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OAK RIDGE INSTITUTE OF NUCLEAR STUDIES

MEDICAL DIVISION RESEARCH REPORT FOR 1962

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FOREWORD

In April 1962 the Board of Directors of ORINS appointed me chairman of the Medical Division to succeed Marshall Brucer who retired because of impaired health in December 1961. The staff of the Division has adapted generously during the period of transition, and is continuing to work effectively and enthusiastically. More than the usual amount of time has gone into planning and program organization. During the summer a 10-year plan of projected research was written jointly by members of the senior staff and was reviewed by the Board of Directors of ORINS and by the Division of Biology and Medicine of the Atomic Energy Commission. This 10-year projection is serving as a basis for more detailed planning for the immediate future, but in some areas of the program it already appears that expansion should be more rapid than was anticipated.

The present report includes specific research activities and does not reflect the full activities of the staff. Other areas of research, not reported, involve long-term programs for which further data were collected during the year.

The staff is indebted to Elizabeth Anderson, Technical Editor, John Flora, Medical Illustrator, and Rush King, Photographer, for help in preparation of the report.

Gould A. Andrews, M.D.

RADIATION EFFECTS AND TREATMENT

Summary of Clinical Total-body Irradiation Program (G. A. Andrews, B. W. Sitterson, D. A. White, R. M. Kniseley, and F. V. Comas)

Since 1957 patients with a variety of malignant disorders have been studied after total-body irradiation. Initially a series of moderate to high doses were given and attempts to graft homologous marrow were made without success. In a few patients with acute leukemia, temporary remissions were obtained from irradiation alone. The special total-body Cs^{137} irradiation facility was completed in 1959.

During the last two years only a limited number of selected patients have been given high doses. Most studies have been on a series of patients given usually 50 r or 100 r total-body irradiation in single doses at the rate of 0.74 r per minute. Hematologic, chemical, and immunologic studies, and clinical observations have been made. Patients with lymphocytic leukemia and lymphosarcoma have obtained some clinical benefit with regression of lymphadenopathy and splenic size. Until this study systematic laboratory evaluation of single-dose treatment has not been made, and base-line information of this kind is important for comparing in the future other kinds of total-body therapy such as with internal isotopes, neutron irradiation, and the like.

Summary of patients irradiated through 1962:

High Dose (200 to 940 r)	No. Pts.
Acute leukemia	13
Subacute granulocytic leukemia	2
Chronic granulocytic leukemia (trans. to acute)	1
Miscellaneous disseminated neoplasms	5

Low Dose (50 to 100 r)	No. Pts.
Acute leukemia	2
Subacute granulocytic leukemia	1
Lymphosarcoma (including Hodgkin's)	14
Chronic lymphocytic leukemia	14
Chronic granulocytic leukemia	6
Miscellaneous disseminated neoplasms	2

Influence of Vascular Anoxia on Radiosensitivity (F. Comas)

Work on the influence of vascular anoxia on radiosensitivity was continued from the previous year. (The Research Report for 1961 described preliminary studies concerning the determination of the degree of hypoxia obtained by occlusion of the vascular supply and the changes in DNA synthesis of the bone marrow and tumor of nonirradiated rats, subjected to anesthesia, laparotomy, and arterial clamping.) In 1962 results were obtained that answer the question: Is there the same degree of change in radiosensitivity, due to vascular anoxia, for bone marrow, and for an experimental tumor? A decrease in radiosensitivity (a "protective effect") under anoxia was expected. If it were the same, or if the protective effect were more marked for the tumor, no useful applications could be envisaged for a technique of irradiation under anoxia. If, however, the protective effect is greater for bone marrow, one could theorize that irradiation under anoxia should be of value, because in differentially protecting a normal tissue (bone marrow), higher radiation doses could be tolerated, and therefore more irradiation could be delivered to the tumor, with better chances of destroying it.

The experimental setup, briefly reviewed, consisted of irradiating the lower extremities of rats bearing the Walker carcinosarcoma 256 in both thighs. The tumor and bone marrow of the left leg were rendered anoxic during irradiation by clamping the left common iliac artery. The vascular supply of the right leg was left undisturbed. Radiosensitivity was measured by determining the time lag in recovery of normal levels of DNA synthesis by the bone marrow and tumor cells. (It has been previously shown that the log of this time interval is linearly related to radiation dose.) As the radiation dose is increased,the longer it takes for the cells to reach a level of DNA synthesis equal to that of nonirradiated control animals. Although this relationship is maintained for anoxic bone marrow and tumor up to at least 1500 r (the highest dose tested), it ceases to hold true for oxygenated tissues beyond 1000 r, which seems to be the limit of applicability of this technique. By comparing the slopes of the dose versus time delay lines for oxygenated and anoxic bone marrow, and similarly for the tumor, it is possible to quantitate the protective effect of anoxia in these two tissues. It has been

found that for bone marrow the protective effect is 2.0. (It takes double the amount of radiation to cause the same delay in recovery of normal DNA synthesis for anoxic bone marrow, as compared to oxygenated marrow.) The protective effect of anoxia on the tumor is 1.5. (It takes 50% more radiation to cause the same delay in recovery of normal DNA synthesis for anoxic tumor as compared to oxygenated.)

The interpretation of these results points to interesting possibilities and raises some new questions. If the amount of radiation one can deliver to a region of the body containing a tumor is limited by the tolerance of the normal tissues in that region (as is often true), the same effects will be caused on these tissues by doubling the amount of radiation, if this is given under anoxia. The effect on the tumor, however, would be 33% greater (2.0 divided by 1.5 equals 1.33), and therefore a favorable differential effect would be obtained. On the other hand there is nothing to suggest that the same numerical values will obtain for other combinations of tumors and normal tissues; thus answers are so far only partial, and more work along similar lines is required before generalization can be propounded. One could object to the end point used to gauge radiosensitivity. The time delay in recovery of normal levels of DNA synthesis was elected because (1) it had been shown to be a linearly related dose; and (2) it was applicable to both tumor and bone marrow in vivo. However, in the absence of proof, one can not positively claim that this particular effect of radiation is equivalent to cell growth inhibition, which is the main effect sought after in the irradiation of tumors. It could be, although it does not seem too likely, that the changes in radiosensitivity under anoxia could be different if another method to measure radiosensitivity was used. Attempts to clarify this point are planned.

Serine Metabolism in Mice Carrying Homologous Bone-Marrow Grafts
(A. L. Kretchmar and C. C. Congdon*)

The concentration of free serine of liver is low in irradiated mice with homologous bone-marrow grafts as compared to unirradiated animals or to irradiated mice given isologous bone-marrow cells. At seven days after injection of homologous marrow, the concentration is within the range of levels in control animals; at 12 days, the concentration is reduced by about 1/3, at 21 and 35 days by 1/2. Levels in plasma are low at 12, 21, and 35 days. The concentration of serine in muscle is not affected until after day 21, when it is decreased. Study of a speculative model of serine metabolism that relates the serine pools in liver, plasma, and muscle dynamically on

*Oak Ridge National Laboratory

an analog computer suggests that a marked increase in net loss of serine (either greatly accelerated utilization, blocked synthesis, or a combination) can explain the experimental results. This imbalance in serine metabolism would begin on about the fourth to sixth day after injection of marrow. This result is consistent with other findings indicating that the disturbance in host metabolism brought about by a homologous graft begins very early after injection of the foreign cells.

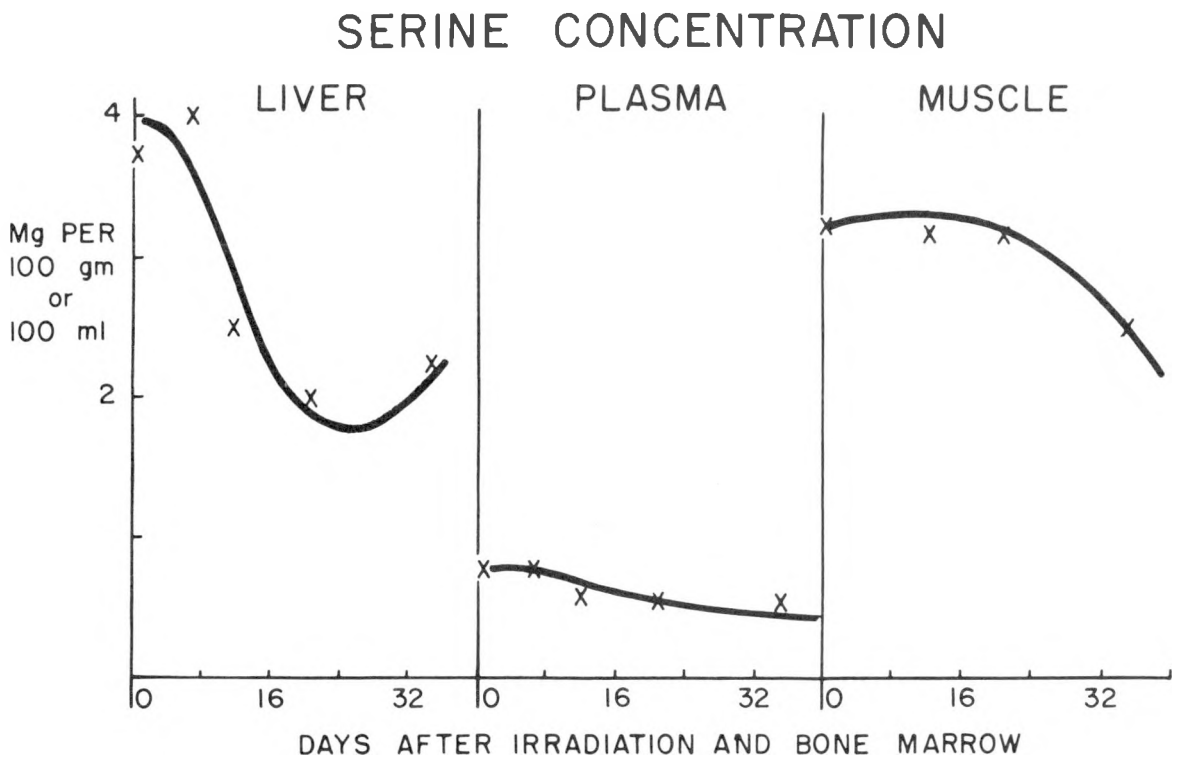

Fig. 1 Changes in serine concentration based upon an arbitrary theoretical model of serine metabolism. The solid lines are taken from the recorded output of an analog computer. The X's are taken from experimental data. The chief point of interest to come out of this particular solution was that a rapidly increasing rate of net loss of serine, beginning at about four days and extending to about 10 days, was necessary in the program.

Studies on Homologous Disease (A. L. Kretchmar)

Cooperative work with William MacArthur of Knoxville College, Department of Biology, has shown that in mice losing weight because of homologous bone-marrow grafts, the nitrogen balance may be positive as it is in normal animals. This paradoxical result suggests that one aspect of the metabolic disturbance of homologous disease is an internal shift of nitrogen within the animal. This shift of nitrogen could be the transfer of tissue and dietary nitrogen into antigen and antibody proteins. The loss of tissue water as its protein is utilized could result in loss of body weight while the nitrogen was retained in antigen-antibody protein. Another possibility is that new types of protein may be synthesized within the host, or proteins ordinarily present in low concentration may be increased. This latter possibility is indicated by the finding, in cooperative work with the Biology Division of the Oak Ridge National Laboratory, that the lysozyme activity in kidney is increased in animals with homologous disease. The function of this protein enzyme of kidney tissue is not known, but it may have an as yet unidentified role in immunologic response of animals to foreign tissues. The observation is especially interesting in view of the known increase in lysozyme activity of kidneys of animals with malignant tumors. The effect of Walker carcinoma S-256 on lysozyme activity of kidney is shown in Fig. 1. This is another observation that links abnormal metabolism in animals with homologous disease to abnormal metabolism in animals with cancer.

Further studies on the amino acid metabolism of mice with homologous disease show that the serine of liver, plasma, and muscle, as discussed in the preceding summary, is reduced even though the animals are eating normally. As expected from the known metabolic relationships between glycine and serine, the concentration of glycine is also reduced in mice with homologous disease, though somewhat more irregularly and at a later time than serine. If the cause of this effect on these two key amino acids in nitrogen metabolism can be uncovered, considerable insight into the biochemical nature of homologous disease can be expected, and this information might be helpful in understanding the weight loss and debilitation that are often associated with cancer.

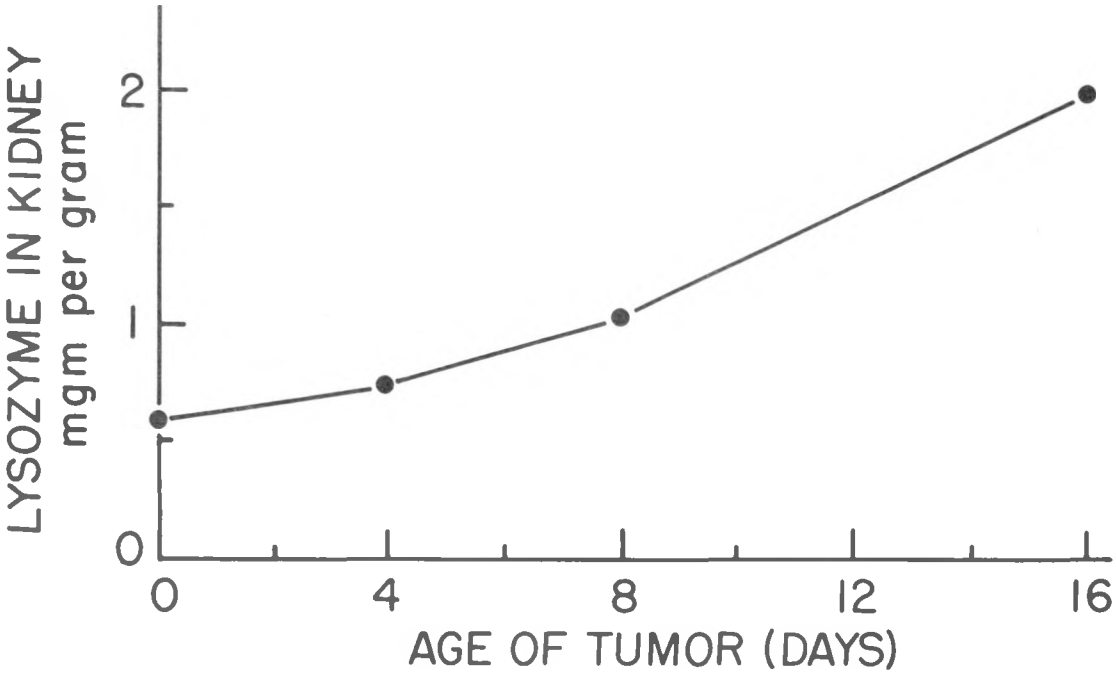

Fig. 1 Lysozyme in kidney of rats with Walker Carcinoma S-256.

Bone Marrow Lipids in Animals Exposed to Total-Body Irradiation
(Fred Snyder, Edgar A. Cress, and Nelson Stephens)

An elevation in total lipids of bone marrow of irradiated rabbits was first reported by Dietz and Steinberg (1) and Bernheim, et al. (2); neither group identified the lipid classes involved. Histologic evidence in man also indicates that fat accumulates in irradiated marrow.

The time curve for the increase in bone marrow lipid of the rat is shown in Fig. 1. Peak values occur about one week ($P < 0.001$) after the 800 r exposure and only slowly decrease with time. Total lipids appear to increase as a function of dose according to the equation $y = bx^a$, since the log of lipid versus log of dose when plotted graphically gives a linear relationship. Table 1 shows the variations in water, lipid, and residue values of control and 800-r rats, eight days after total-body irradiation. The changes in all three compartments after irradiation are highly significant ($P < 0.001$). The increase in total marrow lipids after irradiation is also correlated quite closely with the increase in fat cells seen in histologic sections (Fig. 2).

The effect of total-body irradiation on bone marrow lipids of other species was determined. In these measurements, conditions were arbitrarily chosen to be identical to those used for the rat. Only the rat showed the marked elevation of marrow fat. The dog, pig, and monkey show a decrease in total lipids of marrow after irradiation; however, the small number of animals used does not justify a statistical evaluation of the decrease. A more complete comparison of other species should include a thorough study of other time periods, doses, and marrow sites. On the other hand, the marrow-lipid response in the rat appears to be typical of what is seen in histologic sections of marrow obtained from irradiated patients.

The response to irradiation is much more apparent in areas of marrow that are normally highly cellular, with little fat. In support of this statement, our data show a much more striking change in the marrow of rat femurs than in the femurs of rabbits. These femurs are not very active hematopoietically as compared to those of rats, but instead contain a high amount of fat.

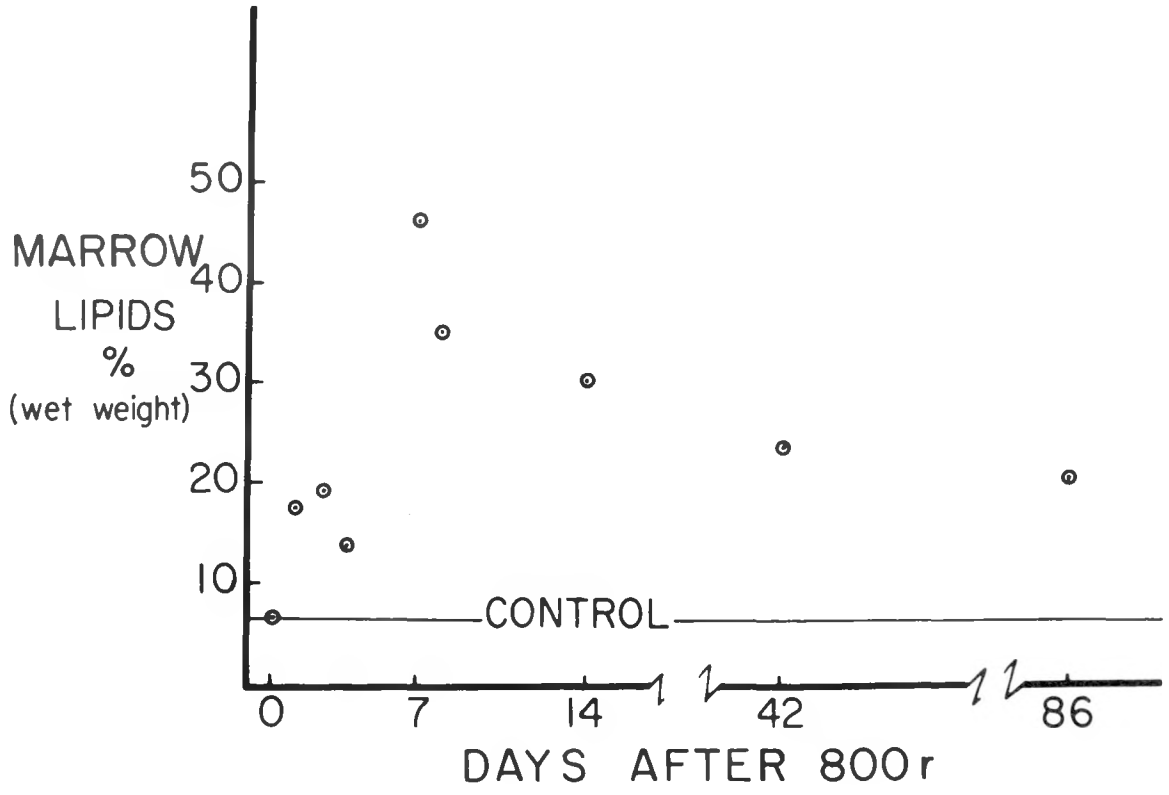

Fig. 1 Total lipids of bone marrow (wet weight) after 800 r total-body irradiation. Each value represents pooled samples from the following number of rats: 0 days = 182 rats (range 5.5 - 9.3% for 7 pooled groups); 1 day = 4 rats; 2 days = 8 rats; 3 days = 8 rats; 7 days = 8 rats; 8 days = 65 rats (range 33.1 - 46.3 for 5 pooled groups); 14 days = 8 rats; 42 days = 8 rats; 86 days = 4 rats. The probability for chance occurrence was less than 0.001 when the test of significance was applied to the difference between the mean of the control group and the 800-r (8 day) rats.

