum amylase activity in dogs showed a slight increase after their salivary glands were irradiated (Table XVIII), but the increase was not as distinct as that found in man.

In order to obtain a further insight into the mechanism of radiation-induced increase in amylase activity, in vitro studies will be carried out in which radiation effect on the secretion of amylase by slices of salivary glands in buffer will be investigated (6). This experimental technique will also be used to carry out dose-response studies.

TABLE XVI

SERUM AMYLASE LEVELS IN CANCER PATIENTS
RECEIVING PARTIAL-BODY IRRADIATION

PATIENTS			DOSE*	AMYLASE LEVELS (Somogi Units/100 ml)		POST
Case No.	Sex/Age	Diagnosis	(Rads)	Pre-irradiation	Post-irradiation+	PRE
	M/41	Ca Mouth Floor	--- (200)	144 ± 6	200	1.4
	M/51	Ca Hypo-pharynx	--- (265)	157 ± 6	900	5.7
	M/57	Ca Tonsil	--- (235)	76 ± 5	360	4.7
108	F/66	Ca Colon	300 U (355)	258	5, 150	20.0
110	M/51	Melanoma	300 U (385)	81 ± 6	1, 380	17.0
92	F/69	Ca Breast	150 L (5)	142 ± 6	145	1.0
94	F/67	Ca Lung	150 L (13)	180 ± 0	180	1.0
102	M/49	Ca Lung	200 L (18)	140 ± 20	450	3.2
101	M/76	Ca Colon	257 L (-)	153 ± 21	150	1.0
95	F/66	Ca Colon	300 L (10)	130 ± 18	170	1.3
100	M/75	Ca Colon	300 L (11)	278 ± 22	360	1.3
106	F/58	Ca Colon	300 L (14)	109	95	0.9

*U: Upper body (above xiphoid). L: Lower body (base of neck to pubis).
( ): Doses to head and neck.

+ Maximum values after irradiation.

TABLE XVII

URINARY AMYLASE LEVELS IN CANCER PATIENTS
BEFORE AND AFTER RADIATION THERAPY

PATIENTS			DOSE*	AMYLASE (SOMOGI UNITS/HOUR)		POST
Case No.	Sex/Age	Diagnosis	(Rads)	Preirradiation	Postirradiation+	PRE
98	F/45	Ca Colon	200 W	177	1, 200	7.1
107	F/58	Ca Colon	200 W	106	740	7.0
99	M/45	Ca Rectum	200 W	186	346	2.4
96	M/42	Ca Colon	100 W	255	1, 368	5.4
105	M/13	Ewing's Sarcoma	100 W	56	362	6.5
108	F/66	Ca Colon	300 U	235	2, 562	10.1
110	M/51	Melanoma	300 U	194	2, 490	12.9

