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OAK RIDGE INSTITUTE of NUCLEAR STUDIES

Medical Division

Report for 1960

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P. O. Box 117 Oak Ridge, Tennessee

MEDICAL DIVISION

Report for 1960

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CLINICAL RESEARCH

Introduction

Studies on the effects of radiation in man have continued to occupy a prominent place in the clinical research program during the past year. The new total-body irradiation facility was completed and this permits giving a more uniform radiation dose over a wide range of dose rates. Emphasis has shifted from attempts to obtain successful grafts of bone marrow in patients given large doses of total-body irradiation to studying the effects of small doses of radiation given to the whole body in various diseases. This program has been broadened to include immunological studies by the addition of Dr. Nazareth Gengozian, an immunologist, to the staff in midyear. Special studies of the effects of local port irradiation on normal bone marrow are being done in patients receiving radiation therapy for nonhematologic diseases.

Applications of radioisotopes in the study and treatment of hematologic disorders in general continues to be of great interest. Special projects started in this area during the year include studies on isotopically labeled blood platelets and plasma cells.

The long-range programs pertaining to diagnostic and therapeutic uses of internal radioisotopes have been pursued. In addition to their research value, these programs give basic training for resident physicians. They also provide the basis for most of the scanning, which constitutes a program in itself. Continual attempts have been made to improve scanning equipment, techniques and interpretation, and to extend the applications of this procedure.

The outpatient service has grown considerably both in scope and number of patients. Dr. David A. White has had the major responsibility for this service, which is an integral part of the over-all clinical program.

Dr. Frank Comas, radiotherapist, has been assisted by Dr. Ra-Ving Samapurnavanitya, visiting scientist from Thailand, in the teletherapy program, which includes special clinical studies in this field in addition to contributing to the clinical program in general.

Dr. Herbert B. Gerstner joined the staff on a temporary basis in midyear and has been most helpful, especially in problems of radiobiology.

Dr. Orren W. Hyman, Jr., served a temporary appointment as postresident assistant in radiology during the first part of the year. During the remainder of the year, the diagnostic radiology has been done by several radiology residents in the short-term residency training program; they have performed this work in addition to their other duties.

Again the clinical staff has relied heavily for consultant help on a number of Oak Ridge physicians whose cooperation contributes greatly to the clinical program. These consultants include Dr. Robert R. Bigelow, Dr. William W. Pugh, Jr., Dr. Avery King, Dr. Betty M. Cooper, Dr. Robert P. Ball, Dr. Paul Spray, Dr. Dana W. Nance, Dr. Earl Eversole, Jr., Dr. Raymond A. Johnson, Dr. Dexter Davis, and Dr. Henry B. Ruley. Dr. Eidson Smith and Dr. Robert Newman of Knoxville, and Dr. George Minor of Charlottesville, Virginia, have continued their contributions in consultant capacities.

Dr. Flora Pascasio and Dr. Frank Oda performed a major part of the work in providing clinical care for patients in addition to conducting specific research studies before the termination of their appointments at midyear. Dr. Helen Vodopick (Mrs. Francis Goswitz), Dr. Francis Goswitz and Dr. Robert H. Johnson joined the staff on July 1 with one-year appointments as post-resident assistant in medicine, research fellow and resident in clinical investigation. They have taken over the primary duties involved in caring for patients in addition to special research projects.

Dr. Teruo Nagai and Dr. Hiroshi Saito, visiting investigators from Japan, have participated actively in clinical rounds although their primary work has been in special research areas.

A major contribution to the clinical program has been made by the nursing staff under the supervision of Mary E. Sutliff (Mrs. Adren R.). In addition to providing nursing care for the patients, their help in performing special studies and collecting data from patients has been invaluable.

The resident physicians staff, representing various medical specialty fields, has performed excellent work in caring for the patients and has been a source of stimulation in the research and teaching programs.

SHORT-TERM RESIDENT PHYSICIANS

George B. Carter	Massachusetts General Hospital
Robert Dingle	Boston City Hospital
Robert Dinsmore	Massachusetts General Hospital
Elwin Donnelly	Massachusetts General Hospital
F. Eskandari	Cleveland Metropolitan General Hospital
Juan Fayos	Tumor Institute of Seattle
Murray Janower	Massachusetts General Hospital
John Little	Massachusetts General Hospital
William McCoy	Massachusetts General Hospital
Sigmundur Magnusson	Boston City Hospital
Yosh Maruyama	Massachusetts General Hospital

RESIDENT IN PATHOLOGY

Raymundo Villalva	St. Mary's Hospital, Knoxville

RESEARCH PARTICIPANT IN PATHOLOGY

Ben Dowdy	Southwestern Medical School

VISITING RESIDENT

Emma Lappat

ABBOTT FELLOWS

James Densler	Meharry Medical College
Robert Overholt	University of Tennessee Medical School

nitrogen mustard therapy was given again. There was temporary benefit, but about one month later he was admitted to the Medical Division hospital for the first time because of progression of his disease. At this time there was evidence of metastatic lesions in the brain, soft tissues of the scalp and chest wall, parietal pleura bilaterally, and in the bones. The patient was treated with another course of nitrogen mustard with only temporary benefit. A month later all areas of disease previously mentioned had progressed and additional lesions were present in the scalp, chest wall and bones, and the patient had developed a partial paraplegia. Just before the irradiation, blood counts showed a hemoglobin of 12.9 mg, hematocrit 38, WBC 6100, platelet count 107,500.

He was given 400 r mid-line air dose total-body irradiation at a rate of 1.53 r/minute using the cobalt-60 source and technique used previously in treating patients with acute leukemia. There was brief improvement in his symptoms and slight decrease in the size of soft tissue masses, but by the tenth postirradiation day there was again evidence of advancement of the disease. The beneficial effect of the total-body irradiation on this malignancy was only slight and very brief.

Patient 2 was a 30-year-old woman with a poorly differentiated malignant neoplasm (probably neuroblastoma). She had previously received external radiation therapy to a mediastinal mass with striking decrease in the size of this lesion. At the time of admission to ORINS Medical Division, chest X rays showed multiple discrete nodules of metastatic tumor measuring up to 7 cm in diameter scattered throughout both lung fields. Bone marrow aspiration showed no invasion of the marrow by malignant cells. The patient was given 360 r mid-line air dose total-body irradiation at a rate of 1.53 r/minute, using the same cobalt-60 source and technique used in the first patient.

Just before the irradiation 200 cc bone marrow was aspirated from the patient. While she was receiving the radiation therapy the marrow cells were labeled with tritiated thymidine. The marrow was given back to the patient intravenously 30 minutes after the irradiation was completed. (Details of this study are reported separately.) From subsequent routine peripheral blood counts there was no evidence that this marrow altered the patient's course.

Six days after irradiation chest X rays showed a marked decrease in the size of the tumor masses and this regression was still present on the thirteenth postirradiation day. By the seventeenth day after irradiation, however, chest films showed the lesions to be increasing in size again. Thus this dose of total-body irradiation did have a definite effect in producing regression in the size of the tumor masses, but the improvement proved to be of only short duration.

Patient 3 was a 12-year-old girl with Hodgkin's disease of three and one-half years duration. She had received local port irradiation therapy to the left cervical and mediastinal areas with good response

early in her disease. A few months later enlarged hilar nodes appeared and responded to treatment with Chlorambucil. Except for anemia, there was no evidence of recurrent disease during the next two years. About one year before her admission to the ORINS Medical Division the disease became active again and progressed in spite of therapy with nitrogen mustard, mannitol mustard, and X-ray therapy to the spleen. The last three months before her admission to the Medical Division, she required frequent blood transfusions for anemia.

When admitted to the Medical Division hospital, the patient had enlarged lymph nodes in all the peripheral lymph-node areas with a particularly large mass of nodes in the right cervical region. The spleen was markedly enlarged and chest X-ray films showed widening of the mediastinum, enlarged hilar nodes, and multiple nodular densities up to 1 cm in size scattered throughout both lung fields. Blood counts showed hemoglobin 6 g, hematocrit 21.5%, WBC 9250, and platelet count 170,000. The bone marrow showed hyperplasia involving both red cell and granulocytic series with increased hemosiderin, consistent with a hemolytic process, and no evidence of Hodgkin's disease in the marrow.

It was felt that this patient was a candidate for total-body irradiation therapy, especially since she had a healthy identical twin who could serve as a bone marrow donor. She was given 300 r mid-line air dose, total-body irradiation at a rate of 0.74 r/minute, using the new total-body irradiator with cesium-137 sources. During the ensuing six weeks, there was no significant change in the size of enlarged lymph nodes or spleen and no improvement in the appearance of the lesions demonstrable by chest X-ray films. Thus there was no demonstrable beneficial effect of the total-body irradiation on Hodgkin's disease in this patient.

The thrombocytopenia and leukopenia resulting from the radiation became quite profound in this patient and on the twentieth post-irradiation day she was given an infusion of bone marrow with her identical twin serving as donor. Clinically, there was no definite evidence of a successful graft of the infused marrow as judged by the degree of depression of the white blood cell and platelets and the time relationship of their subsequent recovery.

The changes in the peripheral blood cells for the three patients are shown in Figs. 1, 2, and 3.