Table 1

THE COMPOSITION OF RAT FEMUR
MARROW 8 DAYS AFTER 800 r TOTAL-BODY IRRADIATION

	Number of rats	Lipid %	Water %	Residue %
Controls	182	7.2 ± 1.4	65.6 ± 1.0	27.9 ± 1.0
		(7)	(5)	(5)
8 days after 800 r total-body irradiation	65	38.4 ± 5.6	39.0 ± 6.7	22.3 ± 1.2
		(4)	(3)	(3)

The numbers preceded by ± are standard deviations. The numbers in parentheses represent the number of pooled groups used for the determination of the mean value. The lipid expressed on the basis of dry weight was 19.0 ± 2.2% for the control groups and 63.1 ± 4.5% for the irradiated groups. The probability of chance difference between the control group and the irradiated group for lipid, water, and residue compartments was less than 0.001 when the test of significance was applied.

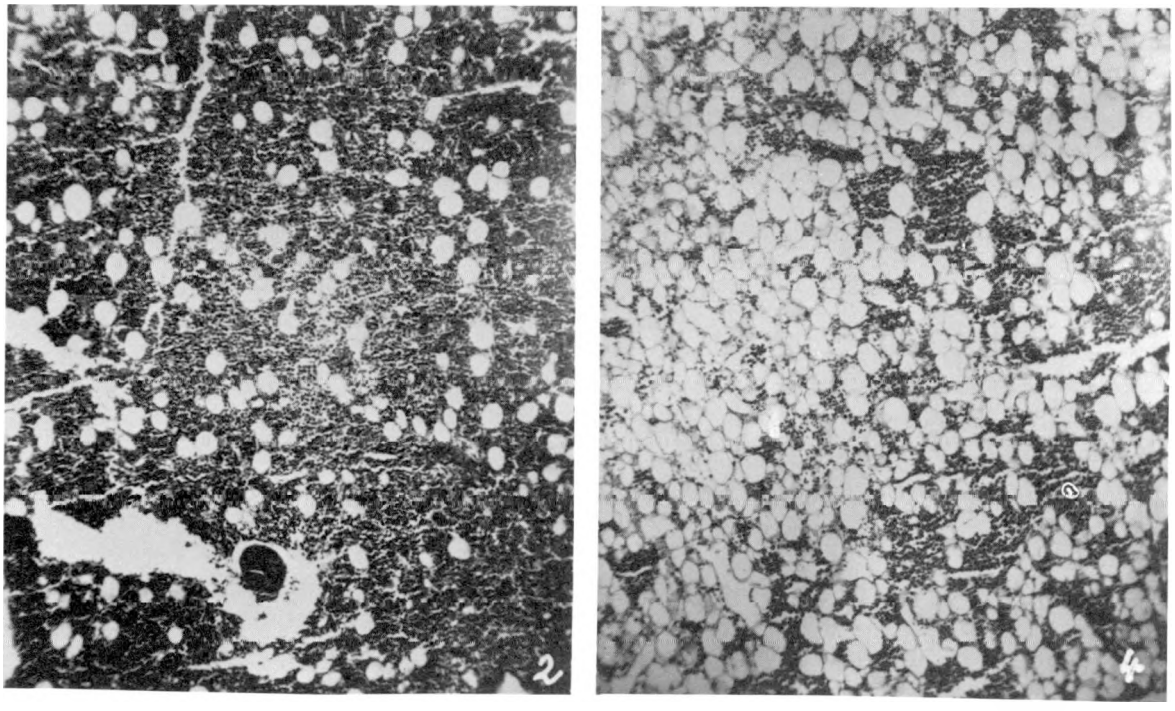

Fig. 2 Histologic sections of femur marrow from control (left) and irradiated (8 days after 800 r) rats (right). The sections were stained with hematoxylin and eosin.

Thin-layer chromatography (Fig. 3 and Table 2) has been used to demonstrate that bone marrow lipids of rats are primarily triglycerides; gas-liquid chromatography of the fraction revealed that palmitic and oleic acids account for more than 80% of the fatty acids. Minor lipid components present in the control and irradiated marrow are glyceryl ethers, cholesterol, fatty acids, and phospholipids. Cholesterol esters were not found.

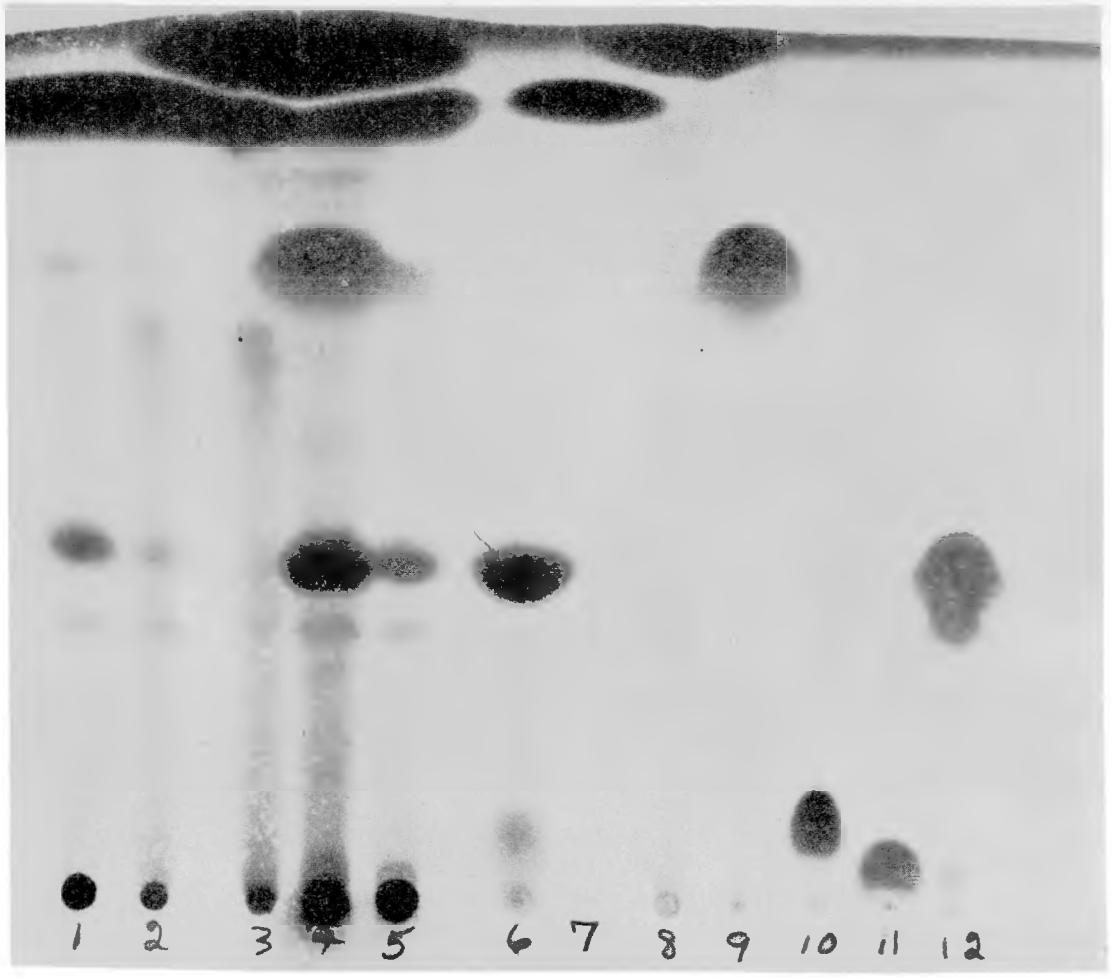

Fig. 3 Thin-layer chromatogram of bone marrow, adipose tissue, plasma and liver lipids: 1) control marrow, 2) 800-r marrow, 3) perirenal fat, 4) plasma lipids, 5) liver lipids, 6) cholesterol, 7) triolein, 8) cholesterol stearate, 9) oleic acid, 10) batyl alcohol, 11) monopalmitin, 12) 1,3-dipalmitin. The thin layer of silica was applied to an aluminum plate and after chromatography was visualized by spraying with concentrated H_2SO_4 and heating. The origin is located immediately above the numbers.

Table 2

THIN-LAYER CHROMATOGRAPHY ANALYSIS
OF RAT BONE MARROW TOTAL LIPID EXTRACT

	Control	800 r*
% of total esterified fatty acids as triglyceride	85	91
μg triglyceride/mg marrow	53	384
μg phosphorus/mg total lipid extract	5.94	0.85
μg phosphorus/mg bone marrow	0.35	0.32

*8 days after total-body irradiation

In the studies on rats, irradiation was shown to increase the triglycerides of the marrow cavity at the expense of water. The reciprocal relationship of water and lipid in the marrow confirms the earlier work of Dietz and Steinberg (1). In evaluating the mechanism of lipid increase, the role of oxidation and mobilization must be considered; however, the absence of any change in the neutral glycerides of plasma suggests that transport of glycerides as such from other sites is not an important factor. An initial investigation of plasma free fatty acids revealed little change in this pool at 1, 3, 5, and 8 days after 800 r total-body irradiation. The possibility that a humoral agent may be a responsible factor for at least part of the lipid response is suggested by preliminary evidence, which shows lipids of the femur to increase after irradiation of only the head; however, additional experiments are required for more definitive proof.

Fatty acid oxidation in irradiated bone marrow is being studied with a technique previously described (3). The data obtained demonstrate that a marked decrease in the oxidation of palmitic-1-C^{14} acid (as the albumin complex) occurs as early as one day after total-body irradiation. This decrease is still apparent even when the amount oxidized is corrected for fat-free and dry tissue. The quantity of fatty acid incorporated into triglycerides is concurrently being measured in these experiments.

At the present stage of experimentation we conclude that the biosynthesis of triglycerides and reduced oxidation of fatty acids in the marrow cells are both intimately involved in the fatty degeneration of marrow following total-body irradiation. Analysis of chemical reactions within specific cell fractions is expected to yield more definitive conclusions on the nature of the mechanisms involved.

Acknowledgements

Special assistance in this study has been obtained from Dr. Anton Lindner for histological preparations, from Dr. F. Comas for dosimetry measurements, from Sister Maria Benigna, Charles Dickinson, and Catherine Snyder for participation in some of the fatty acid oxidation experiments.

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Glyceryl Ethers as Protective Agents Against Irradiation Leukopenia
(Fred Snyder, E. A. Cress and D. Litton)

We have identified the presence of glyceryl ethers in bone marrow using thin-layer chromatography (1). Of the minor lipid components found in bone marrow, the glyceryl ethers are perhaps the most interesting as related to irradiation effects because of their reported dramatic stimulatory effect on hematopoiesis. The early work of Marberg and Wiles (2) with yellow marrow was the first to indicate that the monsaponifiable fraction of marrow lipids had a hematopoietic stimulatory factor present. More recently, protection by glyceryl ethers against the leukopenia in irradiated subjects was reported by Edlund, and by Brohult and Holmberg (3, 4). Our studies have the purpose of obtaining detailed information about the metabolic significance of these compounds and to assess their value in preventing or retarding irradiation damage. A considerable number of experiments has been completed in which various dose levels of highly purified glyceryl ethers (selachyl acetate, selachyl alcohol, batyl alcohol and batyl diacetate) have been administered to rats given total-body irradiation (800 r and 300 r).

Intraperitoneal injections of batyl alcohol and selachyl acetate (1 to 10 mg per rat for 20 days) have shown the most striking changes upon the irradiation leukopenia; oral intubation and dietary experiments are also planned for these compounds. Our current work indicates that the glyceryl ethers are most effective in rats given less than 300 r total-body irradiation, although at higher levels of irradiation the glyceryl ethers appear to cause a more rapid recovery of the leukocytes. When the total-body irradiation dose was reduced to 150 r, the batyl alcohol and selachyl acetate (10 and 1 mg/rat) showed a significant effect on the initial decrease of white blood cells that occurs in unprotected irradiated rats (Table 1).

Table 1

LEUKOCYTES FOUR DAYS AFTER 150 r TOTAL-BODY IRRADIATION IN RATS GIVEN INTRAPERITONEAL GLYCERYL ETHERS

Glyceryl Ether	G. E. Mg/day	No. Rats	Av. WBC/mm^3 Blood	Std. Deviation	Probability
Normal	0	13	11300	2277	-
150 r	0	18	4800	1045	<.001
Batyl alcohol + 150 r	0.1	4	2563	-	-
"	1.0	4	5300	-	-
"	10.0	19	7600	2172	<.001
"	20.0	5	5770	-	-
Selachyl acetate + 150 r	0.1	4	3788	-	-
"	1.0	4	4900	-	-
"	10.0	13	8581	2906	<.001

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The Medical Division Cell Preservation and Storage Facility
(Karl Hübner, N. Gengozian, and R. M. Kniseley)

During 1962 a device was designed by the Linde Company to the specifications of the ORINS Medical Division for the controlled freezing and preservation of human and animal cells. The apparatus, which consists of a BF-3-2 Linde Freezer and a special freezer controller, has been delivered and initial tests have been carried out. Thermocouples are employed to record the rate of freezing and to control by means of an amplifier circuit a valve that supplies liquid nitrogen to the freezer. The rate of freezing is chosen on a control panel and opening and closing of the valve supplying the liquid nitrogen regulates the rate of fall of temperature. The freezing chamber contains a specimen rack that sits within the insulated box; a fan at the base disseminates the vaporized liquid nitrogen throughout the chamber. A heater element is also included in the design. See Fig. 1.

The critical point in freezing viable tissue occurs when the sample reaches the heat of fusion, usually around -4 to -6°C. During the period of heat of fusion (-4 to -13°C.), it is essential to maintain the rate of cooling at about 1°C/min. Faster or slower rates are detrimental to cell viability. Even though the new unit has a controlled-rate freezing feature, various samples and sample sizes behave differently when passing through the heat of fusion. Therefore, the operator must acquire skill in manipulating the controls and maintain the best possible cooling curve by monitoring the recorded temperature on the Brown recorder.

Sealed glass ampules containing from 1 to 10 ml samples have been preserved and samples of leukocytes up to a volume of 85 ml have been preserved in metal storage containers. The medium consists of tissue culture medium, or plasma, and including in final concentration 10% dimethyl sulfoxide. Specimens preserved have included whole blood, leukocytes, bone marrow, suspensions of lymph nodes, and splenic cells from a variety of patients. Viability studies on the thawed cells have been made using tritiated thymidine, tritiated cytidine, and C^{14} uracil. Preliminary results show that there is DNA and RNA synthesis, but at the time of this report quantitative data concerning viability are not yet available.

The unit will be used in preservation of human leukocytes for supportive care of leukopenic patients, in the preservation of marrow samples for homologous transplantation attempts, for a source of supply of a single-cell source for sequential immunologic experiments : for example, splenic cells from one patient, providing a uniform source for a series of chamber culture antigen-producing experiments.

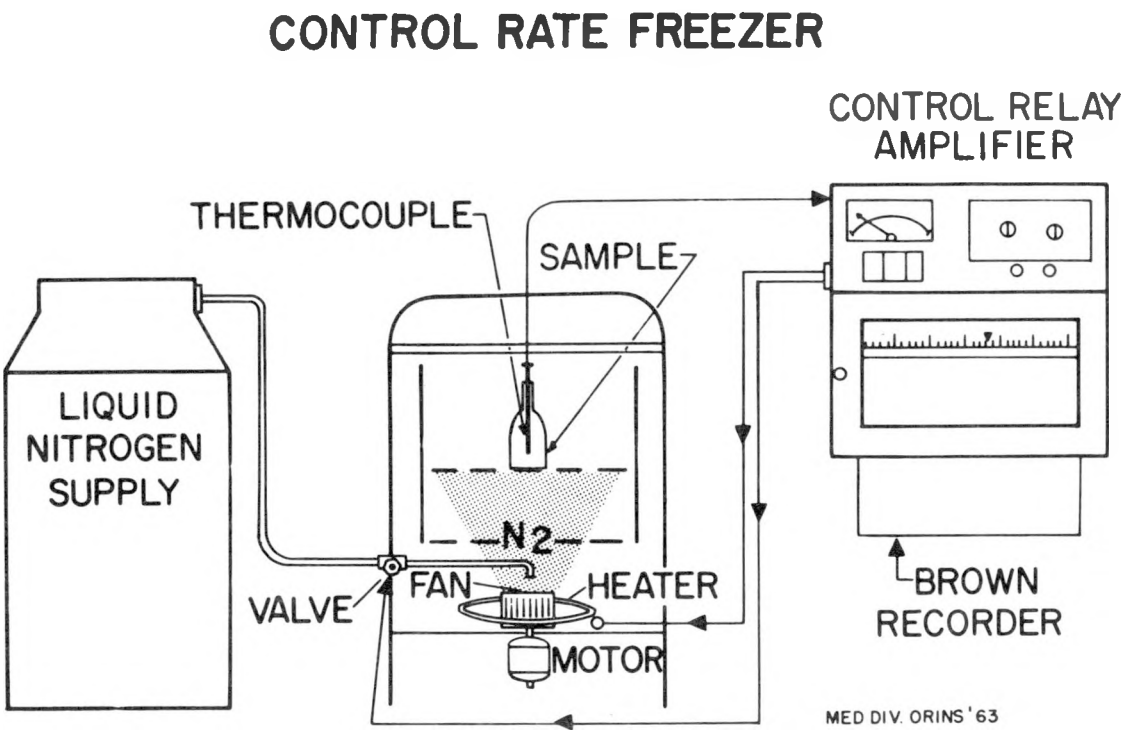

Fig. 1 Diagram of apparatus for controlled freezing and preservation of human and animal cells.