*W = Whole body; U = Upper body

+Maximum values after irradiation

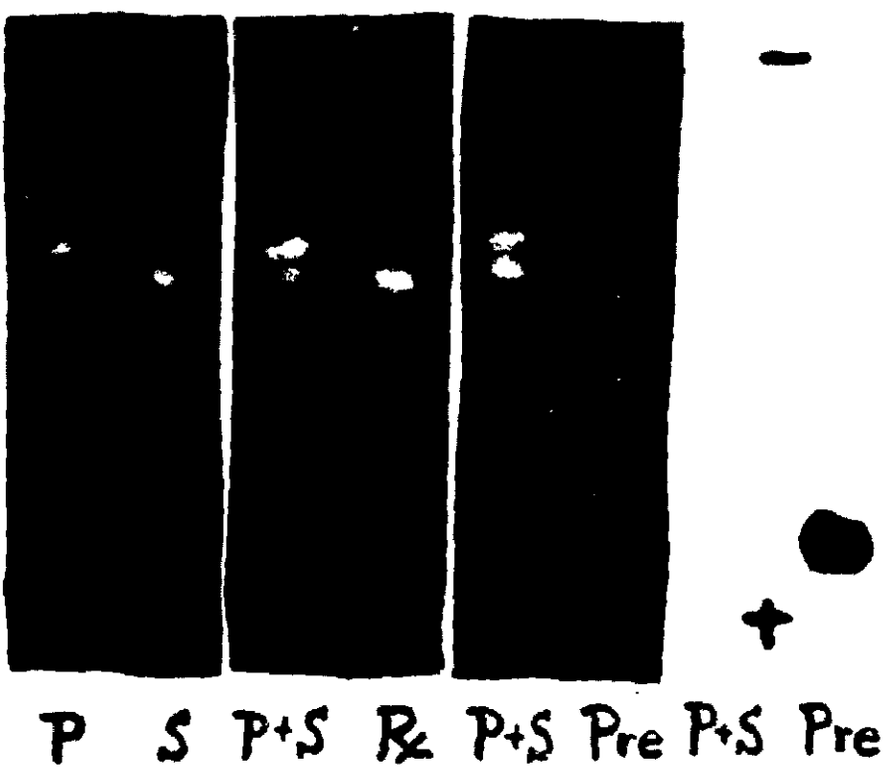

Figure 10. --Agar gel electrophoretic separation of human salivary and pancreatic amylase.

P: pancreatic amylase, S: salivary amylase, P+S: mixture of pancreatic and salivary amylases, R_X: postirradiation serum, Pre: preirradiation serum.

3 μl of serum samples from Case No. 108 (300 R upper body) were used for electrophoresis at 300 volts for 2 hours. Amido black staining of serum proteins is also shown in the slide at extreme right.

TABLE XVIII

SERUM AMYLASE LEVELS IN DOGS BEFORE AND AFTER IRRADIATION

Dog No.	Dose (Rads)	Amylase (Somogi units/100 ml)		
		pre	1st day post	2nd day post
1.	480 to salivary glands	1,550	1,620	1,800
2.	480 to salivary glands	1,130	1,540	1,575
3.	480 to salivary glands and 100 to abdomen	1,280	2,100	1,800
4.	480 to salivary glands and 200 to abdomen	1,280	1,575	1,575

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PSYCHOLOGICAL EVALUATION OF THE EFFECTS OF TOTAL- AND PARTIAL-BODY RADIATION

Seven additional patients were evaluated during 1970-1971. (See DASA 2599 for the most recent report of this ongoing study.) Biographic data for these seven subjects is given in Table XIX. This brings to 43 the total number of patients who have undergone assessment for the effects of total- or partial-body irradiation on their cognitive-intellectual functioning and emotional reactions. In terms of the characteristics of the overall sample, the addition of the new patients will serve to improve the ratio of whites to Negroes, to increase slightly the average educational attainment, and to decrease the average age. The trend noted in the 1969-1970 report toward recruiting patients in comparatively better physical condition has continued.

The methodology and timing of the 13 testing occasions continues to follow the format outlined in previous reports. During the 1-week period of initial evaluation, personality and intelligence testing is carried out and the patient is oriented to the procedures to be followed prior to and following sham and actual irradiation as well as to what will be expected of him during the 6-week post-radiation study period. In addition to the usefulness of the data collected during the initial week of study (usually three separate testing periods), it is clear that this time spent with the patient is an important aspect of the later high levels of motivation to cooperate which all patients exhibit. Verbal behavior elicited with standardized instructions, depression rating scales, and the Trails performance test continue to be regularly collected on each of the scheduled occasions.