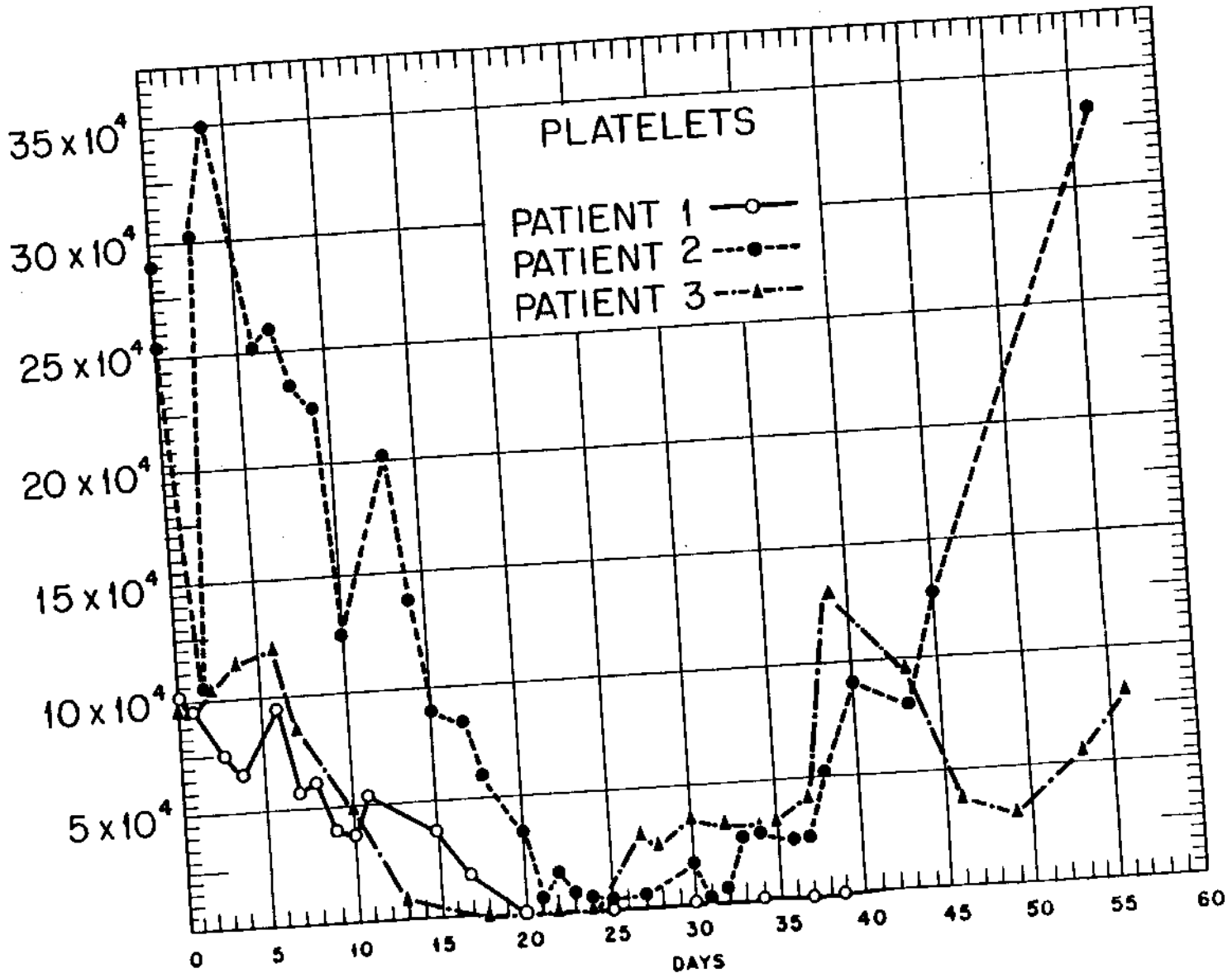

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Figure 1. Platelets

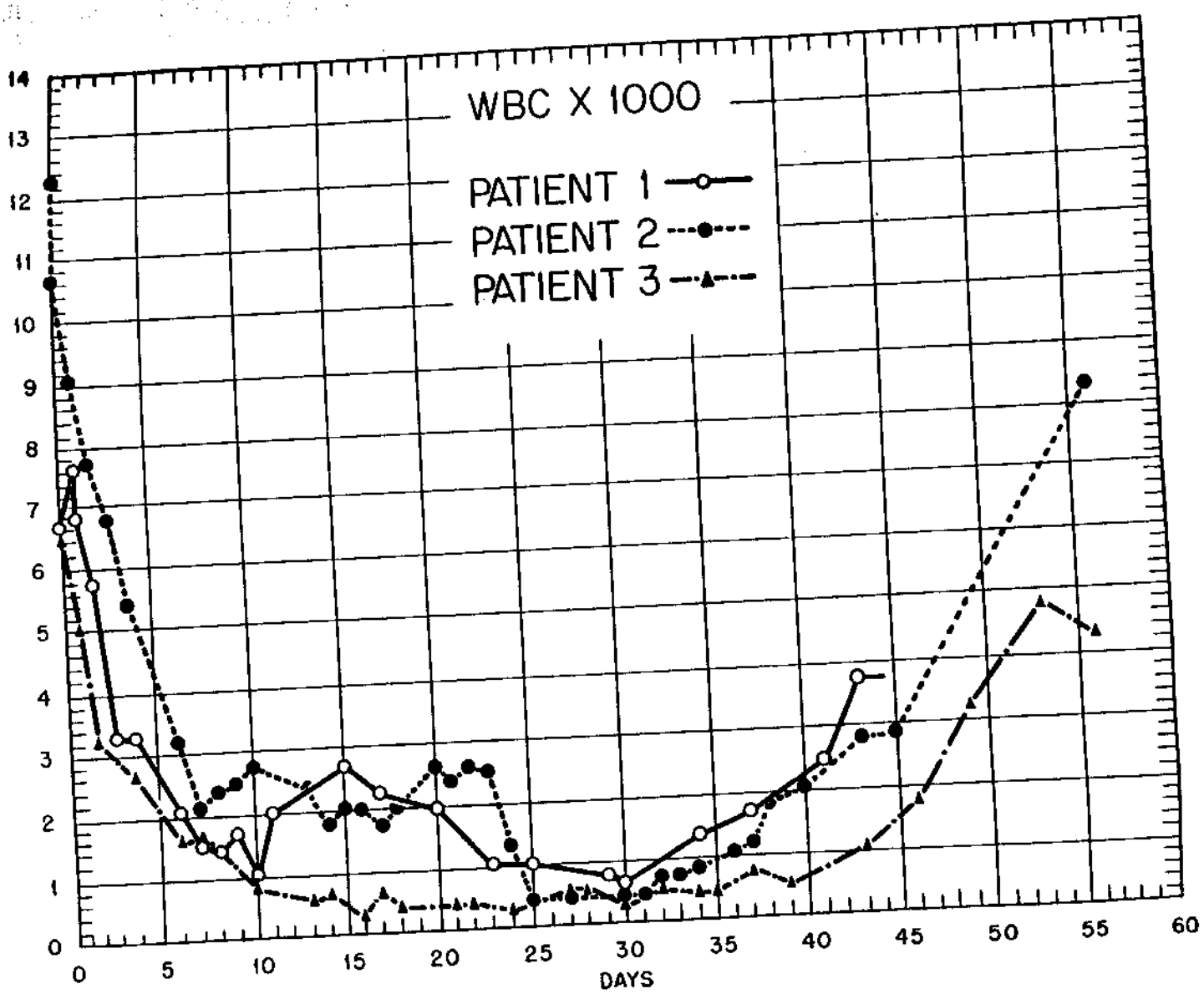

Figure 2. White Blood Cells

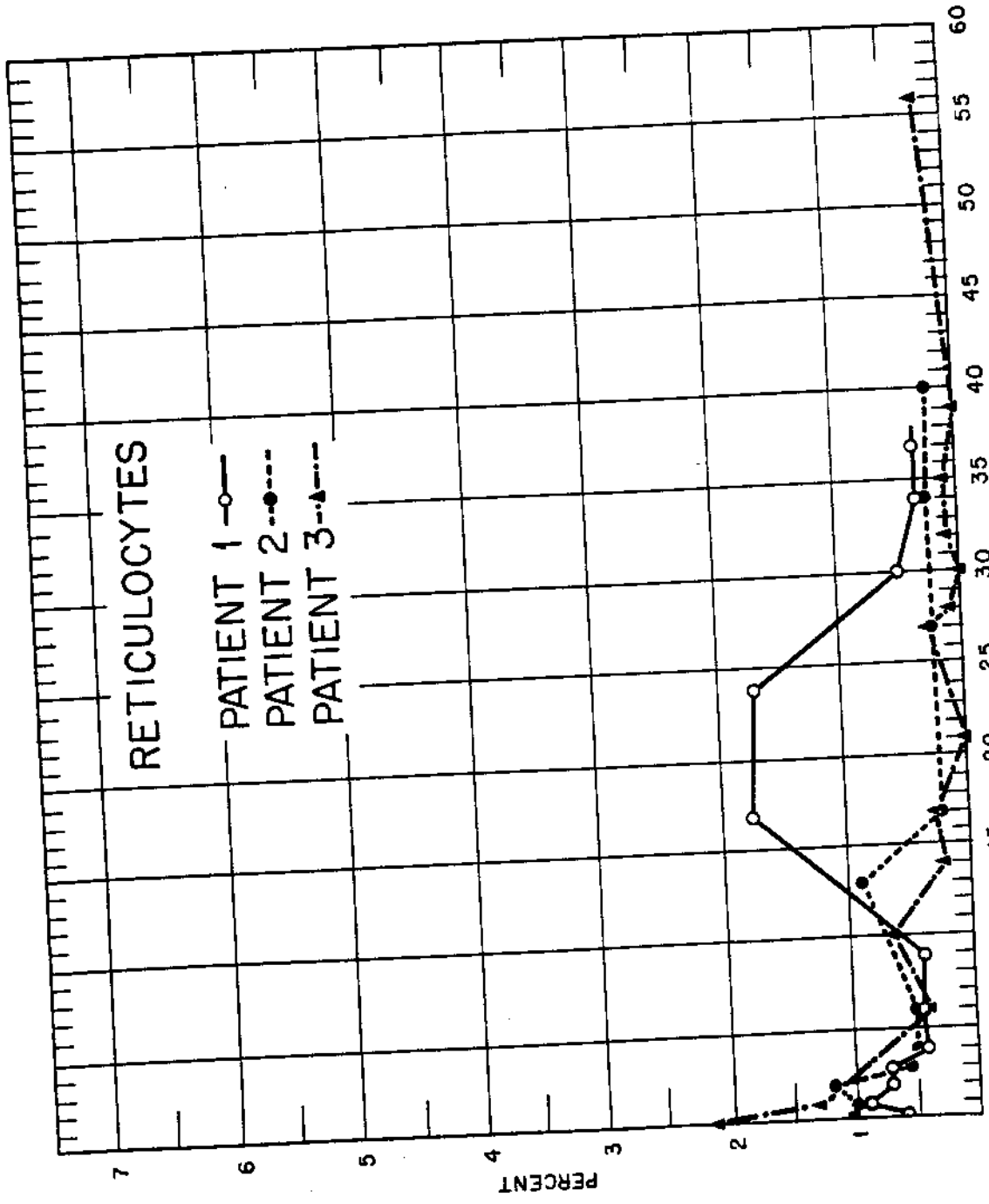

Figure 3. Reticulocytes

Blood platelets: The general pattern of changes in blood platelets was similar for all three patients, as shown in Fig. 1, but there were some individual differences within the over-all pattern. In Patients 2 and 3, there tended to be a slight rise in platelets during the first week, followed by a decline to very low levels. Patient 1 did not show an early rise but rather a gradual decrease from the beginning. The maximum depression in platelets occurred between the eighteenth and twenty-first day in all three patients. However, there was a sharper drop in Patient 3 so that her platelet count was down to 10,000 as early as the thirteenth postirradiation day. The count for Patient 2 was considerably higher than that of the other two patients originally and, therefore, during the period of fall her absolute values were considerably higher than those of the others. In her case the period of maximum depression persisted for about 12 days followed by a rather abrupt increase thereafter, beginning on the thirty-third postirradiation day. Maximum depression in Patient 3 also persisted for about 12 days. However, inasmuch as her counts fell to 10,000 as early as the thirteenth day, recovery started about the twenty-fifth day, a week earlier than that of Patient 2. In the early part of the recovery period in Patient 3, there was a plateau persisting for about 10 days followed by an abrupt rise to a level of 130,000. Instead of continuing upward, however, there was another fall during the next ten days with a gradual rise thereafter. The fact that recovery in Patient 3 started earlier in the postirradiation period and suffered another decline after having reached levels of 130,000 might be interpreted as an abortive period of recovery resulting from a temporary successful graft of donor bone marrow. But, as seen in Fig. 2, there was no such abortive recovery in the total white blood cell count as might have been expected if a temporary successful graft of bone marrow had occurred.

In Patient 1 there was no significant increase in platelets from the maximum depression. During the latter part of his course he was in extremely critical condition and died on the forth-fifth postirradiation day.

White Blood Cells. As shown in Fig. 2, the white blood cell count fell rapidly during the first postirradiation week to a level of about 2,000 in all three patients. In Patients 1 and 2, a plateau then occurred with the counts remaining at this approximate level for an additional two weeks. A further drop then took place with maximum depression occurring between the twenty-fifth and thirtieth days, with gradual recovery beginning about the thirty-second day and progressing thereafter. In Patient 3, however, the plateau during the second and third postirradiation weeks was not seen and instead there was a steady decline; the level of maximum depression was reached on the sixteenth day, about ten days earlier than in the other two patients. In this regards the white blood cells followed the same course as the platelets in this patient. Unlike the platelets, however, recovery did not also begin earlier than in the other patients, but rather the period of maximum depression persisted longer and it was not until about the forty-third day that gradual recovery began.