Experimental Studies on a Small South American Primate* (N. Gengozian and J. S. Batson)

Studies on the use of a small South American primate, Tamarinus nigricollis, in the laboratory are being continued under sponsorship of the U. S. Air Force Aerospace Medical Division. During the past year evaluation of various biological parameters in the tamarin was performed in an attempt to define some base line for members of this species. This was done primarily in anticipation of radiation sensitivity studies, and also for comparison of their values with the well-documented data existing on the rhesus (Macaca mulatta), the primate used most extensively in the laboratory. Our results have shown no major differences in the hematologic picture of these animals when compared to published data on the rhesus; indeed they showed a remarkable similarity in the mean values, range, and standard deviations of several parameters, particularly in the white blood cell counts and differentials. Part of the data obtained in our laboratory on the tamarin are shown in Table 1.

To ascertain the feasibility of using the tamarin in a study of radiation effects and the application of various therapeutic measures, a small group of animals was exposed to total-body gamma radiation to determine their radiosensitivity. Only animals maintained in our laboratory for at least three months were used in these studies, each animal being caged individually two weeks before irradiation and thereafter. The tamarins were exposed to doses ranging from 100 r to 600 r with a dose rate of approximately 4.1 r/min. Blood samples for hematology were collected one day before irradiation and at intervals of 1, 4, 7, days and weekly thereafter until death. The 30-day mortality of the tamarins exposed to 100 r, 200 r, 300 r, 400 r, 500 r, and 600 r is shown in Table 2. All seven animals exposed to 400 r or more died within 8 to 14 days after irradiation. Deaths also occurred at doses of 100 r and 200 r, although at a later date. The single animal surviving at 300 r for 30 days died 49 days after irradiation. In general the time of death was a function of the radiation dose administered. The apparent radiosensitivity of this species of primate as suggested by the mortality data is also manifested in the hematologic data, an example

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Aerospace Medical Division, Air Force Systems Command, United States Air Force, Brooks Air Force Base, Texas.

Table 1. ANALYSES OF VARIOUS BIOLOGICAL PARAMETERS IN TAMARINUS NIGRICOLLIS*

Parameter	Number of Observations	Mean	Standard Deviation	Range
Red blood cells (x 10^6/mm^3)	163	6.66	.910	4.47 - 9.35
Hemoglobin (g/100 cc)	162	16.0	1.4	11.4 - 19.1
Hematocrit	162	54.8	4.4	41 - 65
White blood cells (x 10^3/mm^3)	162	15.0	5.9	6.8 - 45.9
differential, %:				
lymphocytes	163	55.2	15.1	20.5 - 91.0
segmented neutrophils	163	39.2	15.1	4.0 - 72.5
monocytes	163	2.7	2.0	0 - 11.0
basophils	163	1.5	1.3	0 - 7.5
eosinophils	163	1.2	1.2	0 - 11.5
Platelets (x 10^3/mm^3)	31	430	36	232 - 713
Body temperature (°C, rectal)	34	39.3	0.5	38.1 - 40.3
Body weight (g)	41	314	40.2	227 - 436
Serum protein (mg N/ml)	19	12.5	1.3	9.9 - 15.1
Electrophoretic distribution %:				
gamma globulin	19	20	4.4	13 - 27
beta-2 globulin	19	6	1.4	4 - 9
beta-1 globulin	19	16	2.3	13 - 20
alpha-2 globulin	19	8	1.4	6 - 11
alpha-1 globulin	19	<2	0.7	1 - 2
albumin	19	49	5.1	39 - 58

*Values in the upper half of the table were obtained on 21 tamarins bled every two weeks over 12 to 14 weeks. The values in the lower portion of the table were obtained from single observations on individual tamarins, the numbers used shown in the left hand column.

Table 2

MORTALITY OF TAMARINS AFTER TOTAL-BODY EXPOSURE TO CESIUM-137 GAMMA RAYS

Radiation Dose (r)	No. of animals	Time of death after exposure (days)	No. of 30-day survivors
100	9	27	8
200	6	18, 22, 24	3
300	6	13, 13, 14, 15, 17	1
400	4	9, 10, 12, 13	0
500	2	8, 10	0
600	1	11	0

of which is shown in Fig. 1 for animals exposed to only 100 r. Thus, the radiation injury to the hematopoietic system is quite severe at this low dose when compared to values obtained with other primates.

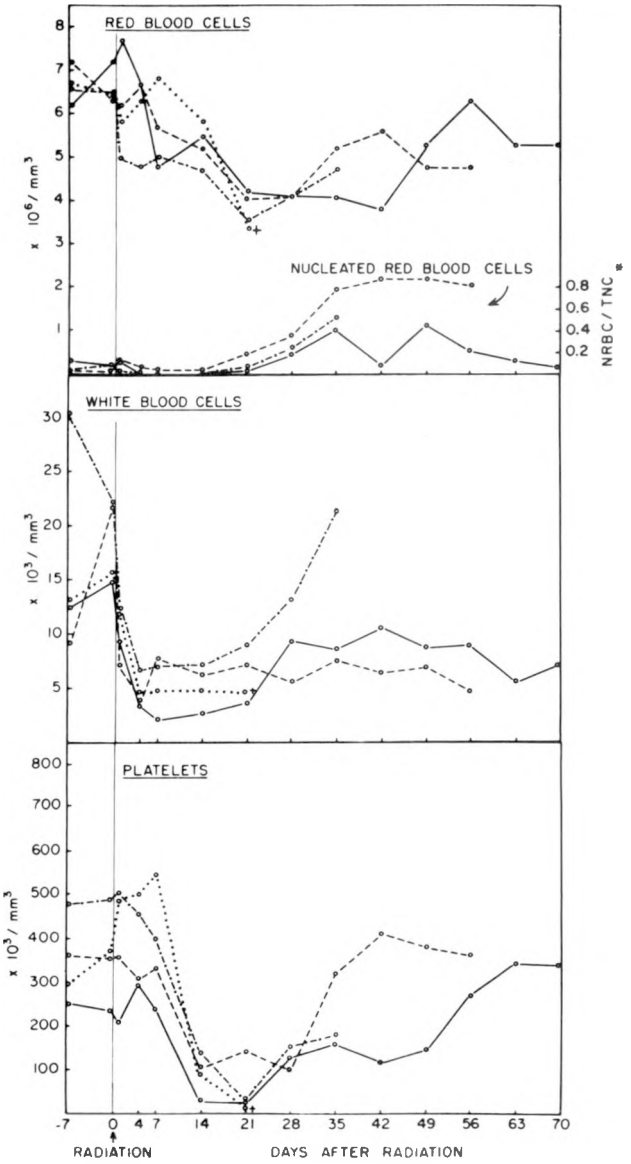

Fig. 1 Hematologic changes in tamarins exposed to 100 r total-body gamma irradiation. *NRBC/TNC indicates the number of nucleated red blood cells per total number of nucleated cells counted in differential analysis of 200 white blood cells.

Studies now under way concern the effectiveness of marrow grafting in promoting survival of the irradiated tamarin and also the effects of radiation on their immune mechanism. Attempts at grafting will also involve the use of hematopoietic tissues from fetal or newborn primates. During the past year, the feasibility of breeding these animals in the laboratory has further been verified by the birth of two sets of twins along with several pregnancies, which unfortunately ended in miscarriage. On the basis of these preliminary breeding achievements, additional funds for establishment of a breeding colony for experimental purposes have been received from the Air Force Contracting Agency. This supplemental program is expected to play a major role in our program objectives on primate immunology and radiation studies.

Negative-Pion-Beam Project (F. V. Comas and R. Cloutier)

The Oak Ridge National Laboratory has proposed building a 900 Mev cyclotron, based on the fixed-frequency alternating radiant principle, the outstanding feature of which would be a very high proton current of about 100 microamperes. If this project is approved it will offer a unique opportunity for studies of biological and medical effects of negative pions. Calculations indicate that large numbers of negative pions would be obtained from the interaction of the proton beam with a suitable target. These pions would be focused and brought into a special medical treatment room and used for irradiation of patients.

Several consultations took place with members of the Electronuclear Division of the Oak Ridge National Laboratory, with the aim of making preliminary plans for the design of the medical room. Several alternatives were discussed concerning the solid angle of pion collection from the target, ways and means of obtaining a flat beam, the question of focusing and bending magnets, and provisions for crossfire irradiation. In addition, Roger Cloutier and Frank Comas visited several institutions where electron and proton accelerators are used for medical and biological work; they exchanged views with and obtained advice from people who have an interest in pion irradiation. Further consultations were held during a symposium on pi meson factories, at which they presented a paper on the possible use of negative pions in biology and medicine.

MEDICAL NUCLIDES

Gastric and Intestinal Excretion of Intravenous Cerium and Yttrium in Dogs* (Granvil C. Kyker and John J. Rafter)

Increased fecal excretion of various heavy metals, which parallels increased intravenous dose, is undefined in mechanism or site. We have observed this relationship to apply for many lanthanons in the range of 10^{-10} to 10^{-5} M/kg. This study includes comparative measurements of the gastric, biliary, and segmented intestinal excretion of cerium and of yttrium in short term tracer experiments terminating at six hours. Simultaneous measurements were done on blood, urine, and related tissues. Biliary excretion was always quite small although liver is the major site of localization. On the other hand, the stomach and each segment of the intestinal tract contributed significantly and almost uniformly to excretion. The concentration of each metal in the various segments of tissue correspond to its excretion. The actual amount of metal was proportional to the intravenous dose although, in percent, excretion decreased sharply. In contrast, the circulating fraction increased sharply both in amount and in percent with increasing dose. The results show that both elements behave similarly, that their alimentary excretion is direct instead of biliary, and that excretion occurs in the gastric as well as in the usually defined excretory parts of the tract.

*Abstract of paper presented at meeting of Division of Biological Chemistry, American Chemical Society, January 14, 1963, Cincinnati, Ohio.

Fatty Liver Due to Lanthanon Chelates (Granvil C. Kyker and John J. Rafter)

Previous studies here have established that acute fatty infiltration of liver occurs regularly in rats after small intravenous doses (2 to 3.5 mg/kg) of any of the first five lanthanons. Elements in the series above samarium do not show this metabolic effect. The chloride of the element was used in most of these previous studies. A wide variety of hormonal factors and a few chemicals prevent the fatty liver. Among the latter, EDTA protected rats against the

effect of cerium but only as the chelate. EDTA could not reverse the effect when given immediately after an injection of the metal as its chloride. A less stable complex such as cerium citrate acted like the chloride.

Although the transport of lanthanons remains unexplained, related evidence supports formation of stable soluble complexes with plasma proteins. The formation of metal-enzyme complexes could explain the acute metabolic effect. Interpretation of such a mechanism would depend on the stability of such in vivo complexes and comparison of series of chelates of different elements has proved fruitful. The stability of a given chelate of the lanthanons increases with atomic number. For a particular lanthanon, its complexes with citric, mandelic, NTA, HEDTA, EDTA, and DTPA acids form a series of increasing stability. Therefore, parallel series with cerium, neodymium, and samarium progressively overlap in stability. Correlation of the stability of a specific chelate with its ability to cause fatty liver reflects the effective stability of the in vivo metal-complex with a critical site in the metabolic pathway leading to fatty infiltration.

For cerium all complexes more stable than Ce-NTA failed to cause fatty liver. Ce-NTA gave some positive and some negative responses and preparations less stable than Ce-NTA caused fatty liver. The strongest complexing agent in the series, DTPA, given separately but immediately after injecting cerium as its chloride, did not reverse the characteristic effect and prevent fatty liver. This was true at each of three molar ratios of metal to chelating agent (Ce:DTPA - 1:2, 1:5, and 1:10). When, however, the same amounts of DTPA were administered first, fatty infiltration due to cerium occurred at the first ratio (Ce:DTPA - 1:2), but the larger molar excesses did give protection. The results of the samarium series are analogous generally with the positive-negative dividing line at citrate instead of at NTA. Another difference appeared for samarium. Separate injections of DTPA before or after samarium prevented the fatty liver at each of the three molar excesses (2, 5, and 10) of the chelating agent. Measurements with the intermediate element are in progress.

In each of the measurements, a radiotracer of the element was used to show the distribution and excretion. As expected it applies that excretion increased and localization in liver decreased with increased stability of the chelate in use. But also fatty infiltration was sometimes prevented when the fraction in liver was not reduced appreciably. This is consistent with other tenuous evidence that the predominant fraction of the dose that localizes in liver may not be

the critical part of the dose causing the metabolic disorder. Results with the intermediate series of neodymium chelates should extend these interpretations for the cerium and samarium series.

The Use of Synthetic Diets in the Study of Fatty Infiltration Caused by Cerium (I. H. Miller and Granvil C. Kyker)

A synthetic liquid diet has proved useful for quantitative study in rats of certain nutritional factors that influence the fatty liver caused by cerium. We have mentioned in previous summaries that this acute biochemical disturbance of lipid metabolism could be prevented by an adequate intake of glucose alone or of total calories and that the metabolism of other major foodstuffs was also very probably affected profoundly. Previous measurements of glycogen and protein in liver and of nitrogen balance indicated a pronounced depletion of glycogen concurrent with fatty infiltration. Interpretation of these observations was, however, limited because cerium causes the loss of appetite regularly caused by the cerium treatment. The depletion of glycogen in liver could have occurred by starvation.

A nutritionally complete synthetic liquid diet containing about 50% total solids, recently described by workers at the National Institutes of Health, proved convenient for use. Glucose is the predominant caloric ingredient. Reducing this ingredient thereby provides an elegant tool for restricting caloric intake without changing the supply of any other nutrient or water.

The diet proved adequate for maintenance of Carworth-Farm Nelson female rats, and these animals gave the same typical fatty liver response to cerium that is seen when regular colony ration is used. Treated animals showed the usual loss of appetite and a severe voluntary restriction of intake for this liquid diet also. The preparation was, however, convenient for forced feeding by intubation, which the animals tolerated well. Different groups of animals, treated identically except for graded levels of glucose in the liquid diet, showed that restriction to 0.4 of the standard formula continued to give some "caloric" protection against cerium fatty liver. In general the levels of glycogen and total lipid in liver showed a reciprocal relationship, the sum of the two approximating 11 to 12 % of the fresh weight. The diet modified to contain no methionine or choline also gave protection, if the reduced glucose was supplied at a level of 0.6 of the amount in the standard formula.

Intralymphatic Administration of Radioisotopes to Lymph Nodes*
(Takashi Honda**, John J. Rafter, and Granvil C. Kyker)

We have administered selected radioactive preparations to 50 or more dogs by intralymphatic injection to compare their localization in lymph nodes. The study applies to the problem of nodes containing minute metastases that are not discernible for removal during surgical treatment. According to radioisotopic characteristics, preparations that localize effectively offer a therapeutic approach by selective irradiation and offer detection by external scanning or internal probing to guide surgical treatment.

Injections were into a lymphatic vessel made visible in the hind foot of a dog by a previous interstitial injection of Indirect Sky Blue between the toes. Through an incision in the dorsum of the foot, lymphatic vessels containing the blue dye are clearly seen lying alongside other major vessels. Soluble preparations (Au^{198}, Ce^{144}, Y^{90}, chelates, etc.) are conveniently injected through a 27-gauge needle fitted to polyethylene tubing (PE-20). Observations at prescribed intervals include measurements of distribution and excretion, scintigrams by external scanning, and, occasionally, radiography.

In general, very small chemical doses (Y^{90}, 10^{-11} M/kg) prepared from a Sr^{90}-Y^{90} generator (Brookhaven) fail to localize adequately and distribute widely somewhat like intravenous doses. Larger chemical doses (stable yttrium, 10^{-7} M/kg) localized better in lymph nodes; however, animals varied considerably. Results with colloidal Au^{198} suggested the use of larger particles. By contrast, ceramic microspheres (50 μ diameter) containing Ce^{144} and suspended in Carbopol (3 M Company) localized largely in the first lymph node of the stream. We are comparing chelates of these elements that differ in stability to find a suitable preparation between these extreme behaviors. Preliminary results from this last phase of study encourage our continued efforts.

*Abstract submitted in December 1962 for program of tenth annual meeting of the Society of Nuclear Medicine, June 1963, in Montreal.

**James Picker Foundation Fellow in Radiological Research.

Radiation Dose to the Human Intestinal Tract from Internal Emitters
(R. L. Hayes and J. E. Carlton)

International recommendations for maximum permissible concentration of various radionuclides in water and air are based on a standard-man model of average behavior. Where the intestinal tract is the critical organ, it is obvious that the individual experience will vary considerably although the average dose may be in close agreement with the dose predicted by standard-man assumptions. The extent of these variations among individuals may be of considerable importance. A previous investigation with animals where the actual in vivo dose from beta emitters was measured served to strengthen this opinion. A continuing program to assess the extent of the dose variation in man has now progressed to the point where some tentative conclusions may be made. Fifty-four subjects have been studied to date. The following points appear to be of possible importance:

(1) The age of the subject does not seem to be an important factor (Fig. 1), although in the group studied intestinal motility did decrease with age (Fig. 2).

(2) A sizeable proportion of the population may experience doses many times in excess of that assumed for the average or standard man. The measurements indicate that about 15% of the general population may experience a dose 3 times that of the standard man and 6% as much as 5 times that of the standard man.

(3) As expected, the dose experience of the population studied approximated that of a Gaussian distribution. The average dose was, however, approximately 70% greater than that predicted for the standard man where a long-lived isotope was involved. For a short half-life activity (12 hr) the average was equal to the standard-man value.

(4) Whether the route of entry of activity is through food (at mealtime) or through water (between meals) does seem to definitely affect the dose received (Fig. 3).

If the results of this population sample are borne out in further studies, possibly some adjustments in the assumptions for the standard man are in order.