A paired-associates memory test has been added to the regular testing protocols during the course of the year at the suggestion of Dr. Louis A. Gottschalk who served as consultant to the psychological aspects of the project. In structure and scoring techniques this new paired-associates test follows the format of the associate learning subscale of the Wechsler Memory Scale (1). In view of the repeated nature of the schedule of testing of these cancer patients, we required a minimum of seven equivalent versions of such a test in order that at least 10 days to 2 weeks would intervene between use of each form. Using recent

TABLE XIX

BIOGRAPHIC DATA OF RADIATION PATIENTS STUDIED IN 1970-1971

Subject	Sex	Race	Age	Marital Status	Dx	Educ	Rad	IQ
105	M	W	14	S	Ewing's Tumor Spine	9th	100T	74
106	F	W	58	Wid	Ca colon	12th	300L	73
107	F	W	58	Wid	Ca colon	12th	200T	101
108	F	W	66	Wid	Ca colon	3rd	300U	60
109	M	W	55	M	Ca colon	8th	300L	89
110	M	W	51	Sep	Melanoma	3rd	300U	77
111	F	N	52	Wid	Ca colon	8th	200T	76

research in this area, pair-associates were selected on the basis of concreteness and imagery value (2), as well as the original Thorndike-Lorge frequency values (3). After initial testing of the seven lists on a sample of both patients and normal subjects, an item analysis was carried out. On this basis, individual items for the lists were then rearranged to maximize the equivalency of all seven tests. The paired-associates test is now regularly employed with all subjects on all 13 testing occasions. It has already shown itself to have several advantages.

1. The test can be given with ease to patients unable to undertake the Trails test due to physical disability. This decreases the likelihood of missing data--a vexing problem during the analysis of longitudinal data.

2. The pair-associates subtests we have developed appear to have a type of face validity which appeals to patients and tends to mobilize their interest in cooperating and performing at maximal capacity.

3. The test appears to be more independent of intelligence than other tests of cognitive functioning which we have considered.

4. We have found that the test can be given under difficult conditions such as in a recovery room when the patient is unable to move, when arms are immobilized, or when nausea causes difficulties in timed tests.

The problem of handling nausea as a factor which influences tests results and increases performance decrement continues to be a provocative and challenging one. To the extent that nausea is an intervening variable at crucial times in the psychological testing schedule, it causes considerable ambiguity in interpreting test results and in reaching a goal of reliably assessing the effects of varying dosages of irradiation on cognitive functioning.

Since the winter of 1967, all subjects have been interviewed very shortly after their release from the cobalt 60 teletherapy unit where they underwent irradiation. As part of the Clinical Depression Rating Scale rating on degree of nausea and vomiting is made on a four-point scale: a zero is scored for no evidence of nausea, and a three is given for nausea accompanied by vomiting sufficient to interfere with ability to speak or perform on psychological tests. As

shown in Table XX, there has been an increase in the frequency with which some degree of nausea is apparent in the period immediately postradiation. Of the 25 patients for whom we have records, 15 exhibited some nausea (ratings of 1, 2, or 3). Of the seven patients studied this year (1970-1971) only one did not exhibit nausea and three (subjects 107, 109, and 111) had ratings for severe or marked nausea.

It is suggested that future patients be interviewed during the initial phases of the study regarding their attitudes toward nausea, vomiting, and loss of control. While some vomiting cannot be inhibited by conscious attempts at control, it seems likely that there are psychological factors and long-standing life-long attitudes which do play a role in whether a patient attempts control. The interview should include data from early childhood on nausea and vomiting, history of frequency of vomiting as a concomitant to fever and other illnesses, vomiting accompanying abuse of alcohol, and general attitudes toward loss of control. These data could then be used in conjunction with our records on varying dosages of irradiation and the records of postirradiation nausea to assess the value of screening for attitudes toward nausea and vomiting as an important variable in personnel selection.

A second approach to better management of this problem is to experiment with posthypnotic suggestion. The Psychiatry Department faculty includes a number of psychiatrists who are competent and knowledgeable in the uses of hypnosis and posthypnotic suggestion. It is possible that the performance decrement due to nausea may be amenable to either complete control or to control of its latency via posthypnotic suggestion.