Reticulocytes. Experience at the ORINS Medical Division does not indicate as clear-cut a sequence of events in reticulocyte counts after total-body irradiation as occurs in platelet and white blood cell counts. In these three patients there tended to be a fall in the reticulocytes during the first week, followed by a slight rise at about the fifteenth day. A further decline then occurred with persistent low counts for the next month. Reticulocyte counts were not followed beyond this time. It appears that this dose of total-body irradiation does not cause a complete disappearance of reticulocytes.

Hemoglobin and Hematocrit. Because of their underlying diseases and the thrombocytopenia resulting from radiation, all three of these patients required multiple transfusions of fresh whole blood and platelet-rich plasma. The hemoglobin and hematocrit values fluctuated accordingly and the effect of radiation alone on these values was obscured.

All three patients developed nausea and vomiting during the latter half of the period of irradiation. The vomiting subsided as soon as the radiation therapy was completed in Patient 1 and 2. Patient 3 vomited twice during the next twelve hours and once daily for the next two days. There were no other specific clinical developments related to the irradiation in the immediate postirradiation period. Epilation occurred in all three patients, having its onset on the seventeenth post-irradiation day in two of them and about the twentieth day in the other. Petechiae and nosebleeds developed in all three patients at the time of the most marked thrombocytopenia.

Progress report on Y-12 radiation accident patients.

Examinations of the patients from the Y-12 Plant radiation accident of June 16, 1958, have been conducted on an outpatient basis at intervals of approximately six months. In general these men have remained well and continued to work full time. There has been slowly progressive decrease in indications by the men of fatigue, muscle stiffness, nervousness and insomnia, although they still persist in degrees varying from patient to patient and without correlation with the amount of radiation received.

Studies of the peripheral blood and bone marrow have shown normal results. Slit-lamp examinations of the eyes have shown no significant changes during the year. Sterility studies based on sperm counts (participation on a voluntary basis) have been limited and inconclusive.

Tests of thyroid function were made in these patients. Protein-bound iodine (PBI), serum cholesterol and resin uptake of iodine-131 triiodothyronine from serum (T3 uptake) determinations were done. The results were all within normal range with border-line low values for PBI and T3 uptake in one patient.

Bone Marrow Labeling. (R. M. Kniseley, M.D. and T. Nagai, M.D.)

The fate of H_3-thymidine-labeled marrow cells injected in attempted human marrow graft. Although bone marrow grafts have been used successfully to repopulate the marrow in irradiated experimental animals, there is limited evidence of successful marrow grafts in human patients. For this study tritium-labeled thymidine was selected since it is regarded as a specific precursor for synthesis of deoxyribose nucleic acid (DNA) and is utilized by bone marrow cells in vivo and in vitro. The present report concerns two patients given labeled donor marrow cells in vitro to trace them after injection.

One patient with disseminated malignant neoplasm was given autologous-labeled marrow (5.1×10^9 nucleated cells containing 2.1×10^8 labeled cells). This was infused intravenously after 360 r total-body gamma irradiation. The second patient, critically ill with aplastic anemia, was given (without irradiation) homologous labeled marrow (2.1×10^9 nucleated cells containing 2.3×10^8 labeled cells) from a normal donor with the same ABO and RH compatible blood groups. In the first patient peripheral blood and bone marrow aspirations were taken at 10 minutes post marrow infusion, every day for four days, and after that at intervals of a few days. In the second patient fewer samples were obtained. Autoradiograms of 4 to 10 films of each sample of bone marrow and of the buffy coat of peripheral blood were prepared and scanned under the microscope very carefully. Labeled cells were discovered in the marrow of both patients 10 minutes after the infusion; cells were also recovered from the peripheral blood. In Patient No. 1 it was possible to detect labeled cells in the bone marrow up to three days and with a questionable cell found at nine days, while the peripheral blood contained labeled cells up to 10 days. In the second patient an unclassified labeled cell was found at two days and at nine days and labeled cells were seen in the peripheral blood at six days. In addition to the usual cell types, two other categories of labeled cells were recorded. The "unclassified mononuclear cells" may be in the granulocytic series. Most are probably promyelocytes or myelocytes, but some could be monocytes or primitive red cell precursors. A second group referred to as "unclassified small cell" may include myelocytes or red cell precursors. Photographic processing impairs cell staining and absolute identification is not always possible. The labeled cells are so rare that no valid estimate of the percentage can be made.

The method demonstrates the feasibility of labeling marrow cells in vitro to follow their fate after injection. The cells are well labeled and the washing procedure used will remove unincorporated thymidine adequately. The method is laborious and the yield of labeled cells is disappointingly small. However, the results indicate that injected marrow has survived for a time. Since cells synthesize DNA only in preparation for mitosis and since cells beyond the myelocyte stage do not divide, the presence of labeled band and segmented forms indicate that the injected cells were capable of both mitosis and maturation. We have no evidence that the injected marrow continued with proliferation and repopulation in the recipients.

Fig. 1. Labeled band neutrophil found in the peripheral blood three days after marrow infusion.

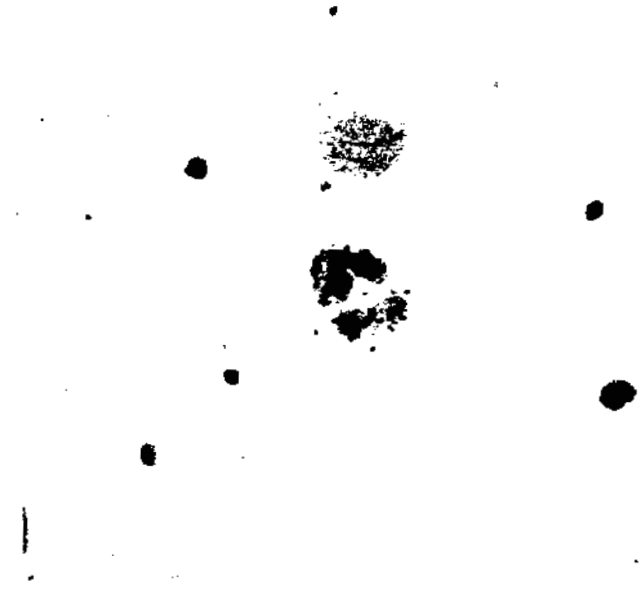

Fig. 2. Labeled segmented neutrophil found in bone marrow three days after marrow infusion.

Recent Projects. (R. M. Kniseley, M.D.)

Radiation effects. A considerable body of data has been collected on seven patients given intensive port irradiation. The study involves the collection of bone marrow samples from the site of irradiation and from control sites before, midway, and after the course of therapy. Qualitative cell changes, hemosiderin deposition, and in vitro uptake of tritium-labeled thymidine studies are being carried out. A detailed report will appear in the Report for 1961.

Bone marrow studies. Short-term tissue cultures of bone marrow from patients with multiple myeloma and certain lymphatic leukemias have been exposed to selected labeled precursor compounds: formate, uracil, thymidine, methionine, cytidine and sulfate. The uptake in these cells is being studied by microscopic single-cell autoradiography. A detailed report will be presented in the Report for 1961.

An Evaluation of Methods for the Determination of Creatine and Creatinine in Human Urine. (Elizabeth Rupp)

Several laboratories have measured creatine and creatinine excretion by the persons exposed to total-body irradiation in the Y-12 accident. There has been agreement that irradiation did not induce changes in creatinine excretion. The quantitative results from different laboratories have been in satisfactory accord. The results with creatine, on the other hand, have not been similar and we have found no change in creatine excretion, whereas another investigator reports an increased excretion of creatine.

In an effort to resolve these differences, a study of the methods used has been undertaken. Several methods that have been proposed will be studied carefully. Two procedures will then be selected and both applied to a re-evaluation of our earlier results with regard to creatine excretion after total-body irradiation.

The Jaffe reaction for creatinine, as modified slightly from Taussky (1954), is being investigated as the first step in this re-evaluation, for the determination of creatine depends upon its conversion to creatinine.

So far, we have studied 1) the stability of color in the Jaffe reaction; 2) the effect of varying mole ratios of picric acid and NaOH; and 3) the dependence of the Jaffe reaction on NaOH concentration during the formation of alkaline creatinine picrate. We have found, in confirmation of reports by other workers in the field, that the optical density of alkaline creatinine picrate is independent of the picric acid concentration above a certain value, which is exceeded in all the methods proposed. The color yield, however, and also the rate of development of the complex formed in the reaction are sensitive to the NaOH concentration. The same colored compound is being formed, but the

reaction between creatinine picrate and NaOH is a multiple order reaction sensitive to NaOH concentration.

Using the optimum conditions for the reaction, we constructed a standard creatinine curve for the Klett-Summerson colorimeter.

We ran absorption spectra, using the Beckman Spectrophotometer, on alkaline picrate and on alkaline creatinine picrate to determine the "difference spectrum" (alkaline creatinine picrate minus alkaline picrate). The best wave length for measuring alkaline creatinine picrate was 510-520 millimicrons. We constructed a standard curve with the Beckman (at 510 and 520) as a check for the Klett colorimeter curve. As reported by others, we found that the absorbance was a linear function of creatinine concentration.

After determining the extent of interference of certain carefully selected substances in the Jaffe reaction, we compared the effectiveness of ether extraction with that of iodine oxidation followed by chloroform extraction in preventing interference by these substances in the Jaffe reaction. We obtained good results with the iodine reaction. However, with the ether extraction, traces of ether remaining in the sample are very difficult to remove and interfere upon addition of alkali.

We are now measuring creatinine in urine specimens, and testing the recovery of added creatinine from these specimens using the conditions which we found to be optimum for this reaction. We are also testing the procedures, slightly modified, of Taussky in removing interfering substances from urine.

The work is still in progress.

Changes in Serum Uric Acid Levels in Patients Given 50 or 100 r Total-Body Irradiation. (Elizabeth Rupp and A. L. Kretchmar, M.D.)

The changes in serum uric acid of patients given 50 or 100 r total-body irradiation are being studied by the spectrophotometric method of Praetorius that takes advantage of the specificity of the enzyme uricase. The limitations of nonspecificity and incomplete recovery of uric acid that are imposed by colorimetric methods are thereby avoided.

To date, three patients with chronic granulocytic leukemia, three with lymphosarcoma, one with Hodgkin's disease, one with metastatic carcinoma of the breast, and eight with chronic lymphocytic leukemia have been followed.

In eight of these 16 patients (two chronic granulocytic leukemia, four chronic lymphocytic leukemia, one Hodgkin's disease and one metastatic carcinoma of the breast) there was an increased uric acid within the first week after irradiation.

In two patients (chronic lymphocytic leukemia and lymphosarcoma) there was a rise in serum uric acid without any early change in levels. The patient with lymphosarcoma, for example, had a serum uric acid between 6.2 and 7.7 mg per 100 ml until the twenty-first day after irradiation when it was 9.7 mg per 100 ml. On the twenty-eighth day serum uric acid was 10.4 mg per 100 ml.

There was little or no change in serum uric acid levels in four patients: the one with Hodgkin's disease and three with chronic lymphocytic leukemia.

In a patient with chronic lymphocytic leukemia, one with chronic granulocytic leukemia and one with lymphosarcoma there was no early change in uric acid but rather a general trend to lower levels (from about 8 mg per 100 ml before irradiation to about 6 mg per 100 ml by 28 days after treatment).