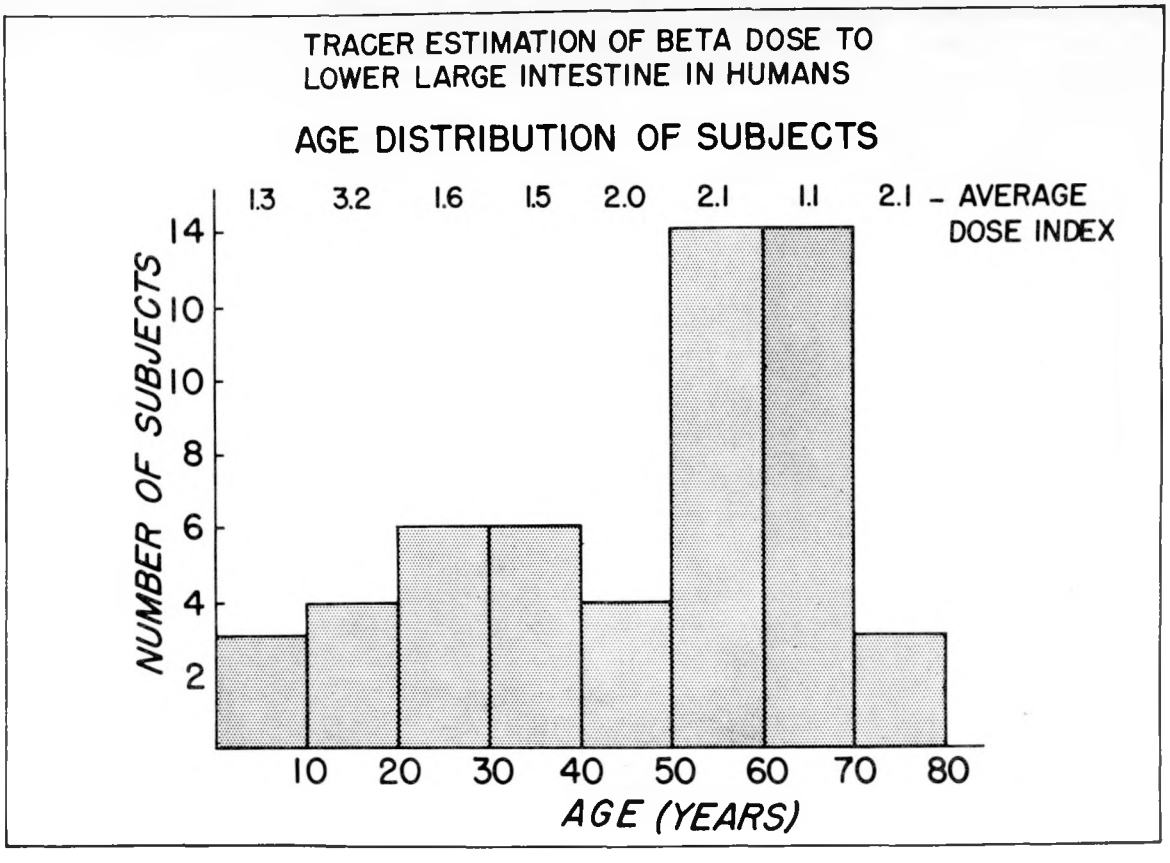

Fig. 1 Age distribution of subjects and dose index by age groups.

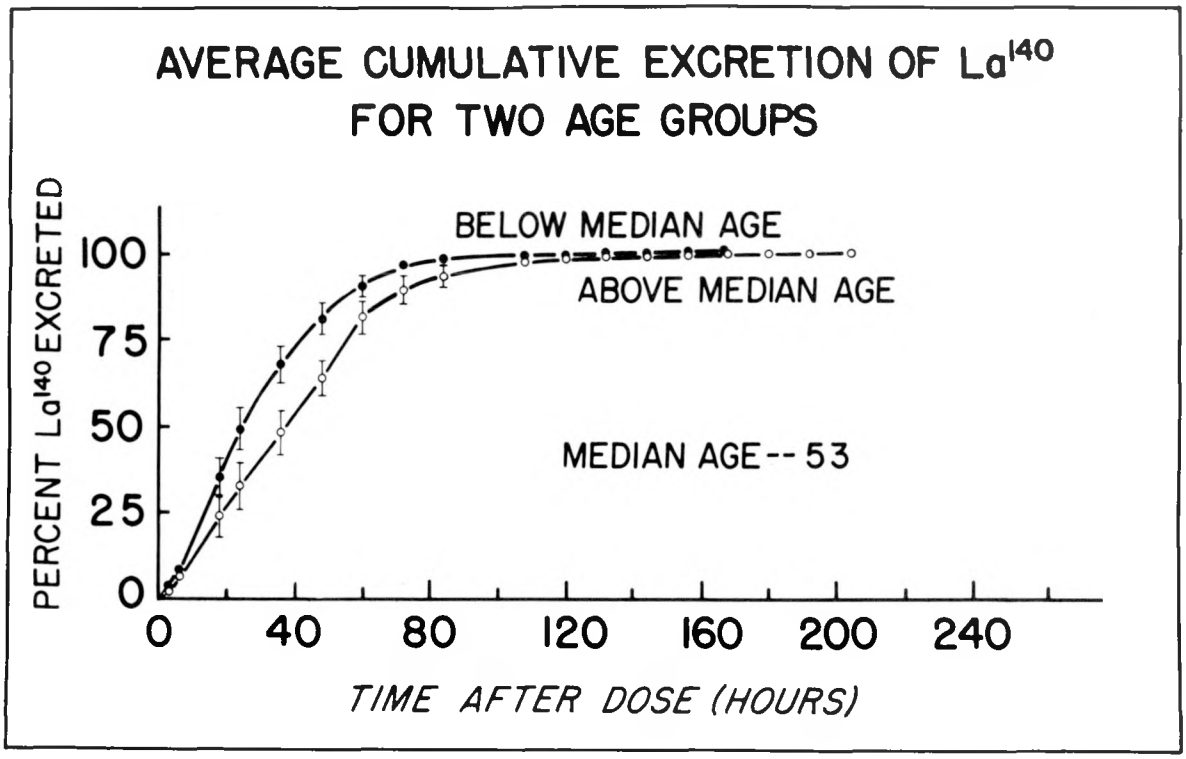

Fig. 2 Average cumulative excretion of La^{140} for a division of the subjects about the median age. Limits on points indicate the standard deviation of the mean.

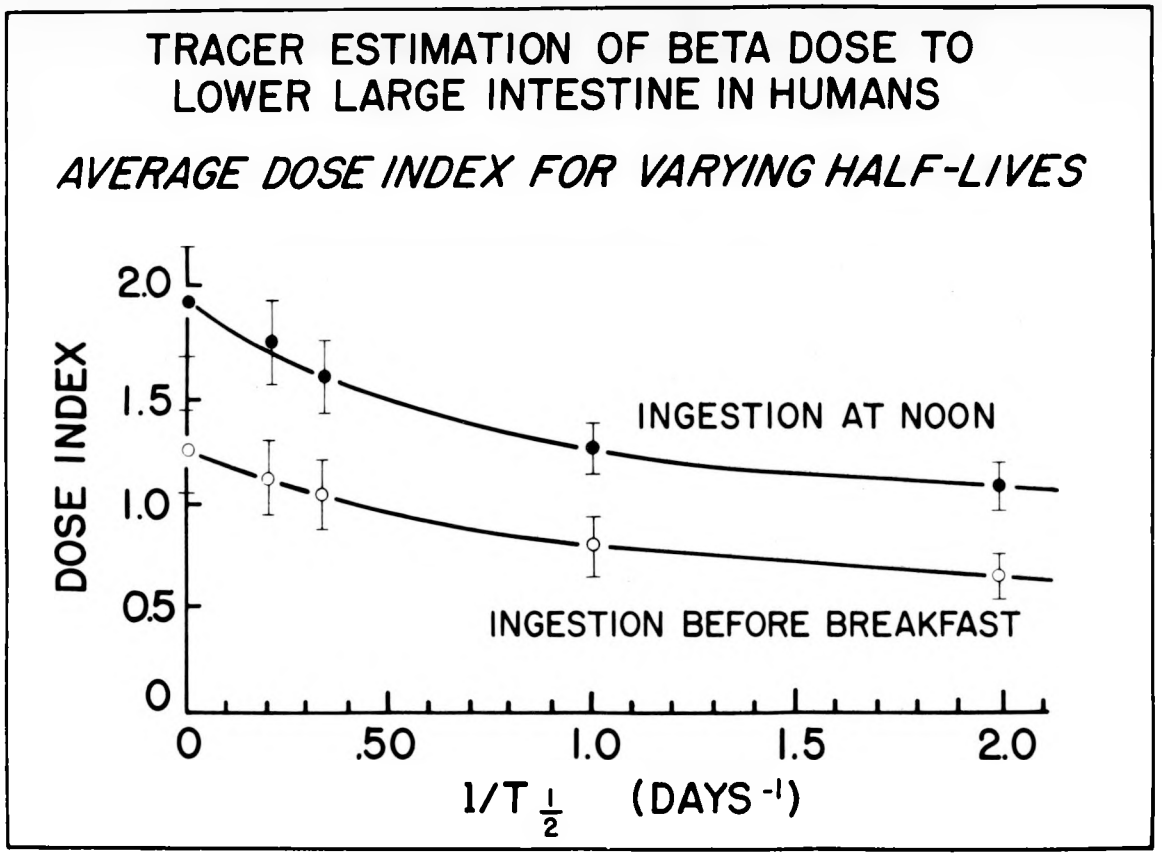

Fig. 3 Average dose index as a function of the reciprocal of the half-life for groups ingesting activity with the noon meal and two hours before breakfast. Limits on points indicate the standard deviation of the mean.

DIAGNOSTIC AND THERAPEUTIC RADIOISOTOPES

Clinical Scanning of Bone Marrow (C. Lowell Edwards, G. A. Andrews, B. W. Sitterson, and R. M. Kniseley)

The hematopoietic marrow is known to vary quantitatively from almost complete aplasia to extensive hyperplasia in different disease states. It is also known to be nonhomogenous at times. Heretofore in clinical evaluation of the marrow we have been limited to surgical or needle biopsy and aspiration studies. Although these are very informative, they provide limited information on the size and distribution of the hematopoietic organ.

It has been known for some time that certain colloids are removed from the blood by the reticuloendothelial cells of the marrow as well as those in the liver and spleen. That the distribution of these reticuloendothelial cells coincides with the distribution of the hematopoietic or red marrow is well demonstrated by autoradiograms on autopsy material. After the injection of the appropriate radioactive colloid, it is possible to demonstrate the distribution of the isotope, and hence the hematopoietic marrow, by external scanning.

Using the ORNL research scanner, we have successfully demonstrated quantitative and distributional changes in the marrow of patients with several diseases. Of the three isotopes used until now, colloidal Au^{198} has proved to be the most successful. While the doses of this isotope required for satisfactory scanning are so high as to interdict its use in persons with a good long-range prognosis, we plan further investigation of other preparations, which we hope will reduce the dose of radiation to the patient. Heat-treated albumin labeled with I^{131} yields a colloidal preparation (as recommended by George Taplin). This shows some localization in marrow, but relatively high body background plus early localization of the radioactivity in the bladder impair results. Colloidal Au^{199}, in a few preliminary trials, has failed to give satisfactory results for reasons that are not as yet clear.

We have scanned the marrow of 18 patients with neoplastic or hematologic malignant disease. The most readily demonstrable variations are as follows:

1) Hyperplastic marrow as in patient C. E. who was diagnosed as having polycythemia for (?) years before coming to ORINS for an apparent erythrocytic leukemia. Here marrow is seen to extend well into the humeri, the femora, the knees, and even to the feet (Fig. 1 a, b, c, d).

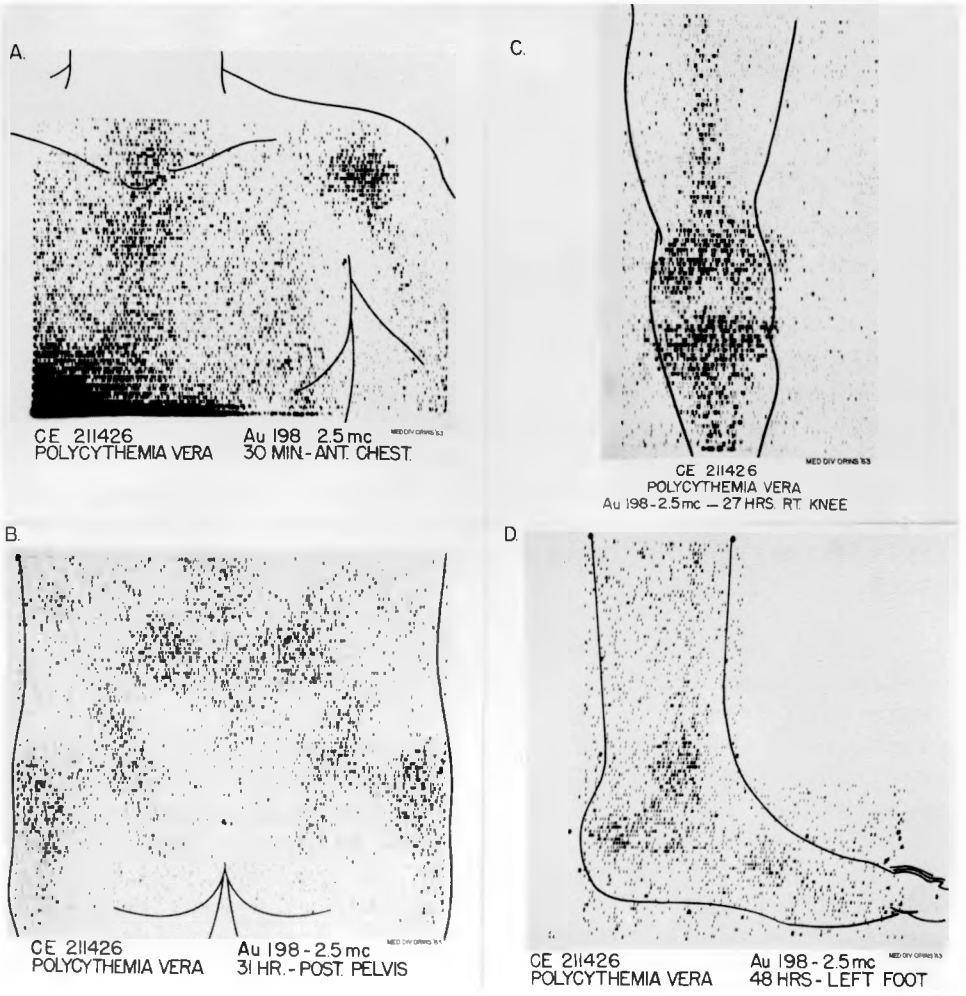

Fig. 1 a,b,c,d Bone-marrow scan showing the expanded marrow organ in a patient with polycythemia vera.

2) Aplastic myelophthisic marrow as seen in D. S. who has had polycythemia rubra vera for 16 years and now is known to have myeloid metaplasia (Fig. 2), shows the absence of Au^{198} in the usual sites.

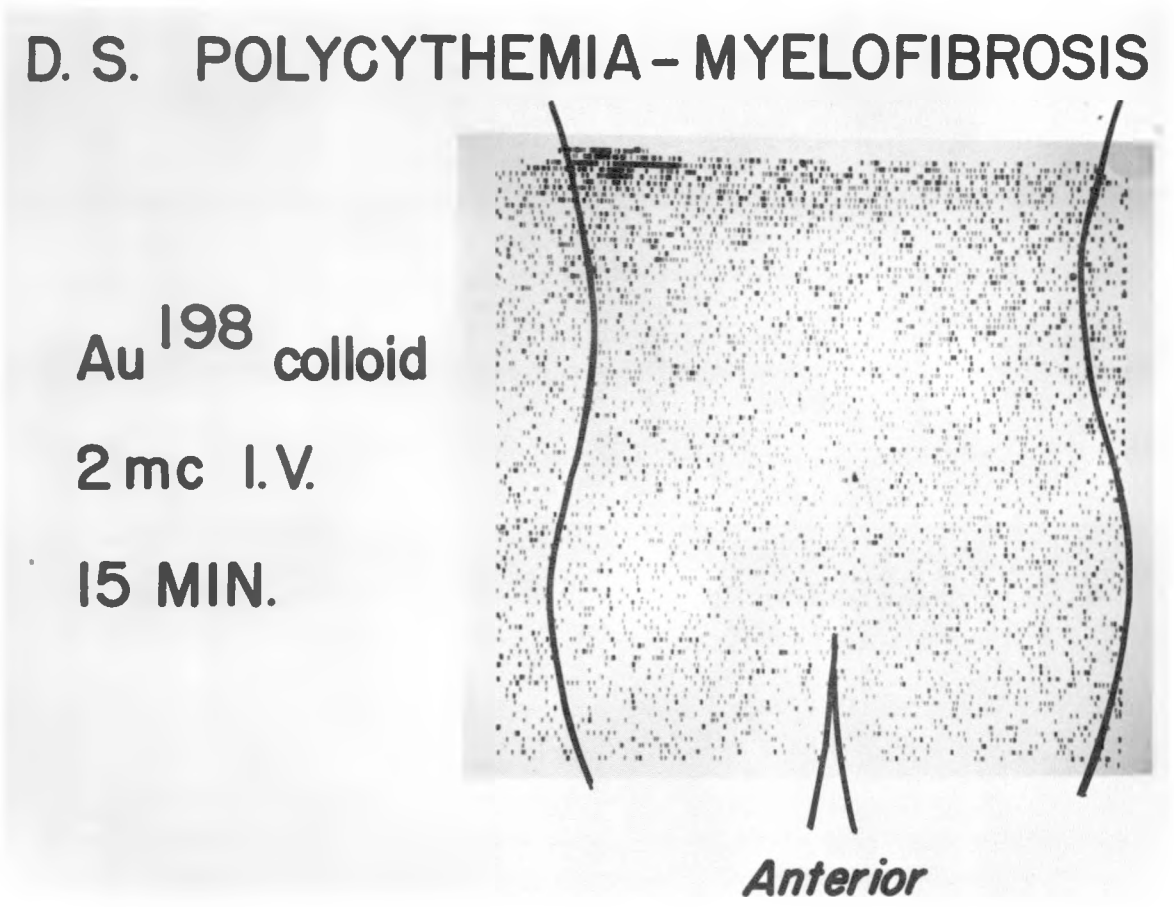

Fig. 2 Bone-marrow scan: myelofibrosis following polycythemia.

3) Local lesions of the marrow as illustrated by patient D. B. who had carcinoma of the breast with metastasis to the pelvis and X-ray therapy to the right side of the pelvis (Fig. 3). Patient C. C. has the diagnosis of lymphosarcoma involving peripheral lymph nodes but with no known bony lesions. Marrow aspiration revealed a few malignant cells in the marrow. The scan, however, revealed the absence of marrow in one-half of the fourth lumbar vertebra (Fig. 4).