An investigation was carried out during the year to assess the relationship of medication and previous fractionated dosages of therapeutic radiation on the initial scores obtained on the Cognitive Impairment Scale. Medications being administered at the time of initial testing were classified as psychoactive or nonpsychoactive. Average scores for subjects in each group did not differ significantly and it is therefore concluded that any elevation of cognitive impairment scores at the time of initial testing is not due to the effects of medication. Patients were also classified into two groups on the basis of whether or not they

TABLE XX

TYPE AND AMOUNT OF RADIATION AND IMMEDIATE POSTIRRADIATION NAUSEA RATINGS

Subj. No.	Rads[1]	Nausea Rating[2]
075	200L	2
078	200T	0
079	100T	0
083	100T	0
084	300U	1
086	100T	0
087	200T	3
088	150T	1
089	200P	0
090	150T	--
091	200T	1
092	150P	0
094	150P	0
095	200T	--
096	100T	0
097	100T	0
098	200T	2
101	257L	1
102	200P	1
103	300L	1
105	100T	1
106	300L	2
107	200T	3
108	300U	0
109	300L	3
110	300U	1
111	200T	3

[1]T = Total-body irradiation

L = Lower

U = Upper

P = Neck to pubis

[2]Based on a 4-point scale with 0 indicative of complete absence of symptoms of nausea and vomiting and 3 indicative of nausea accompanied by vomiting sufficient to markedly impair ability to function.

had had any previous radiation therapy. Average cognitive impairment scores for those with or without previous radiation were almost identical.

The data at this time continue to support the trend reported in 1970 that there is an increase in cognitive dysfunction immediately following irradiation. Using seven occasions (pre and postsham, pre and posttreatment, day 1, day 3, and day 7) and the data of 30 patients, the cognitive impairment scores were again submitted to the Friedman nonparametric test for matched groups. With the addition of the 1971 patients, the data continue to show a significant difference for the column sums of the ranks over all groups, with pretreatment scores ranking lowest and posttreatment scores ranking highest ($\chi^2 = 15.73$, 6 df, $p < .02$). It is also consistent with the 1970 report that the data for 1971 show that the significant postirradiation increase is highly significant for the two Total-Body Radiation groups combined ($\chi^2 = 22.12$, 6 df, $p < .01$) but not for the three combined Partial-Body Radiation groups. The painstaking annual increment of subjects in the study should in the future help to validate the stability of this finding.

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CASE HISTORIES

STUDY NO. 104

PATIENT: A. C.

CHART NO.: CGH No. 128-870

This patient, a 58-year-old Negro woman with known metastatic adenocarcinoma of the sigmoid colon (SP 67-3689) was admitted to Cincinnati General Hospital on 5 June 1970 for partial-body radiation. In October 1969 she received 200 rads of whole-body radiation with a bone marrow transplant. The patient was shammed on 8 June 1970 and treated on 9 June 1970 with 300 rads midline absorbed tissue dose (479 R midline air exposure) of partial-body radiation to the lower body. During the last half hour of therapy, she vomited several times and twice the following day. The patient was discharged 10 June 1970 to be followed in the Tumor Clinic.

She continues to do well, 387 days post PBR.

STUDY NO. 105

PATIENT: P. G.

CHART NO.: CGH No. 151-121

This patient, a 13-year-old white boy, was admitted to Cincinnati Children's Hospital on 23 April 1970, complaining of severe back pain of 6-months duration. A myelogram performed revealed obstruction at D_{12}. Laminectomy on 1 May 1970, revealed Ewing's tumor (Path. No. P 70-1025) partially extradural which was subtotally resected. He received a total tumor dose of 4, 600 R of ^{60}Co from 11 May 1970 to 11 June 1970.

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On 21 September 1970, the patient was readmitted to Cincinnati Children's Hospital for total-body irradiation. He received sham irradiation on 21 September 1970, and was treated with 100 rads midline absorbed tissue dose (154 R midline dose in air) of total-body irradiation the next day. He experienced mild nausea and vomiting for approximately 5 hours following treatment. He was discharged to his home on 25 September 1970, to be followed by the Radiotherapy Department.

He continues to do well 282 days post TBR.

STUDY NO. 106

PATIENT: M. S.