We summarize in Fig. 1 the data for four male patients, all with chronic lymphocytic leukemia selected for illustration because of the similarity of the changes in their peripheral white blood cell counts during the first week after treatment. The serum uric acid levels were similar in J.Q. and H.M., showing a slight rise to levels of 7.5 to 8.0 mg between the second and eighth days and then a gradual return to levels around 6 mg per 100 ml. The changes in G.W. and M.W. were quite different, however, both quantitatively and qualitatively.

We conclude, at this stage of the study, that changes in uric acid of serum may follow small doses of total-body irradiation, but these changes are varied in magnitude and time-course and not obviously correlated with diagnosis, clinical effect of the irradiation, or with changes in the peripheral white blood cell count.

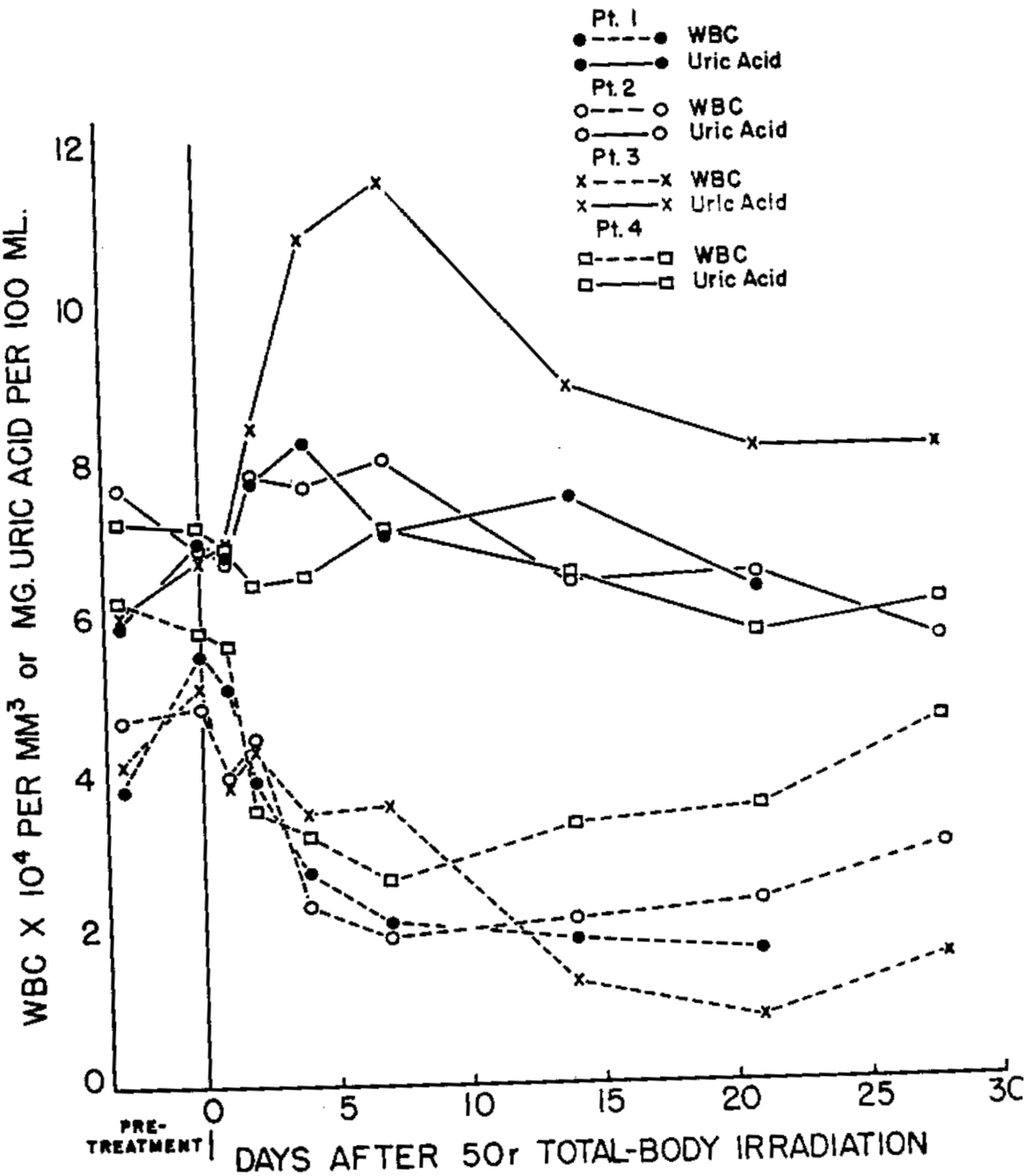

Fig. 1. Serum uric acid and white blood cell count of four male patients with chronic lymphocytic leukemia given 50 r total-body irradiation.

Taurine and Beta-Aminoisobutyric Acid Excretion in Urine from Persons Exposed in the Lockport Incident. (Charles Phipps and A. L. Kretchmar)

Four urine specimens from each of six persons exposed to X irradiation in the so-called "Lockport Incident" were made available to us by the National Naval Medical Center. All persons recovered; but two, S and Lo, had more serious clinical manifestation of injury than the others. Dose estimates were difficult to make and the exposure was not total-body exposure in all cases. Exposure in S was estimated at between 400 and 1500 and in Lo between 400 and 1200 r. The exposure for all subjects was on March 8, 1960 and the urine specimens were collected on March 12, 13, 14, and 15, 1960. For all but one subject the 24-hour volume for March 12 was estimated, since a complete 24-hour collection was not made.

We have reported previously that the effect of irradiation on beta-aminoisobutyric acid excretion is largely confined to the first few days after exposure. Unfortunately, in this group of subjects the first specimen for which reliable volume information is available is on the fifth day after exposure (except subject P, who has a complete specimen on the fourth day). The data for the fifth, sixth, and seventh postexposure days do not suggest any irradiation effect on beta-aminoisobutyric acid excretion as expected since we do not have specimens for the most important first and second days.

The taurine excretion is shown in Fig. 1. We have previously noted that the irradiation effect on taurine excretion in humans appears to take several days to become maximal so that we probably have the important specimens, but without earlier and later specimens, clear trends are not apparent. If it is possible to draw any conclusion from such limited data, it could be noted that (except for the fifth day for S) the two most seriously exposed subjects were excreting taurine at more than 240 mg per day. This compares with data for subjects A (365 rads) and C (339 rads) from the Y-12 exposure who excreted 273 and 262 mg respectively on the fourth postexposure day. On the other hand, there is only one specimen at a taurine level above 220 mg per day among the 12 specimens from the four less seriously exposed Lockport subjects. These data, therefore, support our suggestion based on the Y-12 data that a taurine excretion much above 200 mg per day, particularly four to six days after exposure, is associated with clinically significant irradiation.

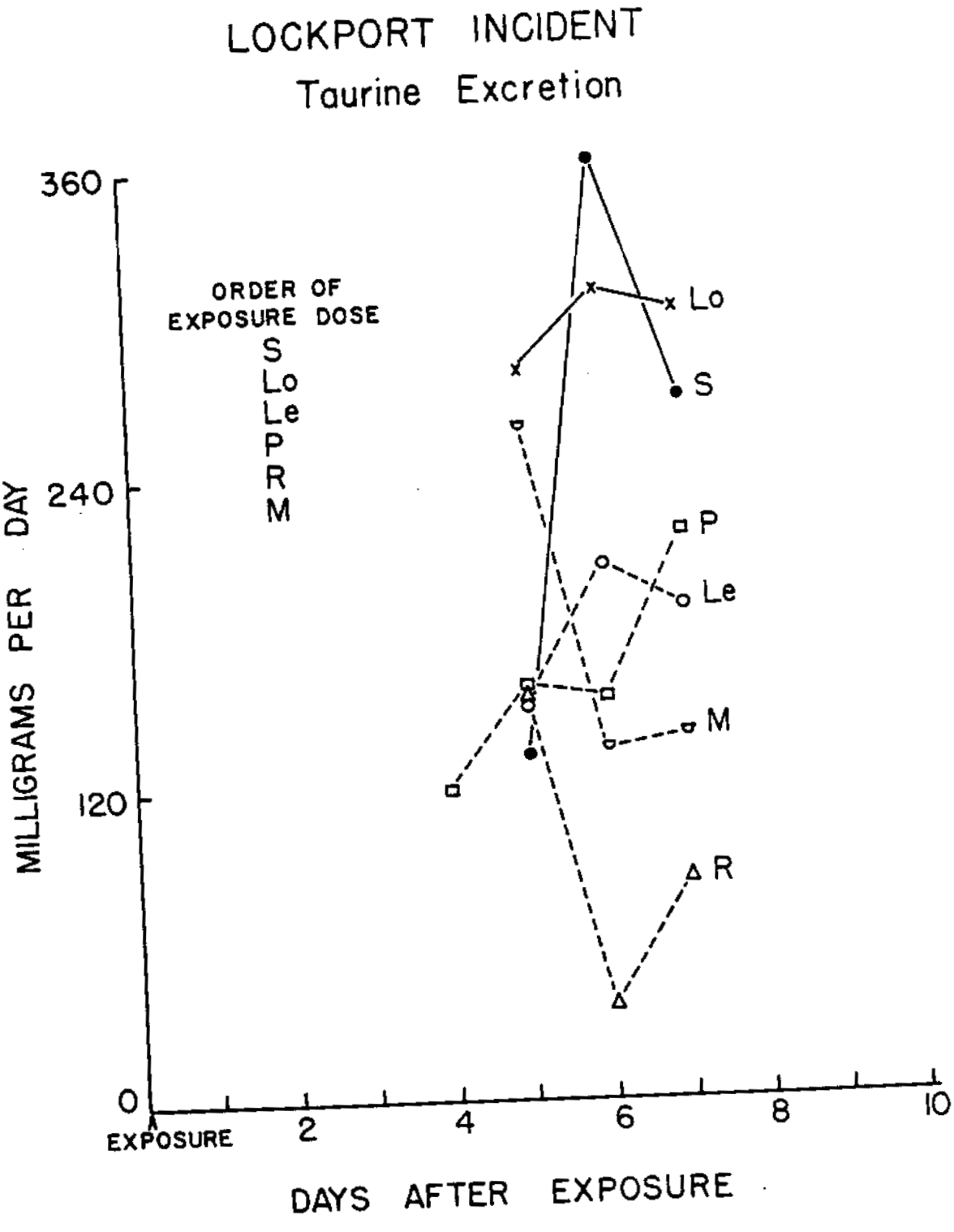

Fig. 1. Taurine excretion in subjects from the Lockport Incident. Earlier specimens were not available.

Effect of Total-Body Irradiation on the Excretion of Taurine and Beta-Aminoisobutyric Acid in Patients with Leukemia. (A. L. Kretchmar and Charles Phipps)

Further information on the effect of irradiation on the amino acid excretion of patients with acute leukemia is summarized in Figs. 1 and 2; for comparison, the results from a patient given nitrogen mustard but no irradiation are summarized in Fig. 3.

In Patient S.W., a 2.5-year-old, quantitative collections of urine were not possible although complete collections were attempted and there is question about the specimen collected on the day immediately preceding irradiation. We have, therefore, expressed the data in terms of taurine or beta-aminoisobutyric acid to creatinine ratio.