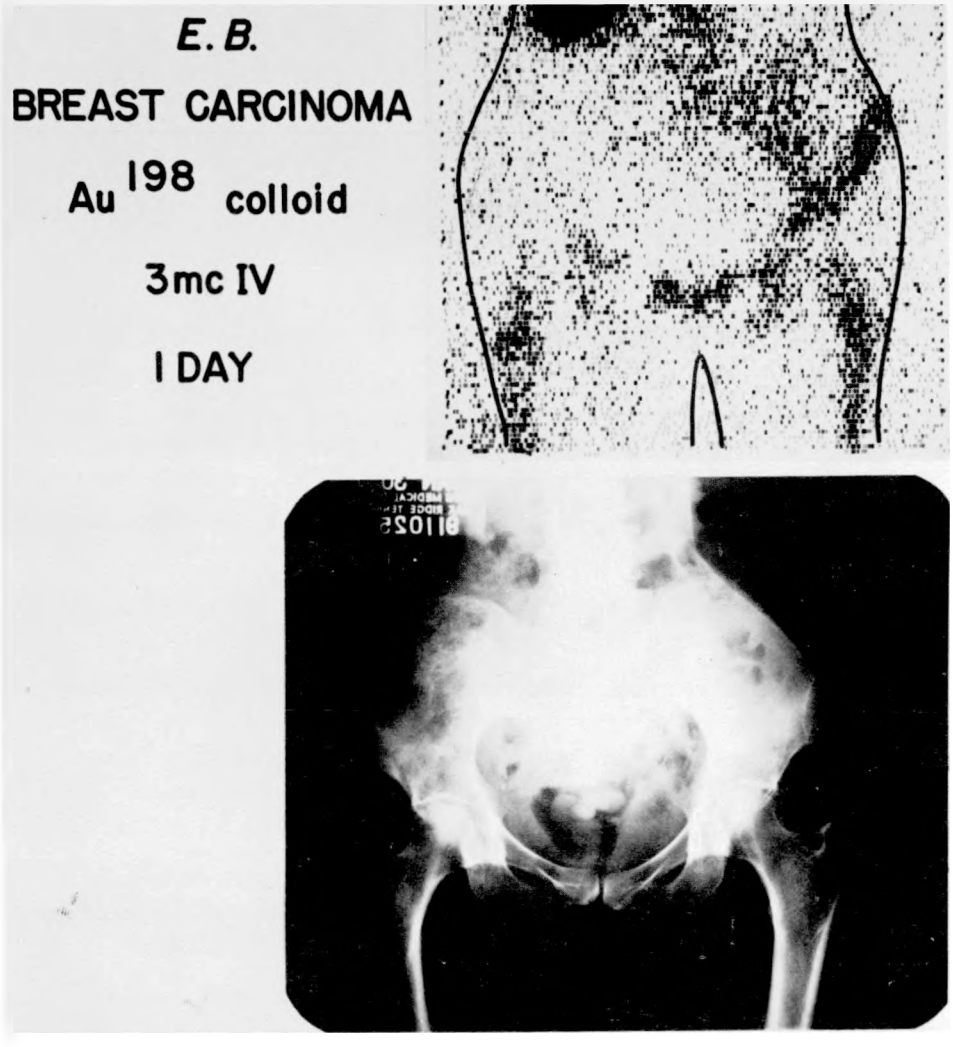

Fig. 3 Bone-marrow scan: local marrow lesion at the site of metastases and radiotherapy.

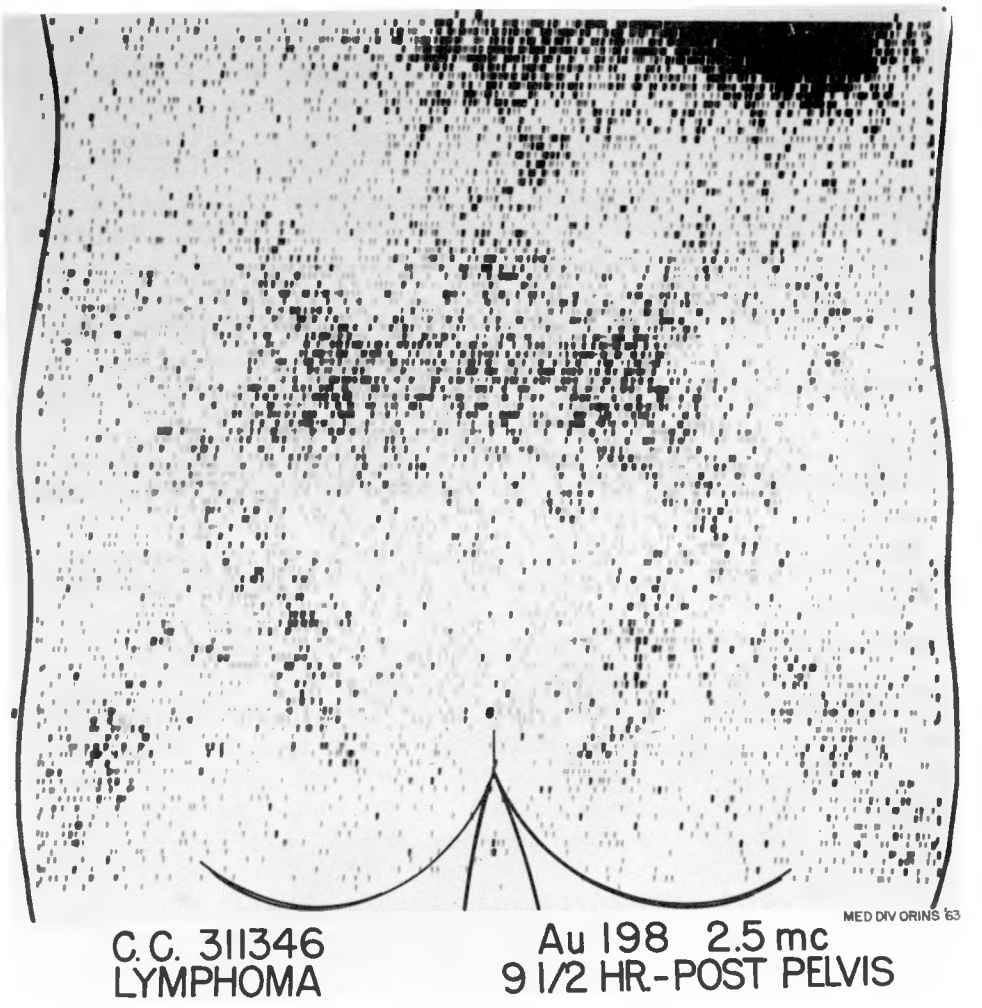

Fig. 4 Bone-marrow scan in a patient with lymphoma. Note the defect in fourth lumbar vertebra.

We have scanned only one case of chronic leukemia, and four cases of acute or subacute leukemia. The scans exhibit striking features, which vary from case to case, and the true clinical significance of these changes is not known and will be further investigated. Patient L. H., with acute leukemia under incomplete control with amethopterin, exhibits extensive activity in the usual marrow areas, and suggests normal or increased size of the marrow organ (Fig. 5 a, b). Marrow aspirates from this patient revealed a densely cellular marrow packed with blastic forms. Patient R. K., with acute leukemia under therapy with prednisone and 6-Mercaptopurine for one month, revealed a densely cellular marrow packed with blastic forms. On scans no definite marrow could be demonstrated. Patient V. S., with acute leukemia under therapy with prednisone and amethopterin but incompletely controlled, revealed a patchy marrow distribution. The pelvis is largely free of accumulated isotope although there appears to be a considerable amount in the marrow of the lumbar and dorsal vertebrae and sternum (Fig. 6). Aspiration of the marrow spaces in the pelvis yielded no marrow on repeated attempts. The aspirate of the sternum, however, revealed a densely packed blastic marrow. These findings were subsequently supplemented by autopsy data that revealed areas of necrotic marrow in the pelvis microscopically resembling the observations on previous aspiration.

Studies of the chest after colloidal Au^{198} has been given have yielded some variable results. In a few patients there has been obvious activity that may be localized in the rib marrow, thoracic wall, or pulmonary tissue. In other patients this is almost completely absent. Further studies should clarify this question.

Our immediate objectives of this study are (1) to find a more desirable agent with which we can scan the marrow while exposing the patient to a reduced dose of radiation and thus broaden the applicability of this procedure; (2) to define more accurately the variations in the hematopoietic marrow as seen by external scanning and correlate these variations with clinical and autopsy data.

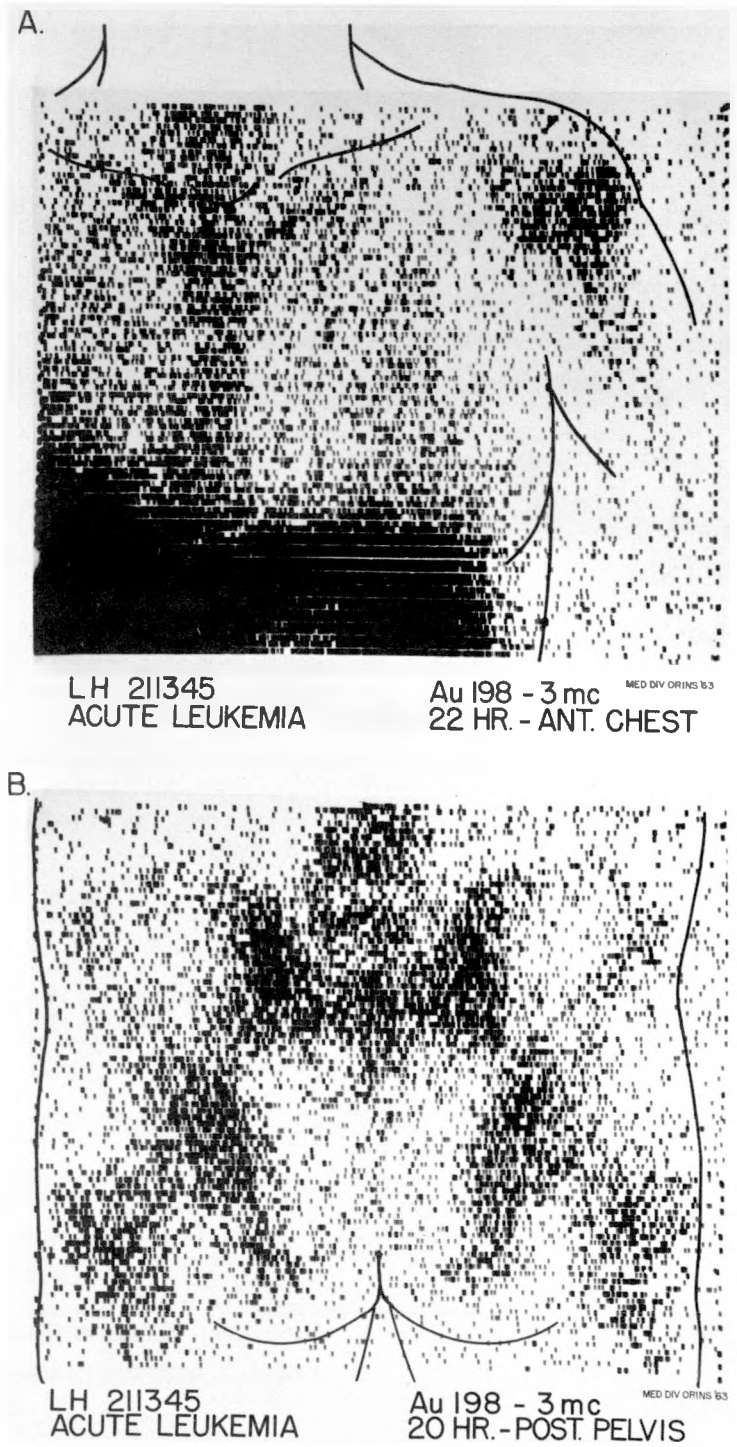

Fig. 5 a, b Bone-marrow scan in a patient with acute leukemia in partial control, showing normal or increased size of the marrow organ.

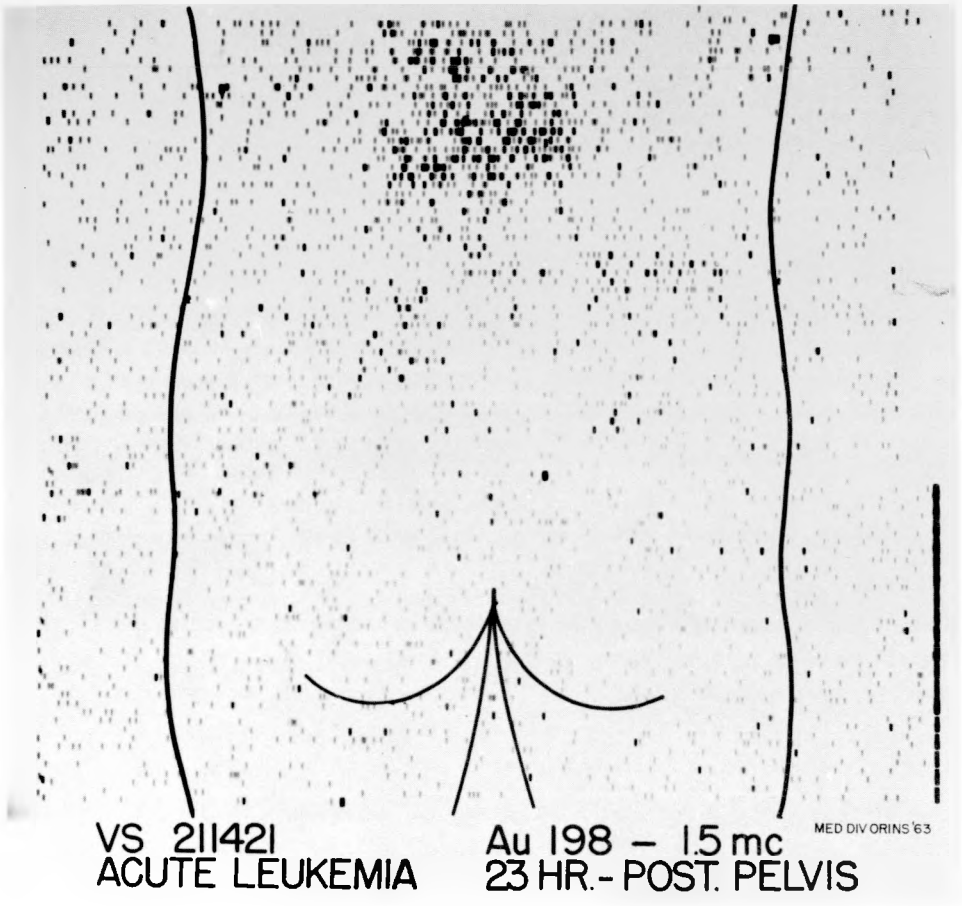

Fig. 6 Bone-marrow scan in a patient with acute leukemia, showing little uptake in the pelvis.

Lanthanum-140 as a Measure of the Completeness of Stool Collections for the Oral Iron-59 Absorption Test (Raymond L. Hayes, J. Elbert Carlton, and Bill M. Nelson)

Tests of a patient's ability to absorb certain orally administered materials may be invalidated by incomplete collection of feces. Lanthanum-140 with an appropriate carrier is not appreciably absorbed from the gastrointestinal tract and may be given by mouth simultaneously with another tracer. Practically all the La^{140} is accounted for in the feces if collections are complete. To date, seventeen persons have been given La^{140} (20 microcuries with 5 milligrams stable lanthanum) with Fe^{59} (2 microcuries and 50 micrograms) as an oral iron-absorption test. Surprisingly, a small but significant amount of Fe^{59} continued to appear in late stool specimens after virtually all the La^{140} had been recovered. The amount was too large to be accounted for by fecal loss of Fe^{59}-labeled red cells. In general, the delayed excretion of Fe^{59} was less than 8% of the dose and was not clinically important for the absorption test. However, in one test 23% of the Fe^{59} dose was collected after 99.9% of the La^{140} was recovered. It had been expected that the fecal Fe^{59} and La^{140} activities would have a constant relationship so that a correction factor could be applied if collections were incomplete. Actually, because of the prolonged excretion of Fe^{59}, the ratio of Fe^{59} to La^{140} rises with each stool collected. The prolonged excretion may be due to transient binding of the iron to the mucosa of the digestive tract, but this remains a speculation.

TRACER AND BASIC BIOLOGICAL STUDIES

Diffusion-Chamber Studies with Human Cells (N. Gengozian)

Human lymph-node tissue, when placed in small lucite diffusion chambers containing a foreign antigen, has previously been shown to produce antibodies against the specific antigen. Attempts to define more clearly some additional variables associated in inducing the cells to form antibody against the antigen (Salmonella typhosa) have shown that the response is dependent upon (1) the dose of antigen relative to the number of human cells placed in the chamber, and (2) the number of cells placed in the chamber. Serum agar tests have now shown that lymph-node cells synthesize at least two different types of human serum proteins, gamma globulin and a macroglobulin. Suitable in vitro inactivation tests with 2-mercaptoethanol have shown the antibody activity to be associated with the macroglobulin protein, the latter identified tentatively by immunoelectrophoresis as the beta 2-M protein. Cultivation of the cells in the chamber has shown a marked proliferative activity of the lymphocytes, as indicated by analyses of mitotic index and by tritiated thymidine incorporation. Differential analyses revealed a striking increase of blast-type cells in cultivation, reaching levels as high as 16% after eight days of incubation in the chambers. There appears to be a definite correlation in the thymidine incorporation, mitotic activity, and appearance of blast cells. Figure 1 shows these changes obtained with two different human lymph-node tissues cultured in diffusion chambers. Thus, although two different sources of tissue were used, the same general types of changes were noted at various intervals after cultivation.

Positive antibody formation has also been obtained with human spleen cells in this system. Several attempts with normal bone marrow and peripheral white blood cells have thus far been negative. Most interesting, however, has been the differentiation of peripheral white blood cells in culture, these showing appearance of blast cells similar to those seen with lymph-node cultures and also incorporation of tritiated thymidine and significant mitotic activity. The time sequence of these changes is comparable to that seen with lymph-node tissue as shown previously in Figure 1.

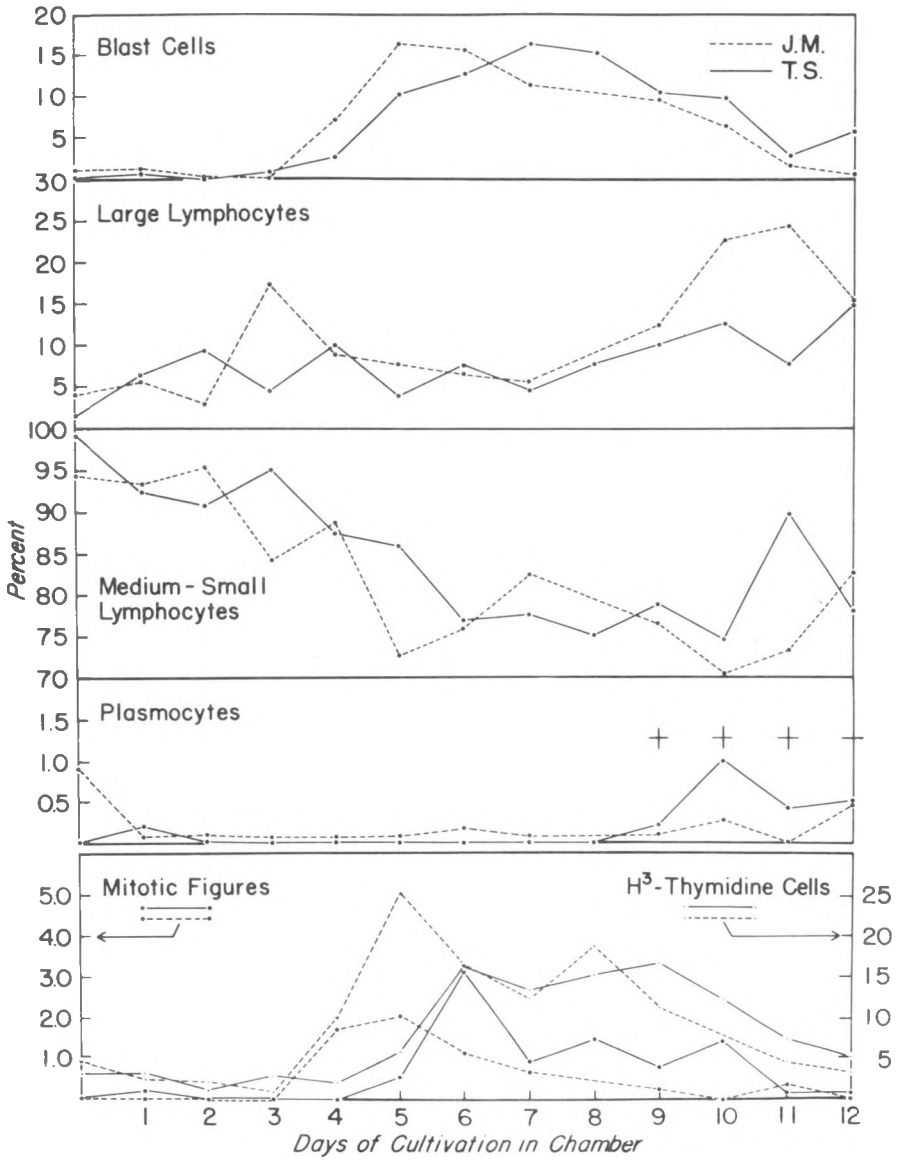

Fig. 1 Diffusion chamber; cultivation of two different human lymph node tissues.