CHART NO.: CGH No. 361-291

On 8 January 1966, this patient, a 58-year-old white female, had resection of an adenocarcinoma of the sigmoid colon (SP 66-56). Due to anorexia, nausea and vomiting, and weakness, the patient was admitted to Cincinnati General Hospital on 26 October 1970, where the presence of liver metastases was confirmed.

She was given sham irradiation on 9 November 1970, and experienced no adverse side effects. On 10 November 1970, she received 300 rads midline absorbed tissue dose (461 R midline air exposure) of partial-body irradiation to the lower half of the body. She experienced one episode of vomiting immediately post treatment and one after her evening meal, otherwise tolerating the procedure well.

A feeding gastrostomy was performed on 4 December 1970; her disease was extensive and following the development of pulmonary emboli, the patient expired on 5 December 1970, 25 days post PBR.

STUDY NO. 107

PATIENT: R. S.

CHART NO.: CGH No. 521-703

This patient, a 58-year-old white woman, was diagnosed as having unresectable carcinoma of the sigmoid colon at another Cincinnati hospital in April, 1970. In June, 1970, due to metastases, the patient was given 4,000 R of ^{60}Co irradiation to the upper lumbar spine and left femoral head. She also received a course of fluorouracil in August, 1970.

On 14 December 1970, the patient was admitted to Cincinnati General Hospital complaining of severe left rib pain in the submammary area, secondary to a roentgenographically demonstrable metastasis. She was given sham irradiation on 14 December 1970. Under general anesthesia five hundred and twenty-two milliliters of bone marrow were aspirated on 15 December 1970 from the sternum and iliac crests. During this procedure, the patient was transfused with two units of whole blood. She then received 200 rads midline absorbed tissue dose (307 R midline air exposure) of total-body irradiation followed by nausea and vomiting episodically over a 3-hour period. One hour post-irradiation, the marrow (containing 3.2×10^9 cells, 95 percent viable) was infused intravenously without sequelae. Her granulocyte nadir, 650 per cu. mm. was reached on day 27 but she never ran a febrile, septic course.

She was transferred back to Daniel Drake Memorial Hospital for followup care on 18 December 1970. Her condition slowly deteriorated and on 14 March 1971, 89 days post TBR, she expired.

STUDY NO. 108

PATIENT: A. S.

CHART NO.: CGH No. 463-480

This patient, a 66-year-old white woman, was admitted to Cincinnati General Hospital on 15 December 1967, complaining of lower left quadrant pain. Exploratory laparotomy and resection of the sigmoid colon performed on 16 January 1968, revealed colloid carcinoma of the colon (Path. No. SP 68-146).

On 25 January 1971, she was admitted for upper-body irradiation for pulmonary metastases. She was shammed on 26 January 1971, and treated on 27 January 1971, with 300 rads midline absorbed tissue dose (485 R midline dose in air) of upper-body irradiation. She was nauseated and vomited for approximately 4 hours following irradiation.

The patient was discharged to her home on 29 January 1971, to be followed in the Tumor Clinic.

Chest X-ray on 23 March 1971, revealed no progression of pulmonary metastases. A complete blood count obtained at this time was normal. On 22 June 1971, 145 days post PBR, the patient continues to do well.

STUDY NO. 109

PATIENT: W. L.

CHART NO.: CGH No. 121-449

This patient, a 54-year-old white man, was admitted to Cincinnati General Hospital on 9 July 1969, with a 3-week history of weakness, malaise, weight loss, and left flank tenderness. On 8 August 1969, a left colectomy was performed for adenocarcinoma of the colon (Path. No. SP 69-2458).

Recurrent colonic carcinoma with generalized abdominal metastases were found at laparotomy (Path. No. SP 71-591) on 16 February 1971. The patient was shammed on 23 March 1971, and treated the next day with 300 rads midline absorbed tissue dose (524 R midline tissue dose in air) to the lower half of the body. Nausea continued for 4-1/2 hours, vomiting for 2-1/4 hours following irradiation. The patient was discharged to his home on 25 March 1971.