It is clear from Figs. 1 and 2 that the increase in excretion of beta-aminoisobutyric acid induced by irradiation is an early effect. We suggest that it is probably related to the destruction of highly radiosensitive cells with breakdown of their DNA. An alternative explanation that has been proposed is that irradiation interferes with DNA synthesis and hence there is failure of utilization of the precursors of DNA synthesis. If the effect were due to failure of utilization of precursor thymine-containing intermediates and with catabolism of unused thymine to beta-aminoisobutyric acid, the decreased utilization is restricted to the first two or three days after exposure, which seems unlikely. Moreover, in both these patients, the beta-aminoisobutyric acid excretion progressively decreased after irradiation to levels that were below the preirradiation levels, while precursor utilization for leukemia cell DNA synthesis undoubtedly was higher before irradiation than during the first 10 days after treatment.

The effect of a large dose of HN_2 in excretion of beta-aminoisobutyric acid is also manifest within the first two days after treatment and has subsided, in the case summarized in Fig. 3, by the fourth day. Again, posttreatment levels go below pretreatment levels.

The effect of irradiation on taurine excretion is different in each of the patients illustrated here. In S.W. (Fig. 1) there is no significant effect of 360 r total-body exposure, a dose sufficiently high to induce an elevated excretion, if the previous results from accident cases can be generalized. We have shown that the level of taurine excretion increases with the ingestion of meat protein and that the level of dietary intake of meat can be closely correlated with the level of 1-methyl histidine excretion. When meat is removed from the diet, 1-methyl histidine excretion drops to trace levels and taurine excretion falls to rather low levels that apparently reflect endogenous metabolism, since very low protein intake does not result in further decrease in taurine excretion. It has been shown in rats that when irradiation is given to animals excreting low levels of taurine because of low dietary protein intake, the effect of the exposure on taurine excretion is nearly

abolished though still demonstrable. In Patient S.W., 1-methyl histidine excretion was at trace levels and we may therefore suspect that taurine excretion was at low endogenous levels before irradiation. Conceivably, increased excretion of taurine was not demonstrable in S.W. for this reason.

In L.H. the increased excretion of taurine was clearly demonstrable. The effect was noted by the fourth day and continued for at least 10 days. On the fourth day this patient excreted 23.4 mg of 1-methyl histidine in contrast to the trace in the urine of S.W.

The effect of mustard therapy in W.J.C. (Fig. 3) on taurine excretion was immediate, and though still elevated at 10 days, was at a lower level than during the first four days after treatment. This is in contrast to the effect of irradiation which, when it was demonstrated, apparently required several days to become maximal.

Studies on irradiated patients and subjects given HN_2 will be continued in an effort to evaluate the significance of these differences.

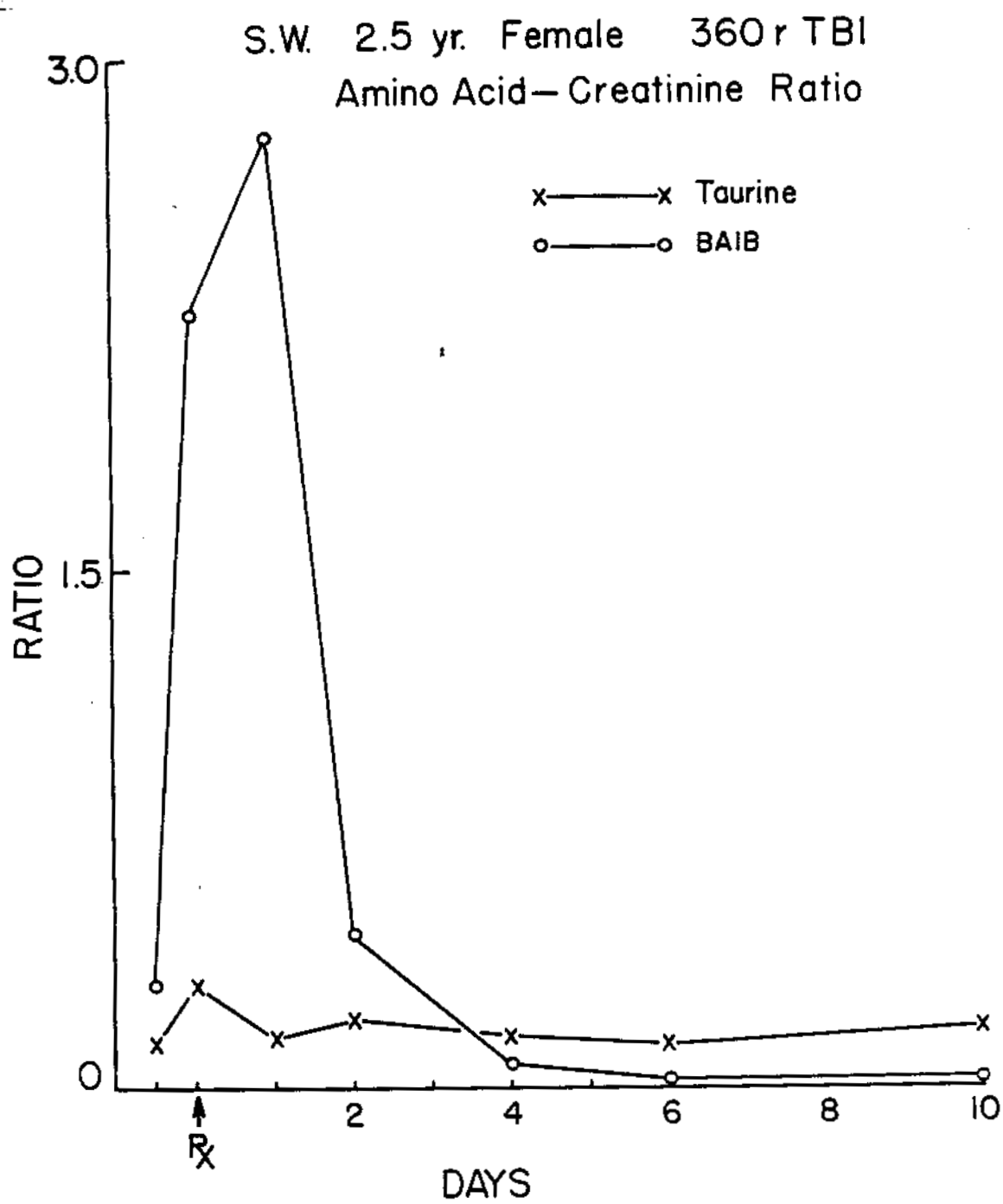

Fig. 1. The excretion of taurine and beta-aminoisobutyric acid by a patient with leukemia given 360 r total-body irradiation.

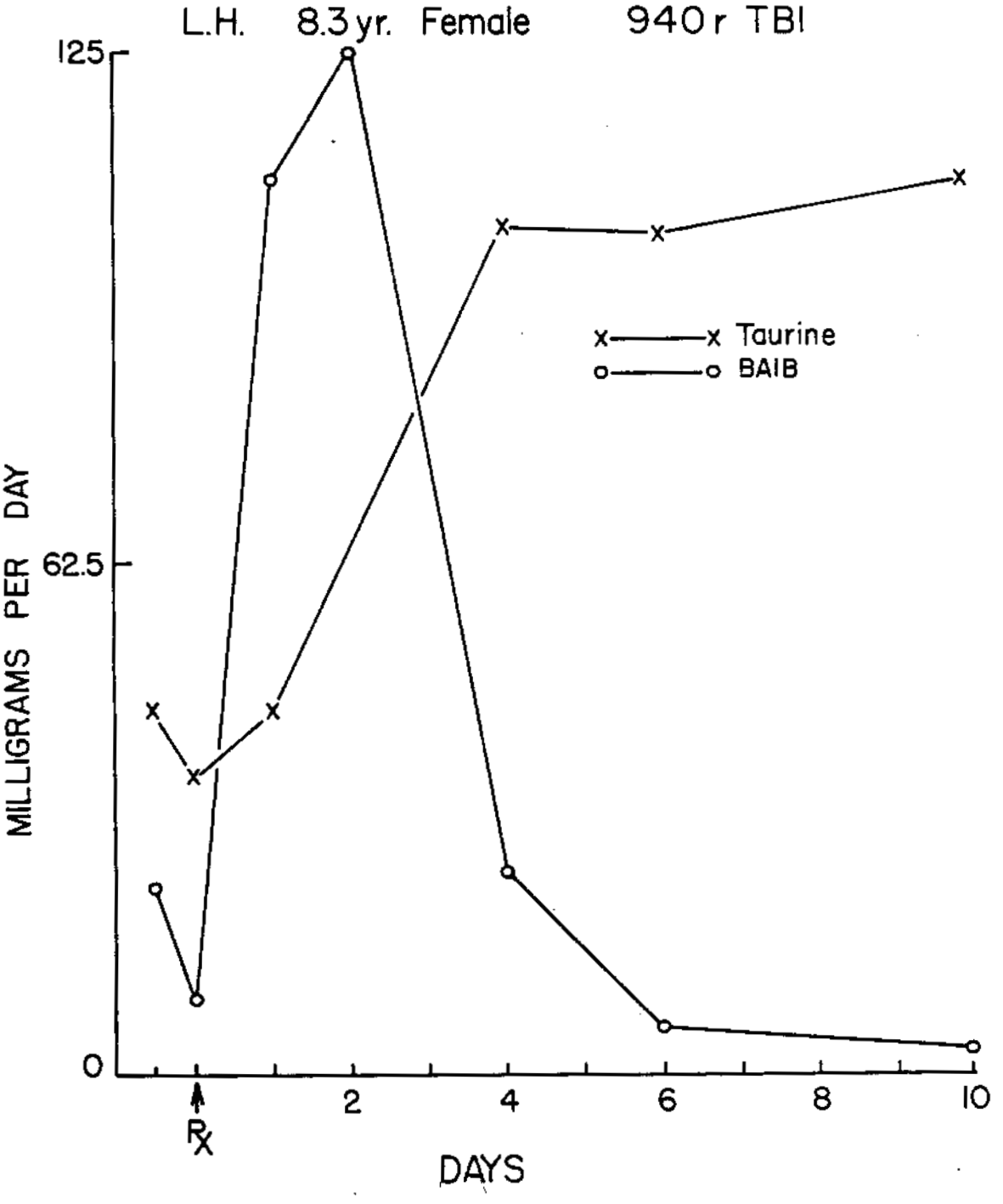

Fig. 2. The excretion of taurine and beta-aminoisobutyric acid by a patient with acute leukemia given 940 r total-body irradiation.