The establishment of a Linde slow-freeze liquid nitrogen controller and freezer at the Institute this past year has now permitted the long-term storage of viable human cells to be used in the diffusion-chamber system. These studies have further substantiated the feasibility of using the diffusion chamber technique for human cells, and with the addition of a storage technique, it will now be possible to study human cellular immune functions under various experimental conditions.

Plasma Disappearance Rate of Vitamin B_{12} in Chronic Myelocytic Leukemia

We participated in a study of the plasma clearance of Co^{57} vitamin B_{12} in three patients with chronic myelocytic leukemia in remission. The experiment was proposed and initiated by Dr. Leo M. Meyer of the South Nassau Communities Hospital of Oceanside, New York, and Dr. Lewis Schiffer of Dr. Cronkite's group at the Brookhaven National Laboratory. Dr. David White of our staff collaborated in the project.

Patients with chronic myelocytic leukemia have elevated plasma levels of vitamin B_{12} and a delayed disappearance rate from the plasma of intravenously administered vitamin B_{12} labeled with radioactive cobalt. It is believed binding sites for vitamin B_{12} are increased in the plasma of these patients as compared to normal subjects. The plasma disappearance rate of labeled vitamin B_{12} becomes as rapid as in normal subjects if the binding sites for vitamin B_{12} are first saturated by a large intramuscular "loading" dose of stable vitamin B_{12}. In an occasional patient with chronic myelocytic leukemia in remission, a normal plasma disappearance curve for labeled vitamin B_{12} is observed without prior administration of the stable vitamin.

In our study we observed the disappearance rates of a tracer dose of Co^{57} vitamin B_{12} (0.13 microcuries Co^{57}) from the plasma of the three patients. The experiment was repeated twice, first 24 hours after a "loading" dose of 1000 micrograms of Barker's coenzyme vitamin B_{12} and then 24 hours after 1000 micrograms of hydrooxocobalamine. Barker's coenzyme may be the form that vitamin B_{12} occurs in the body. Hydroxocobalamine is one of several analogues of vitamin B_{12} that Dr. Meyer is investigating.

In two of the patients prolonged plasma disappearance rates of Co^{57} vitamin B_{12} were changed to normal rapid disappearance rates

after the "loading" doses of Barker's coenzyme vitamin B_{12} and hydroxocobalamine. The other patient had a normal disappearance rate before and after the "loading" doses of these metabolites.

Specific Activity in Radioisotopic Measurements with Goldfish
(Granvil C. Kyker and Barbara Chastain)

Measurements with the procedure summarized previously (ORINS-41, p. 37-8) were extended both for additional convenience and evaluation of the effect on cationic uptake of anesthetic agents suitable for fish. Two agents were compared in Na^{22}-uptake measurements. Ethyl-m-aminobenzoate methane sulfonate (MS 222-Sandoz) is the agent best described for use on fish. It is manufactured by Sandoz, Inc., Basle, Switzerland, for this purpose. Quinaldine was recently mentioned by an observation of chance (Chem. & Eng. News, p. 94, Sept. 10, 1962); quinaldine was found the more satisfactory agent for this use.

MS-222 showed a progressive depression of sodium uptake with concentration. Also the margin of safety between anesthetizing levels (1 part per 15,000 to 20,000 parts) and a lethal dose was narrow. In contrast quinaldine was partially effective at 3 ppm, gave complete anesthesia at 10 to 12 ppm, and was used safely at 25 ppm in measurements throughout three days. Some fish were maintained under anesthesia for a week; their activity returned to normal within a few minutes after being transferred to fresh water. The response to repeated treatment was apparently the same. At low concentrations of sodium (0.45 mM NaCl) there was no significant effect of the agent on the rate or amount of uptake, while at 18 mM some enhancement of uptake occurred with an apparent maximal effect by 12.5 ppm of quinaldine. These observations add much convenience to the previously described procedure and offer interesting applications in the study of cation transport in goldfish.

METHODS

Aluminum Plates for Thin-layer Chromatography* (Fred Snyder)

The substitution of aluminum plates for glass plates in thin-layer chromatography (TLC) offers certain advantages when one is interested in visualizing the components by H_2SO_4 charring. Aluminum plates are heated directly on a hot plate where the rate of heating is controlable while the changes in color are easily and quickly visible. Obviously, there need be no concern over breakage with aluminum.

This note provides information on the source and cost of aluminum, and the way in which the thin-layer of silica should be applied. We have used aluminum sheet alloy 6061-T6 mill finish (4 mm thick) obtained from the J. M. Tull Metal and Supply Company, Inc., Atlanta, Georgia. The price of this aluminum is $2.21 per square foot. The dimensions of the aluminum plates are machine cut in our instrument shop to the same size as the glass plates (20 x 20 cm) normally used for TLC. Aluminum of this type must be thoroughly polished with Brillo soap pads (Brillo Mfg. Co., Inc., Brooklyn, N. Y.) before routine washing and the application of silica layers.

Twenty-five grams Silica Gel G are mixed with 50 ml of approximately 47% ethanol (1:1 v/v H_2O:95% ethanol) in a glass beaker. After mixing for about 30 sec the slurry is poured into a thin-layer applicator designed for the application of a 250-micron layer. The alcohol serves as a wetting agent, necessary for the uniform application of silica to aluminum. We have also found it desirable to use a cellophane tape strip on the bottom surface of the leading edge of the applicator because it is otherwise possible to scratch the aluminum plates.

*Analytical Chemistry (In Press)

A Small-Animal Linear Scanner: Calibration and Use* (Takashi Honda, John J. Rafter, and Granvil C. Kyker)

Many radioisotopes of potential diagnostic and therapeutic interest remain unappraised in the human patient. Such appraisal calls for preliminary study in animals. Evaluation of distribution patterns and excretion rates of a radioisotope by radioassay methods is laborious. External measurement of radioactivity in cross-sectional segments permits much interpretation of the internal behavior of an isotope. Although the information is incomplete, rapid screening and measurements at repeated intervals on the same animal enable dynamic interpretations that are unavailable by destructive testing. We have calibrated a small-animal scanner for radioiodine-131 in the rat and applied it to verify thyroidectomized animals for metabolic studies. Application to various other injected radioisotopes has also proved especially useful.

The linear scanner designed for rapidly screening radioisotopic distribution in small animals uses a chart recorder (Varian Model G-11) to plot externally detectable radioactivity as a function of longitudinal localization in the animal. The 2 x 2-in. NaI (Tl) crystal detector is connected to a medical spectrometer. The adjustable slit between 4-in. lead collimators defines the range of isoresponse patterns. The table speed for the animal is mechanically variable to 20 in./min.

Calibration of the scanner included the isoresponse measurements for I^{131} under various conditions. Three slit widths (1/8 in., 1/4 in., and 1/2 in.) were studied in air, in large and small water phantoms, and in average rats with implanted I^{131} sources. The isoresponse directly above the slit was symmetrical laterally; also the isoresponse curve was essentially flat for lateral displacement of the sources beyond the cross-sectional dimension of a large rat. Collimation longitudinal to the scanner table is inverse to slit width.

The scanner has found application on rats injected by different routes with various radioisotopes including Na^{24}, K^{42}, Sc^{46}, Nb^{95}, I^{131}, and Ce^{144}. Repeated scans at increasing time intervals enable rapid interpretation of the metabolic distribution of a radioisotope as a function of time. Preliminary efforts toward quantitative interpretation were based on planimetric analysis of sections of the profile curve corresponding to arbitrary segments of the animal.

*Abstract of paper presented at annual meeting Southeastern Section, The Society of Nuclear Medicine, March 16, 1962, in Atlanta.

RADIATION PHYSICS AND INSTRUMENTS

Hot Patient Counter (William D. Gibbs)

The ORINS "hot patient counter" has been in routine use for slightly more than one year. It has been used in measuring retention of therapeutic doses of I^{131} in patients.

Studies conducted with phantoms containing known distribution of I^{131} have shown that if the patients are counted while supine and while prone, and these counts are averaged, the accuracy of the result is improved. Therefore this has been adopted as a routine method. Results obtained from 33 patients are shown in Fig. 1.

Other studies with phantoms have revealed that, if the proper portion of the I^{131} scatter spectrum is measured, the results obtained reflect only the amount of I^{131} present and are not affected by the distribution of the radioisotope within the phantom, or by the size of the phantom.

This method has been tried with several patients. Results are shown in Fig. 2. None of the patients included in these data had voided during the first three hours. Therefore the instrument was "looking at" the same amount of I^{131} at each time interval. The only variable for any given patient was the distribution of the I^{131}. When the instrument was "seeing" only the 360 kev primary radiation of the I^{131}, the answer obtained varied with distribution. When the counter "looks at" the scatter radiation, distribution of the isotope in the body is not a factor in determining the result obtained.

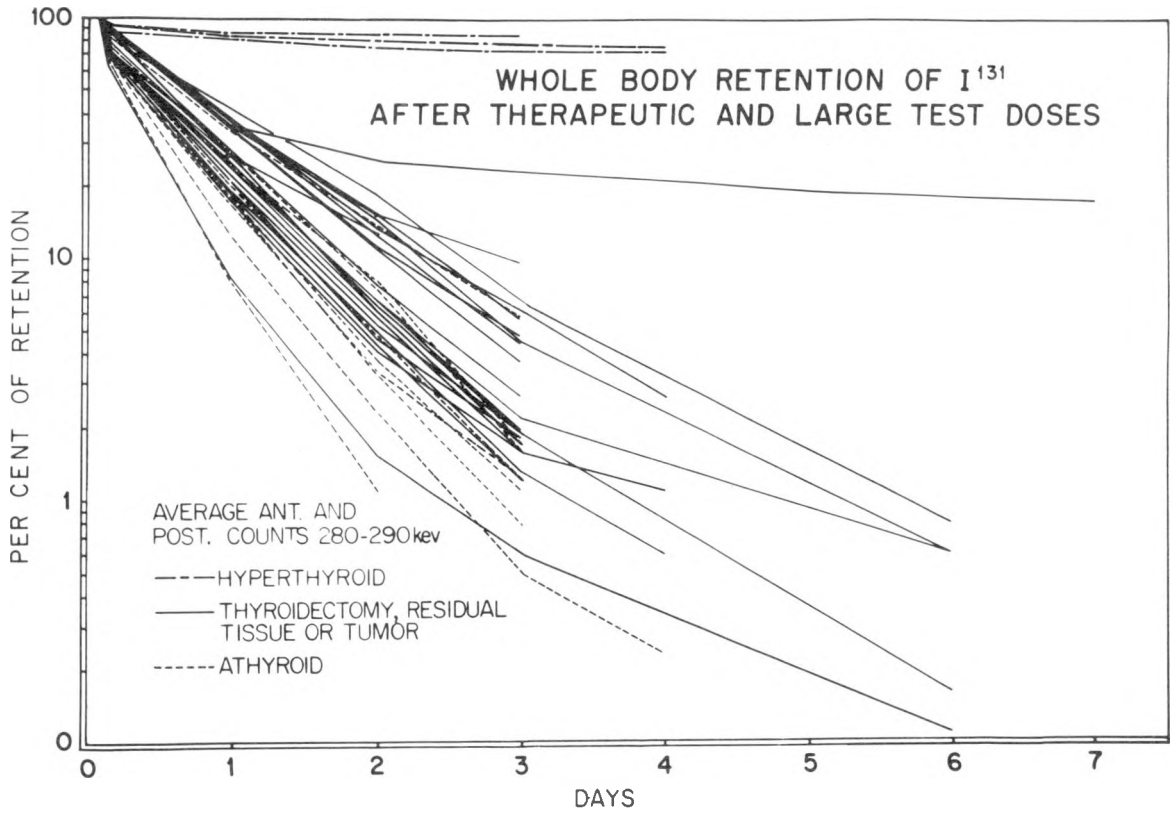

Fig. 1 Retention of I^{131} in 33 patients for periods up to six days. All patients received either 5 millicuries or 100 millicuries.

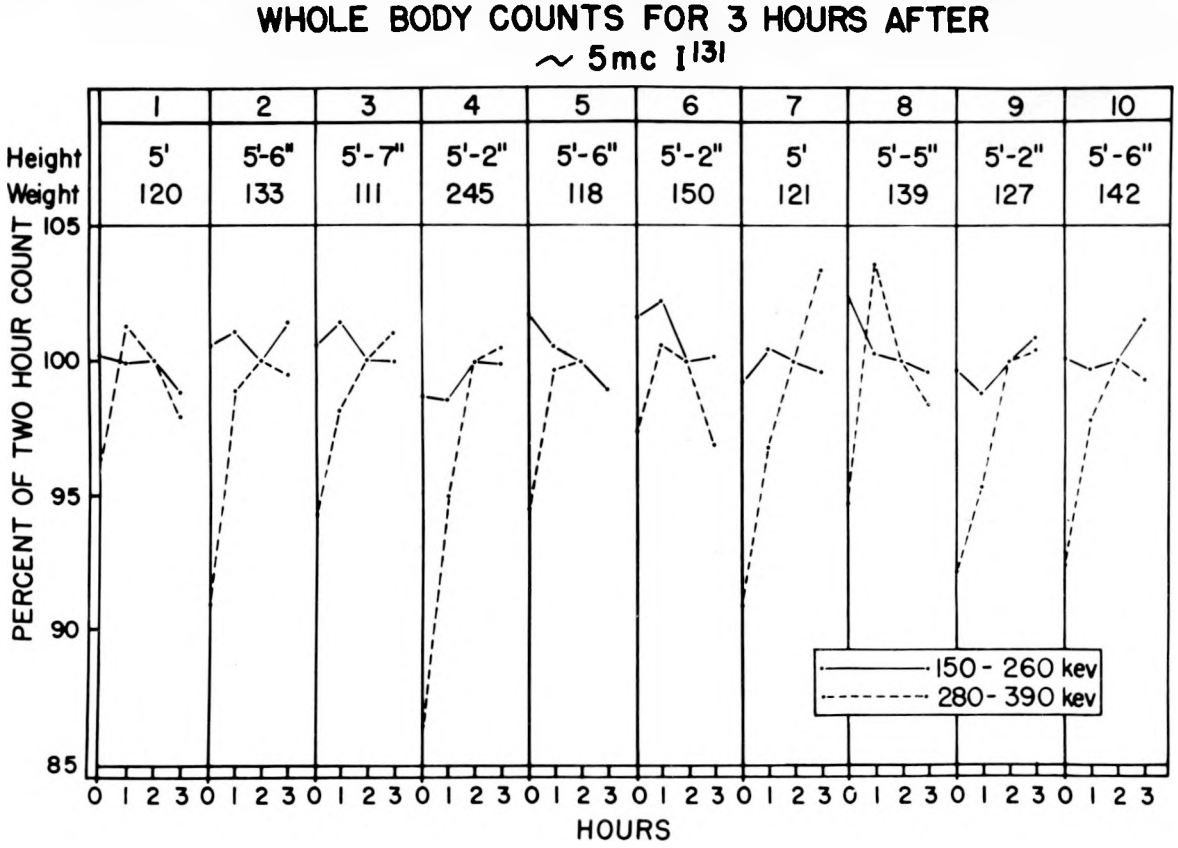

Fig. 2 Results obtained in 10 patients during the first three hours after a dose of 5 millicuries of I^{131} when the analyzer "sees" the 364 kev peak distribution is reflected by large variation in result. When only "scatter" is measured, distribution does not change the result to a large extent.

The Search for Low-radioactivity Concrete (D. A. Ross and A. C. Morris, Jr.)

During the past year we have continued engineering work on the proposed low-range patient counter, and one of the incidental needs has been to find concrete ingredients having very low inherent radioactivity, enabling us to make "cold concrete." This is needed for the walls of the "cave," the low-background room where normal people and tracer-dose patients are to be counted. Here the background radiation must be much lower than that produced by a normal person, for we may be asked to count children or even babies, whose radioactivity could be only a fraction of the minute quantity typical of an adult. Accordingly the walls of the cave must not only prevent external radiation from coming in, they must also contribute practically no background of their own. What this calls for is a dense, thick wall made of "cold" materials.

One way to get a thick shield is to bury the cave in a bank of earth, and this we propose to do, thus protecting the sensitive detecting system from radiations generated in other parts of the Medical Division - for example in the teletherapy section. At the north end of ORINS hospital the middle floor is largely underground (Fig. 1), and our Low-background Facility, containing the patient counter, is to be built as an extension of the middle floor northward into the bank of clay. This annex will need a concrete wall anyway, to hold back the earth and keep out the rain, and it would be convenient if we could make the whole wall of the cave out of concrete, since this is a standard, not-too-expensive material whose properties and fabrication are well understood. A few of the existing patient-counting rooms have actually been constructed this way, but it turns out that ordinary concrete contains enough radioactivity to prevent the attainment of a really low background, and thus, the usefulness of such a facility is restricted. We are anxious to do better.