The patient was readmitted on 16 April 1971, due to gastrointestinal bleeding and dyspnea. X-ray studies revealed metastases involving the stomach. He was discharged to his home on 3 May 1971, to be followed in the Tumor Clinic. On 25 May 1971, 62 days post PBR, his weight was stable and his appetite improving.

On 8 June 1971, the patient was seen in the Tumor Clinic with severe anemia, nausea, and vomiting. His course continued downhill and on 12 June 1971, 80 days post PBR, he expired.

STUDY NO. 110

PATIENT: J. S.

CHART NO.: CGH No. 99-1-77

This patient, a 51-year-old white man, had a malignant melanoma (Path. No. SP 64-1754) removed from his back in June, 1964. He was admitted to Cincinnati General Hospital on 20 August 1970, complaining of several enlarging, tender nodules that had appeared approximately 3 months earlier. Excisional biopsy of two of these nodes revealed (Path. No. SP 70-2963) metastatic melanoma, lymph nodes. ^{60}Co teletherapy was begun on 27 August 1970, and continued until 24 September 1970, to deliver a total tumor dose of 4,500 R. He was also treated with fluorouracil and hydroxyurea until February, 1971, with little effect.

On 12 April 1971, the patient was readmitted for widefield radiotherapy. He was given sham irradiation on 13 April 1971, with no adverse side effects and treated on 14 April 1971, with 300 rads midline absorbed tissue dose (510 R midline dose in air) to the upper body. He had three episodes of vomiting following irradiation. A trans-urethral resection was done on 3 May 1971, due to an enlarged prostate and urinary retention. He was discharged to his home on 10 May 1971, 26 days post PBR, to be followed in the Tumor Clinic.

This patient was admitted 22 June 1971 with abdominal distention and tenderness, anorexia, and weakness. He expired on 27 June 1971, 69 days post PBR.

STUDY NO. 111

PATIENT: M. M.

CHART NO.: CGH No. 235-036

This patient, a 52-year-old Negro woman, was admitted on 29 December 1970, complaining of increasing lower abdominal and back pain for the past 2 months. Sigmoidoscopy on 30 December 1970, revealed adenocarcinoma of the large intestine (Path. No. SP 70-4592). On 12 January 1971, an abdominal-perineal resection, total hysterectomy and bilateral salpingo-oophorectomy revealed adenocarcinoma of rectum with metastases to uterus and right ovary (Path. No. SP 71-125).

Due to persistent right lower quadrant pain, she was admitted to Cincinnati General Hospital on 17 May 1971, for total-body irradiation. On 18 May 1971, she was given sham irradiation. Five hundred and four milliliters of bone marrow were aspirated on 19 May 1971. During this procedure the patient was transfused with 350 cc. of autologous blood. She then received 200 rads midline absorbed tissue dose (309 R midline tissue dose in air) of total-body irradiation followed by mild nausea and emesis. Approximately 1 hour post treatment, the bone marrow, containing 7.4×10^9 cells, 96 percent viable, was infused intravenously without sequelae.

She was discharged to her home on 21 May 1971, to be followed by the Tumor Clinic.

On 19 June 1971, she was readmitted with small bowel obstruction surgically corrected the same day. She was discharged improved to her home on 29 June 1971, 40 days post TBR.

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13 ABSTRACT

The goal of this program has been to obtain new information regarding the pathophysiologic, psychologic, immunologic, hematologic, and biochemical effects of total- and partial-body irradiation in human beings. The patients are irradiated, all of whom have inoperable, metastatic carcinoma but are in relatively good health, provide us with the opportunity to study multiple facets of the effects of radiation in man rather than in experimental animal. As we and many other laboratories have discovered, extrapolation of results from laboratory animals to man to be fraught with error. We have continued our search for a suitable biological dosimeter in human beings. The data contained in this report will suggest several potential biological dosimeters previously considered to be of some value have not fulfilled this expectation.

Biochemical and psychological studies have extended the findings of our previous report in depth and scope. Several new biological dosimeters are under evaluation.

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