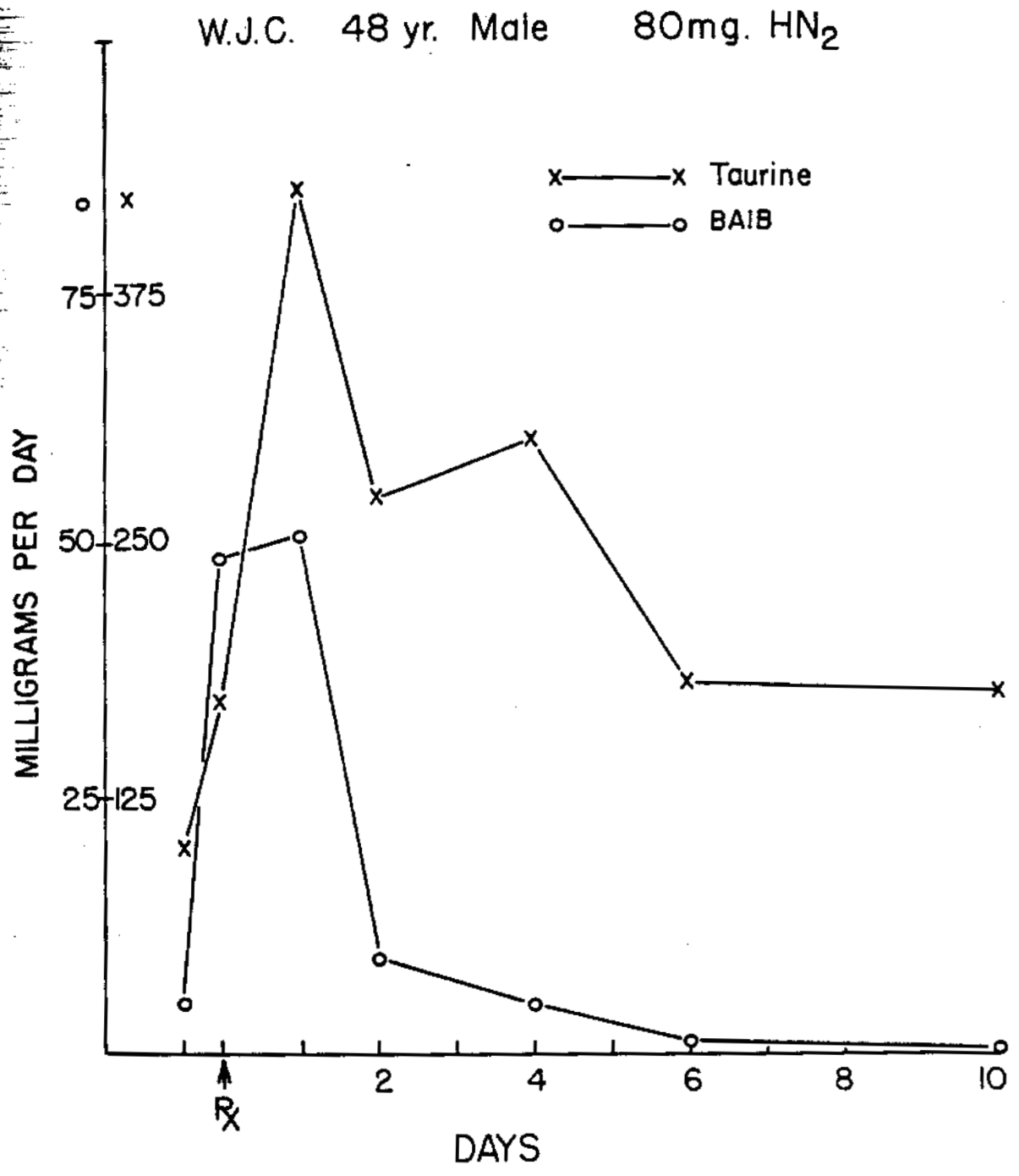

Fig. 3. The excretion of taurine and beta-aminoisobutyric acid by a patient with bronchogenic carcinoma given 50 mg nitrogen mustard in a single intravenous dose.

The Correlation of Liver Weight and Spleen Weight in Bone Marrow Chimeras. (A. L. Kretchmar*)

We have reported (Kretchmar, A.L. and C. C. Congdon, 1961) that bone marrow chimeras exhibit a hepatic enlargement at 12 days after irradiation and bone marrow treatment. This enlargement occurred in mice given genetically compatible cells but was more prominent in animals given homologous cells. Is this enlargement of liver related to changes that also occur in weight of the spleen?

A dominant feature of the histopathology of mice irradiated and treated with bone marrow is hemopoietic regeneration. This is maximal between the sixth and twelfth days after exposure. In animals with homologous marrow grafts there is, in addition, proliferation of primitive lymphoid cells (Congdon and Urso, 1957, Congdon et al. 1958). The spleen rapidly increases in weight during this interval and reaches its maximum size on about the tenth to twelfth day (Congdon, C.C. and I. S. Urso, 1957). There is massive hemopoiesis in these spleens (Congdon, C.C. and I.S. Urso, 1957) as well as the immune tissue (lymphoid) response that occurred in the white pulp of spleens from animals given homologous cells.

Lajtha and Vane (1958) have suggested that the liver makes precursors for nucleic acid synthesis in hemopoietic cells. We found biochemical changes in the enlarged livers of 12-day bone marrow chimeras that could be related to this activity (Kretchmar, A.L. and C.C. Congdon) If the changes in liver size and aspects of its chemistry are related to hemopoiesis, a correlation between spleen weight and liver weight might be expected to exist. We find that this correlation is indeed present.

The experimental conditions were as described in the previous report (Kretchmar, A.L. and C.C. Congdon, 1961). The mice were (C57L x A)F females given bone marrow cells from either the same strain, isologous bone marrow (IBM), or from (101 x C3H)F homologous bone marrow donor mice (HBM). The donor mice were also female. The total nitrogen of the liver was determined by a micro-Kjeldahl procedure (Ma and Zuazaga, 1942).

In Table 1 we summarize data from two experiments. The increased weight of liver (column 5) occurs in mice given isologous marrow but is greater in animals given incompatible cells. The spleen weight (column 6 is also greatest in the latter group and the order of increasing spleen weight and increasing liver weight is the same. There is no significant adrenal hypertrophy. The total nitrogen content (last column) is increased in the irradiated groups and treated with bone marrow; the increase is greatest in the animals given homologous cells.

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* This work was done in collaboration with C. C. Congdon, Biology Division, ORNL.

TABLE 1

Average body weight, organ weights, and total nitrogen content of the liver of (C57L x A)F, female mice 12 days after X irradiation (900 r total-body) and I.V. injection of 40 x 10^6 bone marrow cells. The bone marrow cells were flushed with Tyrode's solution from the femurs of (C57L x A)F female animals (IBM) or from (101 x C3H)F females (HBM).

Group	Expt.	Number of Animals	Body Weight g	% of Body Weight			Total Nitrogen of Liver mg
				Liver	Spleen	Adrenal	
Sham*	I	15	22.6	5.3		0.028	24.3
	II	15	21.7	5.1	0.35	0.021	
IBM	I	24	21.1	6.4		0.023	31.6
	II	25	21.8	6.9	0.67	0.019	
HBM	I	22	21.8	7.3		0.029	32.7
	II	17	21.8	7.1	1.36	0.023	

*Sham irradiated mice given Tyrode's solution instead of marrow cells and caged as for the irradiated (IBM and HBM) groups after treatment.

The coefficient of correlation between the weights of liver and spleen was calculated (Dunn, H.L., 1929) from the data for individual animals. The correlation was better in the irradiated bone marrow treated groups than in the sham control group, and it was better in the HBM than in the IBM group (coefficient of correlation was 0.43, 0.61 and 0.82 for sham, IBM and HBM groups respectively). That this increased correlation is not nonspecific is demonstrated by the experiment summarized in Table 2. A marked splenic enlargement was induced in unirradiated mice by the daily intravenous injection of isologous bone marrow cells. Although these spleens were large, hemopoiesis was not increased and liver weight was normal, in contrast to the massive hemopoiesis in the spleens of the irradiated bone marrow treated mice that show increased liver weight. The correlation between the weight of liver and spleen of the animals in the experiment of Table 2 was similar to that of the sham group (coefficient of correlation 0.39).

TABLE 2

Average body weight, liver weight, and spleen weight of (C57L x A)F female mice after 11 daily injections of 100 x 10^6 isologous bone marrow cells. The animals were sacrificed on the twelfth day after the first injection and were not irradiated.

Number of Animals	Body Weight g	Liver Weight % of Body Weight	Spleen Weight % of Body Weight
5	20.8	5.7	1.07

These results indicate a correlation between the weight of liver and spleen in bone marrow chimeras 12 days after irradiation and injection of bone marrow. This is at about the peak of hemopoietic regeneration after the irradiation induced aplasia. The spleens of bone marrow chimeras are known to participate prominently in formation of blood cells at this time (Congdon, C.C. and I.S. Urso, 1957). This evidence supports the thesis (Lajtha and Vane, 1958) that there is a relationship between hemopoietic activity and liver function. The increase in the nitrogen content of the liver and in the size and basophilia of the individual liver cells (Kretchmar, A.L. and C.C. Congdon) is also compatible with this view. From studies of liver changes in tumor bearing animals (Wu and Bauer, in press; Sherman, Morton and Mider, 1950) and from results (unpublished) that suggest a further increase in weight of liver at the time of proliferation of lymphoid cells in the spleen of animals given homologous bone marrow cells, it might be suggested that there is a relationship between proliferating tissue and liver function in general and that hepatic synthesis of precursor material for tissue components is not restricted to cells of the hemopoietic system.

Immunology (N. Gengozian)

The effect of radiation on the immunologic mechanism of humans represents the primary area of study. The depressive effect of both total-body and high local doses of radiation on the capacity of persons to produce humoral antibodies and evoke a delayed type of immunologic response will be studied on patients receiving such therapeutic treatment. Defining clearly the immune mechanism of the irradiated patient becomes important when homologous hematopoietic tissue is to be transplanted for replacement-type therapy. To further facilitate analysis of the immune system, tissue culture studies will be used in an attempt to analyze the effect of various types of therapeutic treatment on the antibody-forming potentialities of cells obtained from patients. Also, as an adjunct to human radiation-immunology studies, a major experimental program with marmosets is planned, with emphasis on the effectiveness of hematopoietic tissue therapy for radiation-induced injur

Clinical Use of ORNL Research Scanner. (Clinical Staff)

In cooperation with the Oak Ridge National Laboratory, a research scanner designed and fabricated at ORNL was put into clinical use at the Medical Division in January 1960. The greatest improvement offered by this instrument is provided by the new collimator made up of a conical shaped gold insert containing 37 tapered hexagonal holes. This results in high sensitivity while maintaining good resolution. The collimator has a tungsten side shield, which reduces detection of radioactivity from room background and areas of the patient's body other than that on which the detector is directly focused.

The greatest clinical use of this scanner has been in studying patients with metastatic thyroid carcinoma. In these patients the scanner has proved to be definitely superior to the equipment previously used. This scanner records areas of only slight concentration of iodine-131 and in so doing has produced some problems in interpreting the scans. The physiological concentration of iodine of varying degree in various organs and tissues has not been troublesome in interpreting scans previously, because for the most part these areas of relatively weak concentration were not recorded. When the new scanner was first put into use the detection of these new areas posed the question, in some instances, whether these findings represented metastatic disease or physiological concentration of iodine. The significance of some of them has become apparent only by the frequency and consistency of their presence in a number of patients.

Some of these new areas of activity that have been detected are as follows:

Salivary glands and saliva. With the old scanning equipment it was not unusual to see areas of concentration of the iodine in the region of the mouth after large therapeutic doses of iodine-131 (100 millicuries). This finding was shown quite conclusively to result from the salivary content of iodine-131. For some reason, however, the concentration of iodine in the salivary glands themselves was detected only occasionally. The explanation for this is not clear because the salivary glands are supposed to concentrate iodine to a ratio of approximately 25:1 in comparison with the plasma level. With the new research scanner the concentration of iodine in the mouth region is uniformly detected also. In addition, when therapeutic doses of iodine are used, concentration of iodine in the salivary glands themselves is detected in nearly all patients. With smaller doses of iodine-131 the activity in the salivary glands is sometimes, but not always, detected.

Concentration in the neck. Another rather constant finding with the new scanner has been a linear area of increased concentration of iodine-131 extending slightly above and below the suprasternal notch and a little to the left of the midline. A completely satisfactory explanation of this has not been obtained as yet. Possibilities considered

have been iodine-131 in mucoid secretions in the trachea and iodine-131 from swallowed saliva in the esophagus. If either of these explanations is correct, the question arises as to why the entire length of the trachea or esophagus is not outlined rather than just a short segment at the level of the suprasternal notch. In spite of the absence of a definite explanation, the frequency and constancy of this finding in different patients mitigates against its representing a pathological process.