Concrete is made from coarse and fine crushed rock, plus portland cement and water. Our first job was to devise special equipment for detecting minute amounts of radioactivity in these

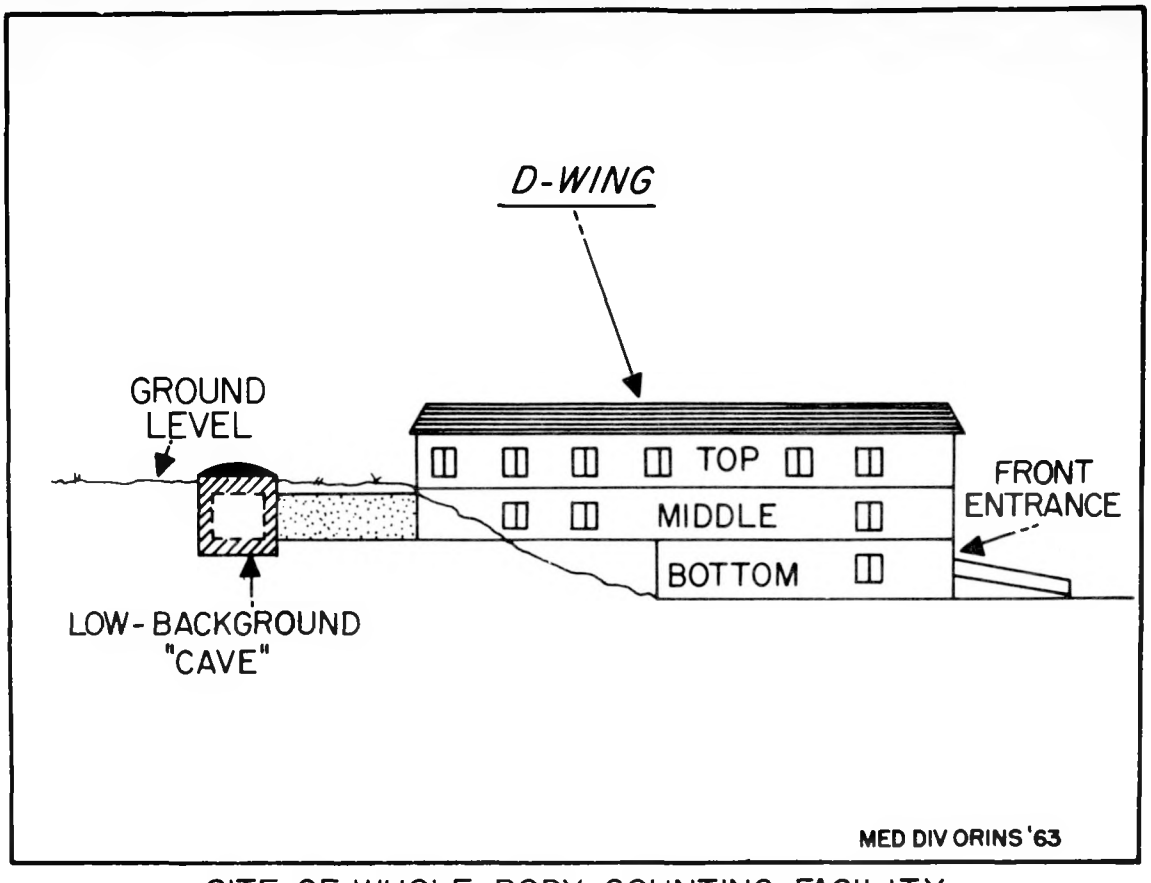

Fig. 1 Proposed location of patient-counting facility, at north end of ORINS hospital.

materials --- in short, a high-sensitivity, low-background sample counter.* Our design (Fig. 2) uses a 5 x 4-in. sodium iodide crystal in a low-background assembly, with the sample surrounding it on all sides except the one facing the phototube. This provides a modified Marinelli-beaker arrangement that brings nearly all parts of a 10-liter sample within about 7 cm of the crystal. Ten liters of sand or crushed rock weigh around 35 lb, and this large quantity helps to present as much radioactivity as possible to the detector. The crystal is mounted in a heavy, shielding box made of low-background steel 6 in. thick, the interior cavity measuring 20 in. square by 24 in. high. The detector's output pulses are processed in a 400-channel analyzer equipped with appropriate print-out and plotting devices. The analyzer and the sample counter will eventually be included in the completed low-background facility, the analyzer being used for both samples and patients.

Radionuclides signal their presence in a sample by making humps in the gamma-ray spectrum, and the locations of the humps are characteristic for each nuclide. The spectrum, therefore, can tell the cold-concrete enthusiast which of his enemies are present. Figure 3 shows the spectra for a number of materials, and at the bottom we have indicated the positions of the more prominent rays for the radionuclides that are likely to bother us most. These are (1) the long-lived, natural elements that have been with us in the earth for millions or billions of years (K^{40}, and the U^{238} and Th^{232} families), and (2) some of the fallout products. The topmost curve shows us, first of all, that the bank of clay where we would like to put the cave is "hot"; in fact it is nearly the hottest of all the materials we have examined. However badly we may need this earth to shield us from the hospital's radiations (teletherapy, X-ray, surgery, hot patients, etc.) we will need further shielding in the cave's walls to protect us from the earth. A good, concrete wall would be the first line of defense, but Fig. 3 shows that if we mix the

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*Before the ORINS testing system could be set up, some of the early samples were analyzed for us at the ORNL Y-12 plant, where a large-crystal, low-background, whole-body spectrometer is in operation. We are especially grateful to Dr. L. M. Scott for his help in this connection. We also wish to thank the Health Physics Division at the X-10 plant for lending us their carefully saved samples of North Carolina dunite (olivine) collected in 1958, thus providing us with older materials to compare with our recently collected ones. This was highly desirable because of the mounting threat --- to our low-background instruments, though not necessarily to our persons --- from fallout. As it turned out, the recently mined dunite shows no sign of fallout; evidently they are quarrying it fast enough so that fallout gets no opportunity to accumulate in detectable amounts.

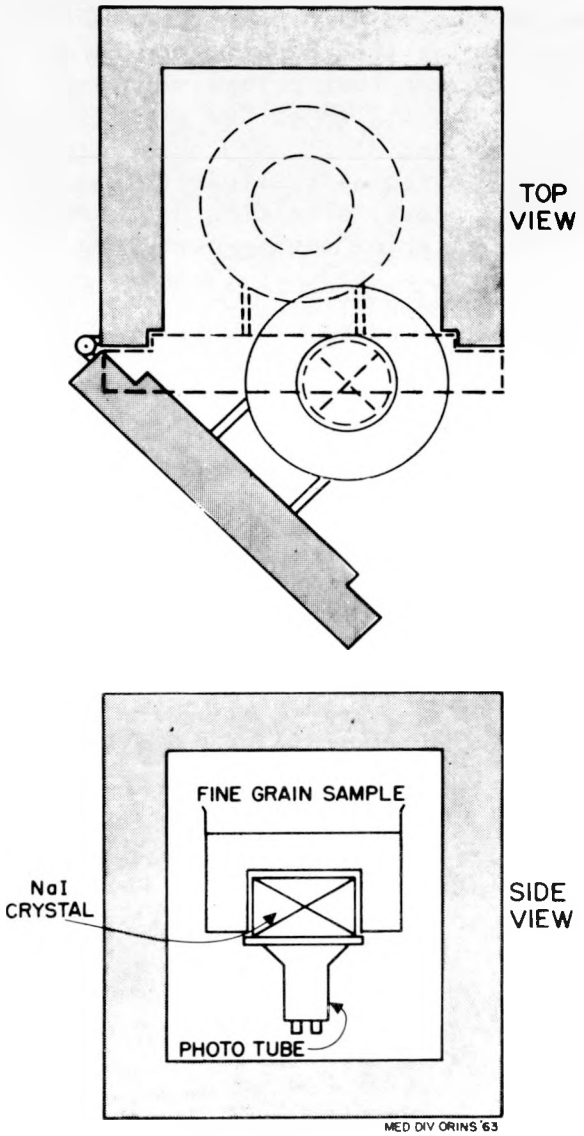

Fig. 2 High-sensitivity counter for large samples, showing the heavy shield and the detector arrangement. The material, about 3 in. thick, surrounds the crystal laterally, and covers its top.

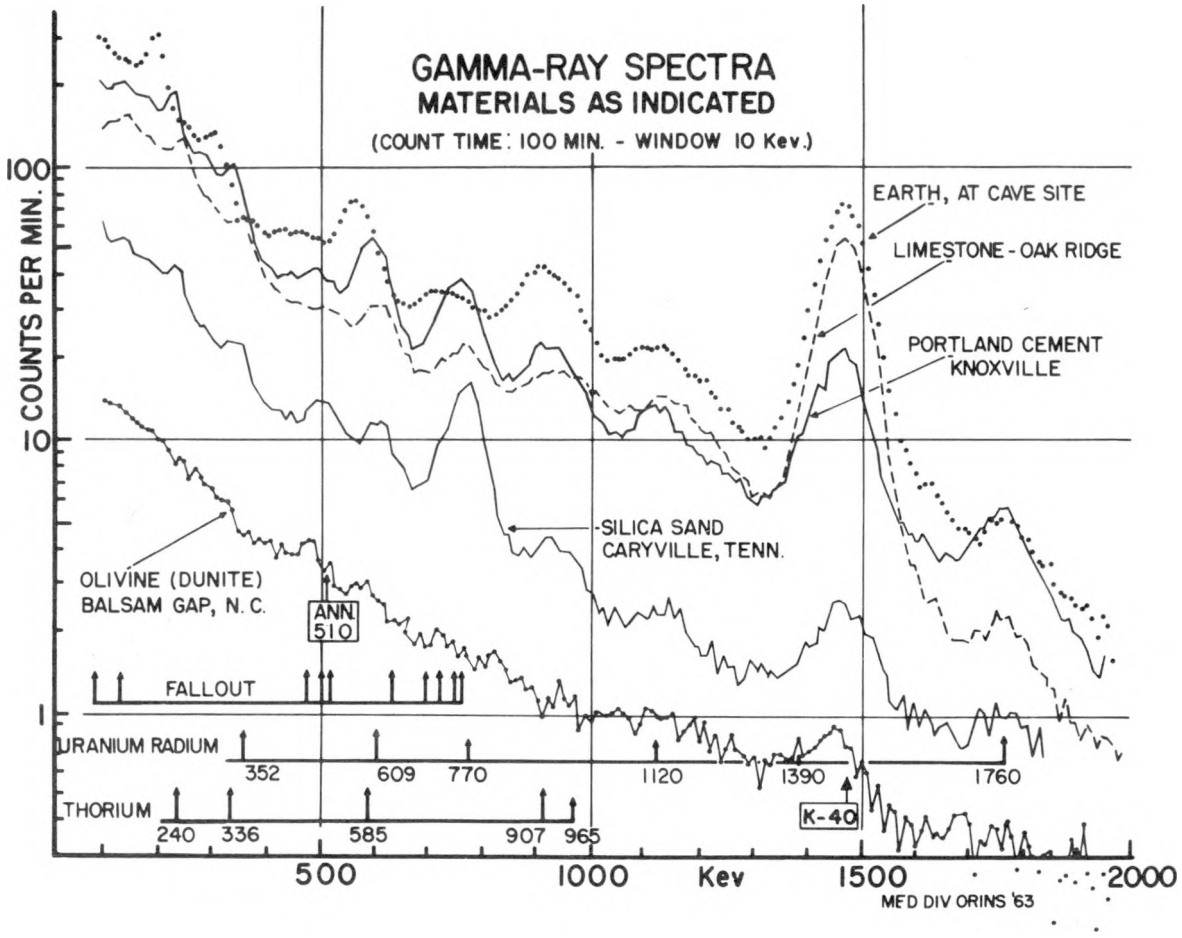

Fig. 3 Gamma-ray spectra for several materials, ranging from the "hot" earth at the construction site to the "cold" North Carolina olivine. The arrows near the bottom show the peak locations for a number of radionuclides of interest.

Fig. 4 Map showing locations where materials were procured.

concrete as is usually done in Oak Ridge, getting the cement from Knoxville and the coarse and fine aggregates from the large limestone quarry near Oak Ridge, the concrete wall will be nearly as hot as the earth. Hence we need to find cooler ingredients. We could, for example, use Caryville sand (Fig. 3) for the fine aggregate, but this would not do a great deal of good, for only one-third of the finished weight of concrete is fine aggregate, and we would still be stuck with the coarse limestone and the Knoxville cement (totaling about 60%), both of which are hot. The latter two items, therefore, are important.

As far as portland cement is concerned, there is not very much that we can do, for all the cements we have been able to find, within reasonable hauling distance of Oak Ridge, are nearly as hot as the Knoxville product. Canvassing Tennessee, we have tested samples from the cement plants at Nashville, Cowan, Richard City, Chattanooga, Knoxville, and Kingsport (see map, Fig. 4), and we have searched as far as Alabama (Birmingham), central Georgia (Clinchfield), north-central Kentucky (Kosmosdale), and southeastern South Carolina (Harleyville). Figure 5 shows the spectra for several kinds of portland cement; we have omitted some of the middle-of-the-road ones to avoid complicating the picture, for the curves overlap extensively. For comparison, and to show what a really stone-cold material looks like, we have put in the curve for North Carolina olivine*, and this makes it clear that none of the cements is anywhere near cold. The Clinchfield product looks like the best for our purposes, but it is "good" only in the sense that others are worse.

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*Two faint humps in the olivine curve are artifacts. The one in the K^{40} energy band (1460 kev) is found still to be present when we take the olivine away, so we conclude that either the sample counter's steel shield or its detector assembly contains a minute trace of potassium. The hump at 510 kev is due to "annihilation radiation," produced by the penetration through the 6-in. steel of hard, cosmic-ray components (mostly μ mesons) and the interaction of their products with the detector. The spike at the left-hand end of the curve is an electrical artifact. These humps constitute the only evidence of "structure" that the olivine spectrum shows, for the other jiggles are no more than what one would expect on the basis of random variations due to poor statistics at these low counting rates. We conclude that even this sensitive detector can't see any radioactivity in the olivine; it is magnificently "cold."

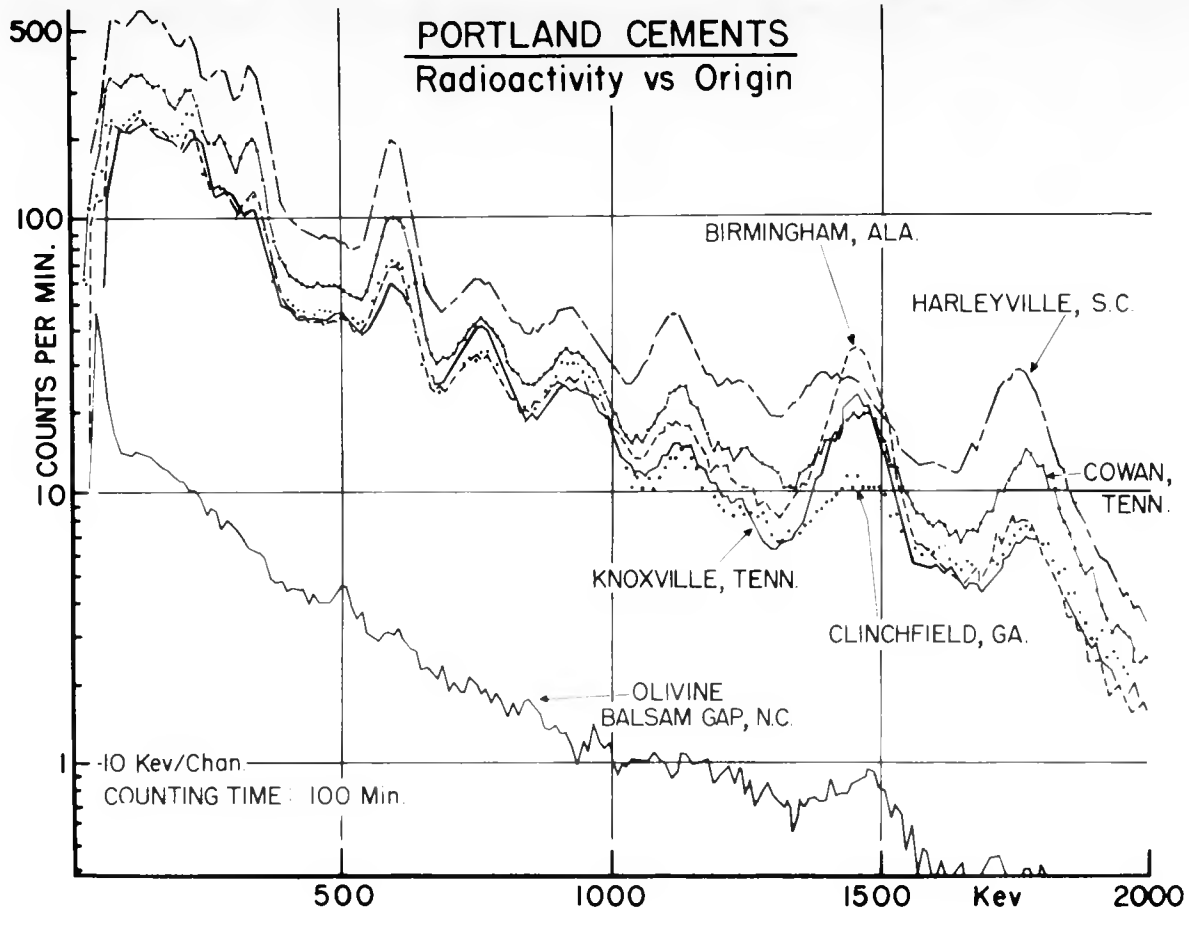

Fig. 5 Portland cements. To identify contaminants, see arrows at bottom of Fig. 3.

Looking at the individual radionuclides for a moment, we see that a prominent contaminant in these materials is K^{40} (1460 kev). To a low-background man K^{40} is an unmitigated abomination, (1) because potassium is very widely distributed in natural materials, and is therefore difficult to avoid, and (2) because the million-and-a-half-volt gamma ray of K^{40} packs such a nasty wallop that an unusually good shield is required to keep it out. Potassium is not our only problem, however, for we can see that members of the thorium and uranium series are often present (see bottom of Fig. 3). Some of these give off rays of uncomfortably high energy, but fortunately they are rather few in number, and the lion's share of the radiation from thorium and uranium and their daughters is not too hard to intercept. The Harleyville cement is unusually high in uranium, which is seen best at 1120 and 1760 kev; Birmingham is high man for K^{40}. All of them contain thorium (900 to 1000 kev).

Fallout products are also present, and there are several of these. Their principal rays are shown at the bottom of Fig. 3; they show up mostly around 500 and 600 kev and in the 700-to-800-kev band. Because of chemical differences the fallout elements do not adsorb equally well on all materials, so sometimes one is prominent and sometimes another. Moreover they decay at different rates, which adds to the variability of their pattern. We would like to avoid fallout, of course, but the fallout elements that we find in these earth materials are somewhat less of a curse than the natural radionuclides because the former are typically rather short-lived, their half-lives being reckoned in weeks or months. If we had to, therefore, we could put up with them (in homeopathic doses) at the start of the cave's career, with the consoling thought that they wouldn't be with us for long. But the natural radionuclides all have half-lives of around a billion years or more, so waiting for them to decay would be a tedious business. The decay of fallout is illustrated in Fig. 6, where a sample of river-bed stone was counted as soon as collected and then again about 3 months later. The fallout peaks had come down considerably, but not the others. Fallout has the additional virtue that most of the energies are not very high. Some of the fallout products are long-lived --- cesium and strontium, for example --- and these are of concern to the biologists; they don't bother us, however, since for reasons of solubility, among others, we don't find them in our earth materials.

The failure to find a cold portland cement has admittedly been disappointing, but fortunately cement constitutes only about 13% of concrete and we could still hope to do something about the aggregates, representing 80%. We concede, however, that we aren't going to get cold concrete; at best it will be "tepid."