Chest findings. With both the old and new scanner, definite discrete areas of iodine-131 concentration have been detected in the mediastinal and hilar lymph nodes as well as the peripheral lung fields. X-ray films of the chest have usually given substantiating evidence of metastatic lesions in these cases. With the new scanner two additional patterns of distribution of radioactivity have been seen. In some patients a diffuse increase in radioactivity in the midline, or mediastinal area, has been seen, and this often merges with an area of increased activity corresponding to the location of the heart. This might well be explained by the body-background activity in the great blood vessels and solid structures in the mediastinum, whereas the adjacent peripheral lung fields containing air have less activity and thus cause the mediastinal area to stand out in contrast. In other patients, however, a pattern of diffuse increase in activity has been seen over the peripheral lung fields with the mediastinal area containing a much smaller amount of activity comparatively. In these patients X-ray films have not shown metastatic lesions, although this does not rule out the possibility of very small, miliary-type metastases. Iodine-131 in the secretions of the smaller bronchioles has been suggested as a possible explanation for this finding. Some credence is lent to this possibility by the definite demonstration of iodine-131 in tracheal and nasal secretions.

Abdominal findings. Abdominal scans made with both the old and new scanner have invariably shown an area of increased concentration of iodine-131 corresponding to the location of the stomach, undoubtedly representing iodine in swallowed saliva and gastric secretions. With the new scanner, however, it is not unusual to detect in addition a pattern of distribution corresponding to the duodenal loop. In some patients an area of diffuse activity has been detected in the right upper quadrant of the abdomen corresponding to the location of the liver. This probably represents the relatively small amount of iodine that is normally conjugated in the liver. With both scanners, areas of increased activity scattered throughout the abdomen are detected. Repeat scans at later intervals show these to change location, and they disappear after cleansing enemas. These are definitely due to iodine in bowel contents. In one patient such an area of activity, originally thought to be due to bowel content, did not move on repeat scans and was ultimately shown to represent a bony metastasis in the ilium.

The improvement offered by the new scanner in the detection of small amounts of radioactivity has led to the finding of areas of

metastatic disease that would probably have been missed otherwise. It has also caused some change in interpretation of the end results obtainable with iodine-131 therapy in some patients. These patients are shown by the new scanner to have persisting areas of very slight concentration of iodine-131 in previously known areas of disease. Further therapeutic doses of the radioisotope seem to be ineffective in eradicating these slight residuals. Thus it seems a point is reached where only a very small amount of residual tissue is left that concentrates sufficient iodine-131 to be detected by this scanner, but an insufficient amount for significant therapeutic radiation effect. It is quite probable that such areas of slight concentration were not detected with the older scanning equipment and, hence, the patient was considered to have no residual iodine-131 concentrating tissue.

Studies of Selected Neoplasms. (Clinical Staff)

Carcinoma of the ovary. The Medical Division has continued to study patients with carcinoma of the ovary. Repeated surgical procedures prove worth while in some patients who at first laparotomy appeared to have hopelessly extensive disease. It is still not possible to predict at the outset which patients will have a rapid course, without much benefit of therapy, and which will be helped by vigorous treatment. The most successful form of therapy is surgery. Postoperative intraperitoneal colloidal gold-198 has been given to quite a number of these patients. Its value in controlling further growth and extension of the tumor is difficult to prove. It does appear to control effusions in some patients. It may contribute to the formation of diffuse adhesions, which add to the difficulty of subsequent operations. External radiation therapy is not used indiscriminately for carcinoma of the ovary but is sometimes directed toward specific lesions previously seen at operation. Intravenous nitroge mustard therapy has been seen to have definite value in selected patients.

Carcinoma of the thyroid. The study of carcinoma of the thyroid has continued to be profitable and revealing, but our current concepts of the disease have altered significantly. A few years ago the clinical staff believed that only a minority of patients with this disease had lesions that would show any significant ability to concentrate the radioisotope, and for all the rest, radioiodine was of no value whatsoever. We now find that in the majority of patients the lesions show at least some "uptake" of the radioisotope. Even if this is not of a level to offer effective therapy, it may allow effective diagnostic studies.

The reasons for this change may involve some alteration in the criteria used by referring physicians in selecting patients to be sent to us -- i.e., elimination of the more anaplastic lesions. Larger factors, we believe, have been alterations in methods of study and improvements in equipment. Much larger test doses are used now than formerly in patients who have had their normal thyroid tissue removed--- currently 1 to 5 millicuries. Efforts to locate lesions are made after most of the unbound isotope has been excreted --- usually at least three days after the dose is given. The much improved area scanner developed at Oak Ridge National Laboratory is the chief instrument used for study, although the linear scanner has been of special value in some situations.

We continue to see encouraging results from our particular approach to patients with papillary and follicular carcinoma of the thyroid without distant metastases. This involves, first, total thyroidectomy and resection of enlarged lymph nodes without radical neck dissection, then, test doses of iodine-131 and further surgery for areas of radioactivity found on scan. Surgery is given up if the functioning tissue cannot be located even with the surgical probe counter, or if the area of activity is nonresectable without excessive functional impairment i.e., lesions involving the larynx itself or located at the site of the

entrance of the recurrent laryngeal nerve. If functional foci are left after all reasonable surgical attempts, therapeutic radioiodine is given. Up to the present, none of the patients in this group have been found to have distant metastases or other evidence of failure referable to the omission of radical neck dissection.

The new detection methods have presented some special problems. Patients previously shown for several years to have "blank necks" are now sometimes found to have foci of radioactivity. This is not because of new lesions but because of better detection. Also, we have found that some areas of iodine-131 concentration cannot be eradicated by iodine-131 therapy --- slight uptake persists and seems refractory to further therapy. This was not entirely unknown previously but appears more frequent with the newer methods.

Studies with iodine-132 substituted for iodine-131 met with serious problems because of the very short half life and difficulties in counting of the very strong gamma emission in vivo. Even so, these studies have given some help in the timing of the therapeutic measures.

Surgery in Patients with Leukemia and Lymphoma (Abstract of paper submitted for publication). (Francis T. Oda, Gould A. Andrews, Robert R. Bigelow, Beecher W. Sitterson and Robert W. Buxton)

During the past year a review has been made of patients treated at the Medical Division who had leukemia or lymphoma and who required surgery. A review of the literature on this subject was also made, which revealed relatively little information on the general topic, although certain specific operations, especially splenectomy, have had considerable attention.

The ORINS report covers 22 operations in such patients. The operative mortality was not excessive, but the incidence of complications was much greater than would be expected in patients without the underlying hematopoietic disorder.

The paper discusses diagnostic surgical procedures, resection of localized lymphomas, splenectomy, and surgery for associated diseases. Consideration is given to preoperative preparation and handling of specific complications. Abdominal operations done for any purpose allow a good opportunity for evaluation of the extent of involvement with lymphoma.

The result of the experience with this problem leads away from a recommendation of extreme conservatism in these patients. Although surgery undoubtedly carries special risks associated with the leukemia or lymphoma, these risks can be minimized by suitable management, and the benefits of surgery can often be achieved. The proper handling of such patients requires balanced judgment and careful timing by both the hematologist and the surgeon, and they need to work together.

Recent Projects. (R. M. Kniseley)

Platelet life-span studies. Four patients with high thrombocyte counts as a manifestation of polycythemia vera or chronic granulocytic leukemia have been studied after injections of sulfur-35. Information on the life span is being collected by measuring in the beta liquid scintillation counter the sulfur-35 activity incorporated into platalets. Detailed results will be presented in the Report for 1961.

Eosinophil life span. One patient having Hodgkin's disease, who developed a high eosinophil count, was given a large dose of sulfur-35. Differential counts of microscopic autoradiograms will be carried out in an attempt to estimate the eosinophil life span. It previously had been reported that autoradiograms of bone marrow in animals show uptake of sulfur-35 in eosinophil series. No results are available for reporting at this time.

Clinical Teletherapy. (F. Comas, M.D.)

Cancer of the bladder. A series of patients suffering from carcinoma of the urinary bladder is being studied with the aim of determining whether an exploratory laparotomy preceding a course of radiation therapy is of value in improving the results in the treatment of this disease. The reasoning is that by exposing the tumor one is better able to determine its size and the presence and location of lymph-node metastases; this permits a more accurate treatment planning than if the size and location of the tumors are determined only by physical examination and cystoscopy aided by X-ray studies. Up to now 11 patients have completed treatment. Of those, four have died with persistent disease and one is living but with known persistent disease. The others remain well to date, but most of them were treated so recently that it is not possible to reach any conclusion about their final survival

Irradiation of the spleen. Seven patients had their spleens irradiated to study the effect of this form of treatment on secondary hypersplenism. The diagnoses were chronic lymphatic leukemia, three cases, and one case each of cirrhosis of the liver, myelofibrosis, lymphosarcoma, and subacute myeloid leukamia, which followed polycythemia vera. It was aimed to deliver a spleen exposure of 2000 rads in 10 days, but in two patients small exposures had to be given because of the large size of the spleen. Therapeutic response was gauged by changes in blood cell values and chromium-51 red cell half-time survival. Six out of the seven patients showed no beneficial effects from this form of treatment, in that the hemolysis continued unabated or the white blood cells and platelets failed to rise. In the seventh patient, who had chronic lymphocytic leukemia, a slight prolongation of his chromium-51 half-time survival was noted. It is not known whether this was due to the treatmen administered or was a spontaneous temporary remission. It is concluded that irradiation of the spleen, even to relatively large doses, is of no value in the treatment of secondary hypersplenism. Nevertheless, some of the patients derived some symptomatic benefit from this treatment because

of the shrinkage of the spleen. Some of them were subsequently treated with corticosteroids or splenectomy, which was usually followed by improvement in the blood values.

Isodose studies. Isodose curves were obtained for all collimator sizes of one of the cobalt-60 machines. They were measured with an ionization chamber attached to a platform, which can be moved in two perpendicular directions by means of Selsyn motors. This machine had been designed and built by Harry E. Kimble, from the ORINS Technical Services Department, some years ago. It was adapted for our use with minor modifications of the original design. Although point by point measurements need to be taken, a complete isodose curve can be determined in one to two hours depending on the size of the field. The instrument is reliable and trouble-free. The accuracy of the curves depends, of course, on the size of the ionization chamber. Two chambers were used, one having an air cavity of 0.2 cc and the other one an air cavity of 1 cc. Although the former gave slightly better definition of the edges of the field, both ionization chambers performed satisfactorily and gave usable information for this specific purpose.

The T3 Tests. (Bill M. Nelson, M.D.)

Clinically useful in vitro technique for investigation of the transport of thyroid hormones in the circulating blood. In 1957 Hamolsky, Stein, and Freedberg introduced a simple test for thyroid function. This consisted of the incubation of whole blood with iodine-131-labeled triiodothyronine (T3); it was shown that the red cells took up increased amounts of tracer T3 from blood of patients with thyrotoxicosis and decreased amounts from blood of patients with myxedema. The method depends upon the strong affinity of certain blood proteins for thyroxin, the weaker affinity of these proteins for T3, and the ability of red blood cells to take up the T3 not bound to protein. Although the hematocrit enters into the calculations of the results, the red cells themselves are not affected by thyroid disorders. The test is of most value in the diagnosis of thyroid disorders, reflecting variations in the levels of circulating thyroxin. It is also helpful in obstetrics, since the low red cell uptake characteristic of pregnancy rises to normal levels very soon after fetal death. Low red cell uptakes are also found after the administration of estrogens, due to an increased capacity of the serum proteins to bind thyroxin and T3.