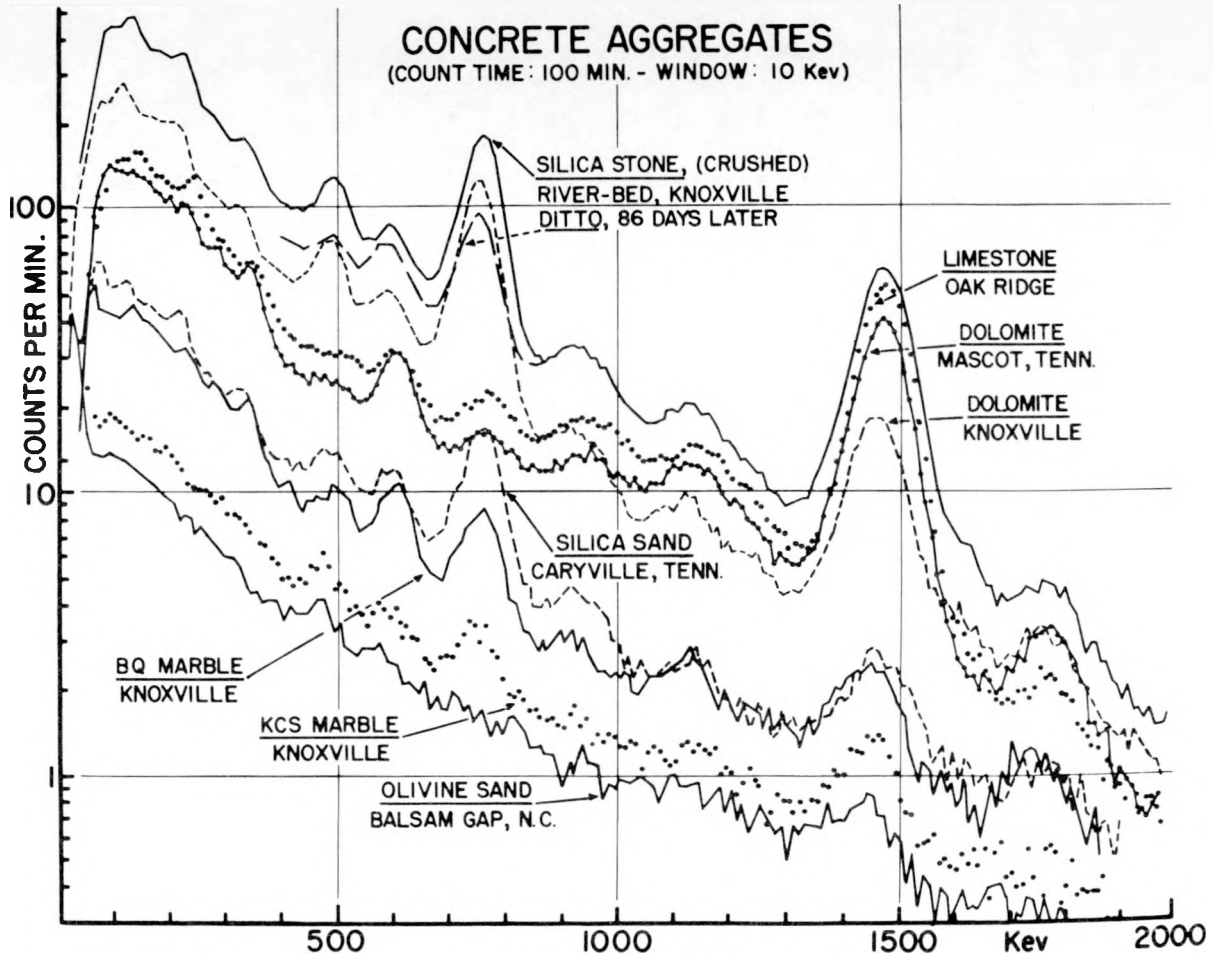

Fig. 6 Concrete aggregates. (See arrows at bottom of Fig. 3.)

A number of minerals and mineral products make good concrete aggregate, and among them we have investigated the following:

1) limestone (largely calcium carbonate), from Oak Ridge;

2) dolomite (predominantly magnesium carbonate), from Knoxville and Mascot, Tennessee;

3) silica of two kinds:

   (a) natural sand, from Caryville, Tennessee, and

   (b) crushed river-bed gravel chiefly SiO_2, from Knoxville;

4) barite or barytes (naturally occurring barium sulfate), from the area around Sweetwater, Tennessee, and also from Del Rio;

5) olivine (a solid solution of iron-magnesium orthosilicates, found almost pure in the rock known at "dunite"), from Balsam Gap, N. C.;

6) iron slag (a blast-furnace scum, mainly iron silicate), from the smelting works at Copper Hill, Tennessee;

7) iron sinters (a heavy clinker material, nearly 70% iron), also from Copper Hill;

8) marble (mainly calcium carbonate), from the Knoxville area.

Figures 6, 7, and 8 show the spectra obtained from the 10-liter samples. As ill luck would have it, the really good materials are expensive. Olivine is superb, for it looks cold even to the severely critical eye of our 5 x 4-in. detector. It makes good concrete, moreover, but it costs about $13 a ton as crude, crushed rock, and perhaps twice as much if properly screened for concrete-making. At the other end of the scale we have Oak Ridge limestone at $2 a ton, screened --- but, relatively speaking, hot. (Fig. 6) Knoxville's river-bed silica (same figure) is even hotter, and the two dolomite samples leave much to be desired. The barites are dense, which gives them good shielding properties, but (Fig. 7) they contain fair amounts of radium and potassium, and particularly of fallout, which clings to some of them like a leech. Moreover they cost about $23 a ton, and a ton isn't very much. We can get washed silica sand (Caryville) for one-tenth of this price, and it is about as good as the best of the barites (Fig. 6). However, Knoxville's "BQ" marble (Fig. 6), at $2.50 a ton, looks a little better than the Caryville sand, and the marble can easily be crushed, washed, screened, and delivered in a ready-made

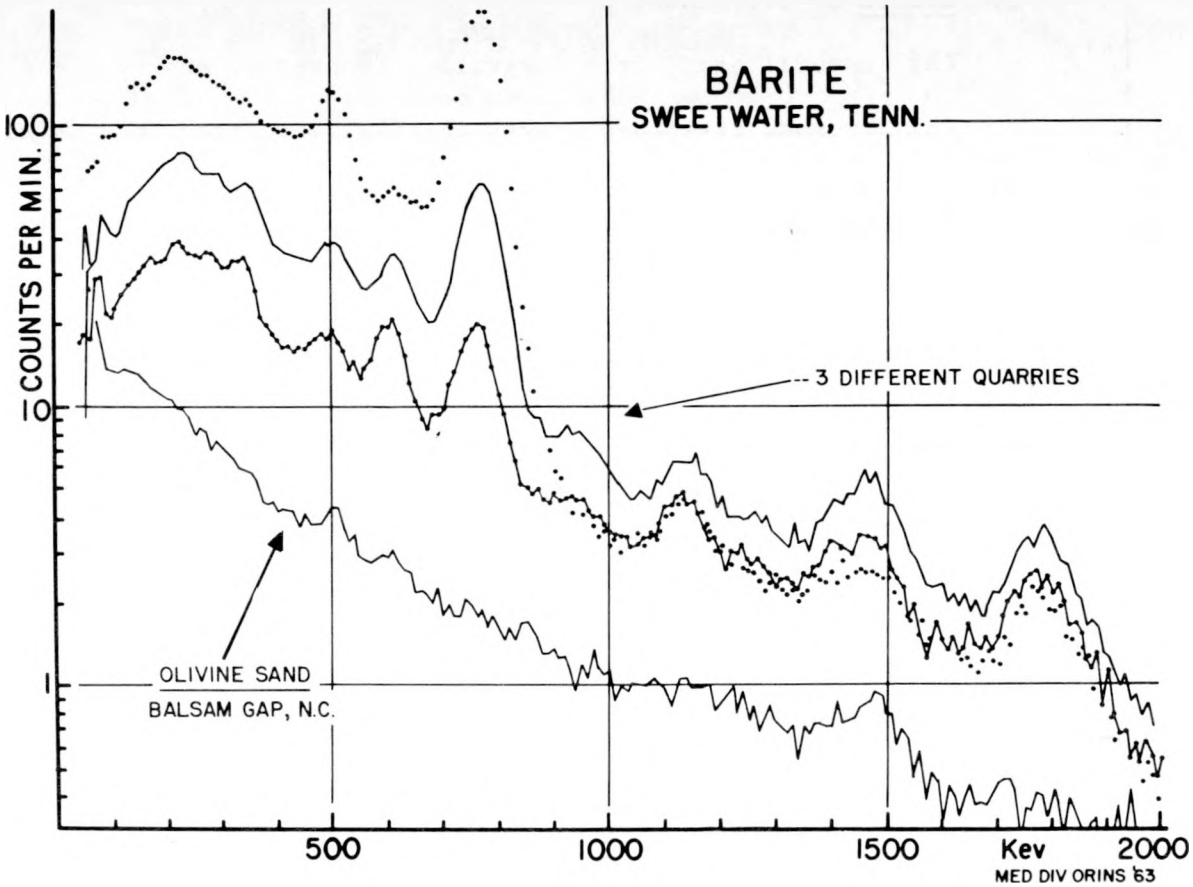

Fig. 7 Barite, from three different quarries near Sweetwater, Tennessee. (See arrows at bottom of Fig. 3.)

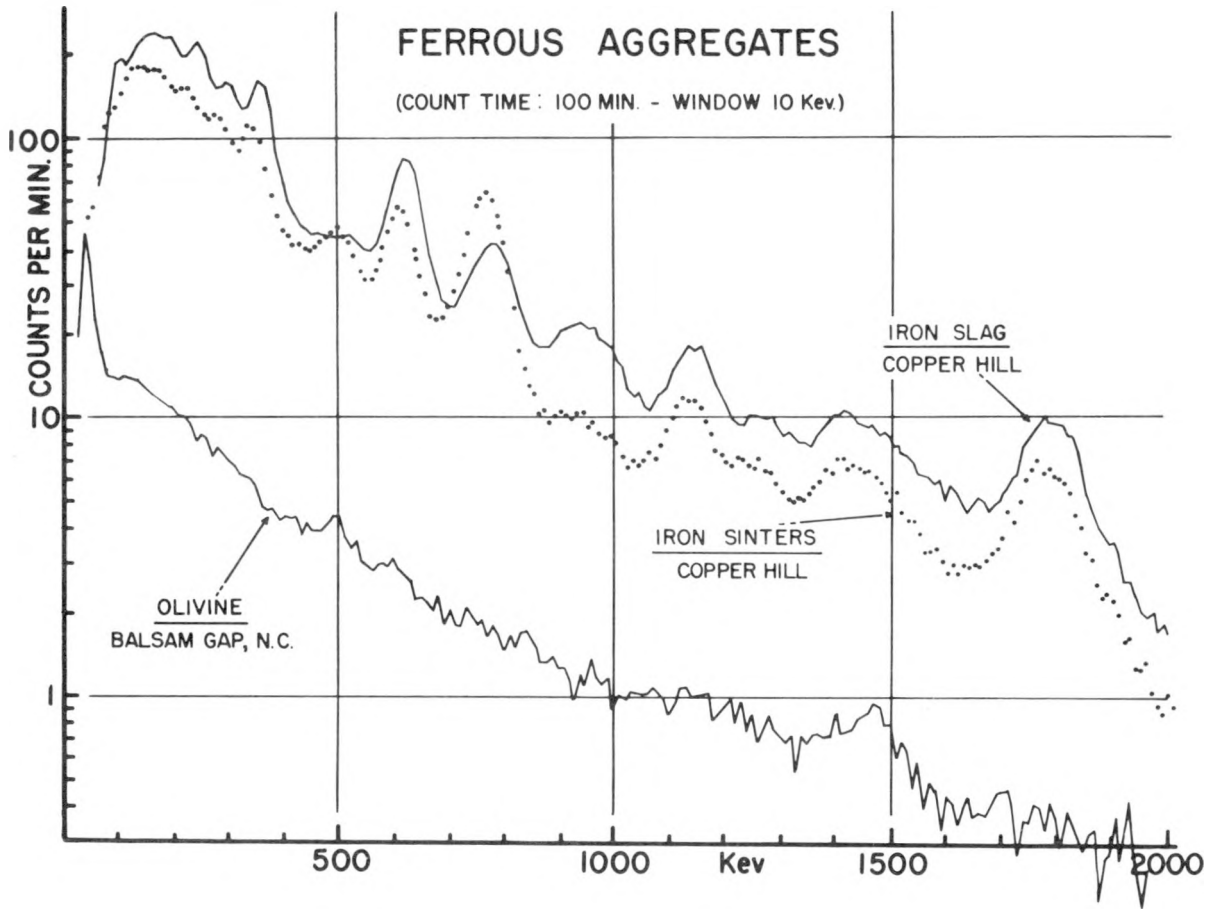

Fig. 8 Iron-rich by-products from a copper-smelting plant. (See arrows at bottom of Fig. 3.)

mixture of coarse and fine, needing only to be mixed with cement and water. A higher grade of Knoxville marble ("KCS", Fig. 6) is almost as cold as the olivine, but it costs $18 a ton. The iron slag and sinters (Fig. 8), which are by-products of copper smelting, looked attractive on paper because their high iron content makes them dense, but they turned out to be too hot, too far away, and too expensive to be competitive. Even the slag, at $4 a ton, would cost considerably more than the BQ marble.

We must crystallize these findings into some practical decision on how we should build our cave. Since we can't get cold concrete, there will have to be cold shielding inside the wall, and the hotter we allow the wall to be, the more inside shielding we will need. We could make the wall of olivine concrete, accepting the high cost in order to obtain fairly low activity, due only to the contained cement. Or we could use BQ marble to build a much cheaper concrete wall, and compensate for its higher radioactivity by increasing the shielding inside. This is not an easy choice, for there are numerous interlocking engineering considerations, but we have chosen the latter alternative. Figure 9 shows the proposed cave structure. "Warm" concrete 12 in. thick will hold back the "hot" earth. Inside this will be a 2-ft-wide gap filled with stone-cold crushed olivine, which we won't need to screen because granule size is unimportant as long as we don't want to make concrete out of it. Inside the olivine fill there will be an 8-ft-cubed steel box, 5 in. thick, and we will take care, of course, to see that the steel is also stone cold. The finishing touches to this massive shield will be provided by two linings, an outer one of 1/4-in. lead and an inner one of 1/16-in. stainless steel.

The patient will be suspended in a horizontal axis of this box, lying on a canvas sling supported by two stainless-steel pipes. A track system will permit us to slide him into the counting position through a small door connecting the cave with the adjoining laboratory. A thin, plastic enclosure will surround the patient, and this permits us to ventilate only a small fraction of the cave's air, leaving about 92% of it "dead." We prefer not to ventilate the cave any more than necessary because ventilation brings with it the hazard of randon-containing air (which deposits solid radioactive daughters) and fallout-containing dust. The eight detecting crystals (NaI, 5 x 4 in.) will be suspended above and below the patient, in the mid plane.

The K^{40} regions of the spectra for the wall materials are shown to the left of the sketch of the room, merely to illustrate the general design policy. We are not ignoring the other contaminants, but each of them makes several "humps" on its spectrum, and without a lengthy discussion they would only confuse the issue. Moreover, as

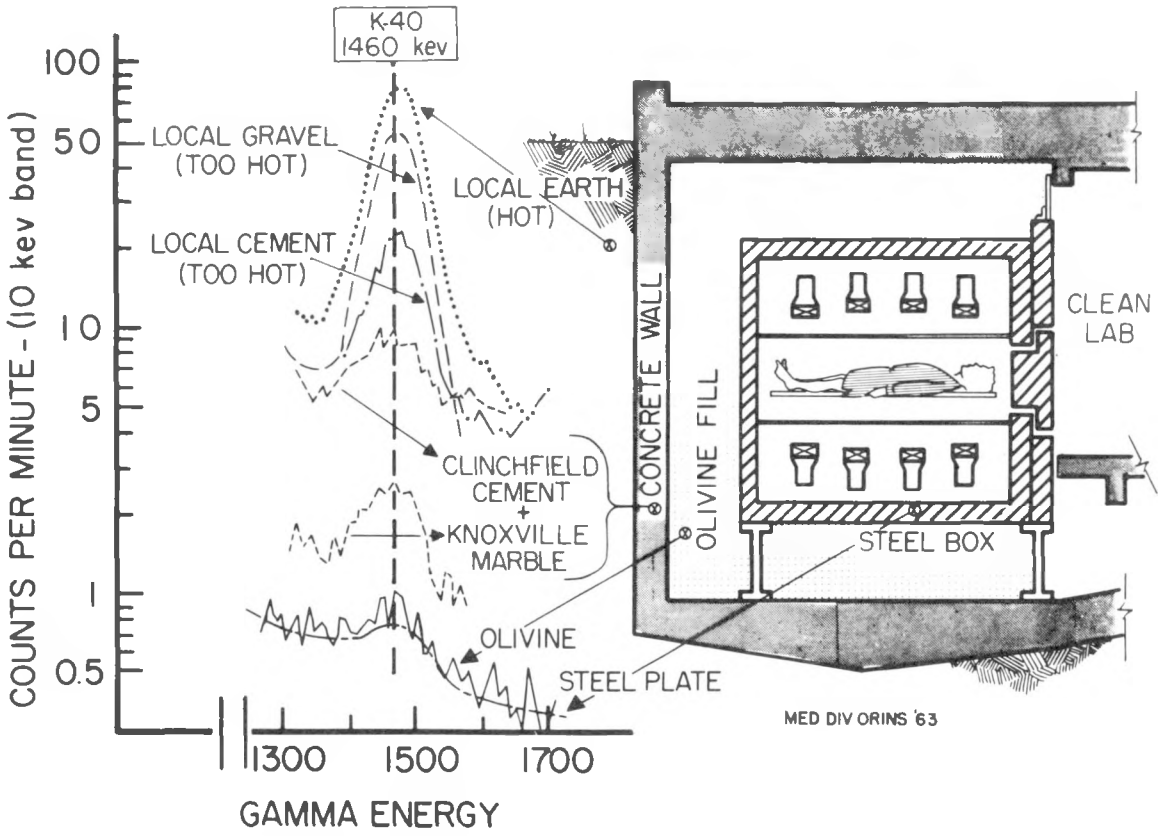

Fig. 9 Vertical section (schematic) through the patient-counting chamber, showing the wall structure. The K^{40} regions of the spectra are shown at the left, to indicate how the inherent radioactivity decreases progressively from the "hot" earth to the "cold" steel box.

mentioned earlier, the majority of their gamma rays have lower energies than potassium, and since such rays are absorbed more readily by the inner parts of the wall, they present, for the most part, a less serious threat. In some studies elsewhere the materials have been assessed by counting all the gamma rays with energies higher than some arbitrary value --- say 100 kev. This, we think, can be highly misleading, for it makes relatively low-energy radiation look more threatening than it really is.

A number of the features of this design are new, for we are trying to profit from other people's successes and avoid their mistakes.

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