At the Medical Division most of our experience has been with modifications of the T3 test that substitute ion-exchange resins for the red blood cells. The use of resin particles obviates irregularities caused by variations in the hematocrit of whole blood samples and permits the storage of frozen sera when the test cannot conveniently be performed as soon as the specimen is obtained. A variety of technical problems has been investigated, including a survey of several types of resins, the effect of temperatures on the equilibrium reaction, and the number of washings needed. We have found the method of Sterling and Tabachnik (J. Clin. Endocr. 21, 456, 1961) to be reliable in distinguishing normal

from abnormal sera, but have had considerable variations in resin uptake values on replicate samples tested on different dates. These discrepancies are most evident when new lots of iodine-131-T3 are obtained from the pharmaceutical suppliers, and certain lots have been found to give very low resin uptake values. Most of the discrepancies, however, are eliminated when the results are expressed as an index with reference to replicates of pooled serum run as controls with each day's work. During the past year our experience with samples from more than a hundred normal and abnormal persons has shown in general a good correlation with the clinical state.

The test is of particular value when the results of the thyroid uptake of iodine-131 and the serum protein-bound iodine are invalidated by the previous administration of iodides. Moreover, serum samples can be mailed to the laboratory and the patient is not given any radioisotope or exposed to radiation.

The simplicity of the method facilitates its use in clinical isotope laboratories.

MEDICAL PHYSICS

D. A. Ross and A. C. Morris, Jr.

Instrument Development.

Total-body irradiation facility. The total-body irradiation facility at the Medical Division was released for clinical operation in May 1960. The purpose of this irradiator is to allow a study of the treatment of leukemia and other diseases with doses of total-body irradiation. In general, the facility consists of a cubical room of 8-foot dimensions, with a centrally suspended bed. Eight 500-curie, cesium-137 sources are in the side walls of this room and are controlled by a pneumatic panel from another maze-connected room. Each source is beamed at the treatment bed from a different angle and is directed to irradiate the whole length of the bed. A means of changing the exposure rate from each source consists of a set of filter slides of varying thickness, which may be positioned in front of each source port. A total of 32 different filter thicknesses (and exposure rates) is possible for each source.

Before releasing this facility for clinical use a series of measurements was performed to study the various radiation parameters. The basic questions to be answered by this series were as follows:

A. What are the spectral characteristics of the background radiation in the total-body irradiation room when its radiation sources are not present?

B. Is there any radiation leakage into this room from adjacent teletherapy rooms?

C. With the sources in ON position is the radiation exposure rate uniform throughout the patient-occupied volume just above the treatment bed? If it is not, can it be made more uniform?

D. What spectral changes result from changes in attenuating filter thickness?

E. What exposure rates are available for treatment with each of the 32 possible attenuating filter combinations?

The measuring instruments gave two distinct types of information in this experimental series: (1) the data having to do with the rate (or quantity) of exposure; and (2) that relating the spectral shape (or quality) of the radiation. For rate measurements a Victoreen Ratemeter with a model No. 605 probe was used as a detector.

Spectral detection was accomplished by a 2" x 2" NaI (Tl) crystal coupled to a single-channel analyzer. A set of eight, 2-millicurie, cesium-137 sources, exhibiting the same spectral qualities as the 500-curie therapeutic sources, were used in the energy measurements. This was necessary because the use of the NaI crystal in the intense therapeutic radiation field would hopelessly jam the electronic analyzing circuits.

The following results were obtained in the investigation of questions mentioned:

A. Natural background radiation in this facility without any cesium sources in place was at a very low level. A slight peak at 1400 kev was probably the result of potassium-40 in the concrete walls. A broad peak starting around 600 kev was probably the conglomerate result of primary and scattered radiation from thorium, uranium, potassium, radium, and other active constituents in the building materials.

B. No leakage was detectable from three cobalt-60 teletherapy units in adjacent rooms turned ON in the direction of this facility.

C. After the radiation sources were in place initial measurements made with the ion chamber suspended over the bed showed a 30 per cent exposure rate fall-off toward each end of the bed when compared with the bed-center reading. Insertion of pyramidal beam-shaping filters in each source port reduced this exposure variation considerably. The exposure variations throughout the patient-occupied volume above the bed surface were not found to be large enough to require further compensation.

D. Spectral evaluations with increasing filter thickness showed an exponential decline in the primary 660 kev radiation from the cesium-137 sources. Primary radiation could not be detected at the higher attenuation levels. Compton scatter regions decreased rapidly in the first few filtrations, but with further attenuation they remained the only detectable exposure component at the highest attenuations.

E. A variation of exposure rate in 32 steps from 286 r/hr to 0.18 r/hr was found possible by using the 32 filter combinations. This information was processed to form a chart relating filter setting versus time versus total dose for use by clinical personnel operating the facility.

With the completion of these initial investigations the facility was released for clinical operation. Subsequent studies will be directed toward exposure rate and spectral investigations in various phantoms.

Whole-body-counting facility. As part of the preparation for building a whole-body-counting facility, in order to carry out a host of experimental and research observations on patients and normal people, and a great deal of time and thought on the part of Medical Physics personnel has been devoted to the various design problems to be encountered in building this installation. The hope is that the detectors will be at least as sensitive as others already in existence -- that is, sensitive enough to record the "normal" radioactivity that is now present in everybody. On the other hand, when radioisotopes are administered to patients, doses are not always restricted to low-level ones, and consequently attempts should be made to provide for hot patients as well as tepid ones. It happens that the hot patients are easier to deal with, for the problem of shielding then becomes of rather minor importance, and consequently pending the negotiations for the low-level whole-body counter, a high-level counter may be set up, using the 3" crystal and RIDL analyzer mentioned in an earlier paragraph.

Designs for the low-level facility center around two main types of detector: 1) a sodium iodide crystal system whose high resolution will permit spectral analysis of the radiation emanating from the patient, and 2) a "tank" detector system having poor energy resolution but considerably higher sensitivity, which permits a shorter counting time. Initial plans emphasized the design of the tank counter but recently it has become likely that only one of these counters can be built, and if there is to be only one the crystal type will be preferable.

A crystal counter will require a multichannel analyzer (200 channels or more) and consequently considerable thought has been given to the type of analyzer to be procured. The earlier analyzers were all of the vacuum-tube type, but within the last year or two transistorized analyzers have become commercially available, and they have now been in service long enough so that some idea as to how reliable they are is now possible. The staff have become pretty well convinced that when an analyzer is bought, it should be of the transistor type, for they are much smaller and require a great deal less power to operate. The power itself is not expensive, but it might well be expensive to get rid of the 2 or 3 kilowatts of heat that a hard-tube analyzer produces. In order to become acquainted with the various commercial analyzers, members of the staff have made a fair number of trips to other laboratories and have talked extensively with the people operating whole-body counting facilities in them. During the past year consultations of this kind were made at Brookhaven, at Vanderbilt, and at the Chicago home plants of both RIDL and RCL. The staff are maintaining an active interest in the progress of these multi-channel pulse-height analyzers, so that they will be ready to start work immediately when the time comes.

Surgical probe for beta emitters. The GM tube surgical probe prototype model was completed this year. The detector unit consists of a small, thin mica-window GM tube mounted in a holder to which various collimators may be attached. The tube holder is about the size of a large cigar and is connected to an electronic supply and audio unit through an 8-foot length of coaxial cable. The tube and holder may be sterilized for surgical use by immersion in Zephren solution.

Collimators for this device include various shapes and sizes for beta detection as well as a heavy tungsten gamma collimator. Three separate audible outputs are available from the electronic unit - pops, peeps are available as well as a "howler" output, which increases the pitch of an audio tone as the count-rate increases.

This GM probe system is very sensitive to beta radiation and a concentrated point containing less than 1 microcurie can be detected. The disadvantage of beta detection in the surgical situation is that it does not give good results from areas of concentration that lie deeper than just below the exposed tissue surface. The heavy gamma shield, on the other hand, does allow a better indication from deeper concentrations but requires a much higher activity level for a good response.

Iodine-125 might be used with this probe. A less massive collimator could be used having a thin shield to exclude beta rays. The 35-kev gamma radiations from this isotope would allow some depth of investigation, pass through the beta shield, and would enter at approximately the proper gamma energy level for maximum GM tube efficiency.

It is hoped that this probe can be given a surgical trial in the near future on both iodine-131 and iodine-125 patients to ascertain its degree of usefulness. If this first model does give good results the electronic circuitry will be reduced in size and perhaps transistorized into a working instrument.

Oximeter. Problems relating to the instrumentation of oxygen tension in small mammals fall into three general areas and it is felt that progress has been made in each.

Fabrication of active and reference electrodes for use in living tissue requires a careful selection of materials. The electrode material must be selected carefully to avoid generation of unwanted electrochemical potentials in the measured medium. Platinum and silver have been used for the active electrode while calomel and silver-silver chloride have been examined as reference elements. Best results were produced when using the platinum electrode in conjunction with either of the reference electrodes mentioned. Another quality of the active electrode is that it must be highly insulated from the surrounding tissue everywhere except at the bare tip. The insulation requirements for these probes are strict because the current flow is low, in the order of 10^{-8} amperes, and because the physical size of the probe for animal experimentation must be kept small. Materials tried for insulation were varnish, polyethylene,

teflon, glass, and embedding plastic. The latter three materials have given acceptable results.

Experimental methods seem to be as numerous as published research papers in this field. Several of the reported methods have been investigated with varying results. Constant DC potentials were applied to the electrodes, which seemed to produce undesirable polarizing effects. Trials with voltage pulses of varying duration were made with or without rest or shorting periods and depolarizing counter-currents. An AC system and pulsating DC system have recently been tried with dubious results. The best method examined so far uses a constant or pulsed DC voltage and our future work will be directed along these lines.

The third area of oximeter development concerns the signal amplifying and recording systems. The signal levels obtained are low and several amplifier circuits constructed in our electronic shop were not found stable enough for this application. A Hewlett-Packard Model No. 412A voltmeter was purchased for this use and performed well. The original Brown recorder was replaced by a Varian recorder, which is less bulky and has given good service.

In general, a number of problems have been overcome this year in the oximeter system, but several other rather imposing ones must be worked out before the system is useful.

Dose checker. A device is needed for making a final check of isotope activity in therapeutic and diagnostic doses just before administration to our patients. This instrument would not have to be highly accurate and would serve only to ensure that the dose level was in the proper range. It should be installed in or near the radiation storage vault where the doses are prepared, and where some are administered.

A device of this type was completed this year. It consisted of three halogen-filled GM tubes mounted inside a standard 8" x 4" x 2" lead brick. The output from these tubes was coupled to a transistor univibrator driving an indicating meter. Sensitivity of this instrument was adequate for diagnostic doses when operated in the electronic shop. However, when moved into the storage vault, the meter was driven to full-scale by the high background present and was useless at diagnostic levels. Either we must design a new detector with heavy shielding or else move the dose checker to another location -- both alternatives having certain disadvantages.

Clinical Evaluation.

"Brain Scanner". The ORNL research scanner, with its gold-insert collimator and heavy, tungsten lateral shielding, was lent to ORINS Medical Division about a year ago and has been used whenever possible since that time not only for brain scans but for routine area scanning

as well. As had been anticipated, the brain-scanning project ran into a number of practical difficulties. It is being carried on, although on a somewhat reduced scale, particularly with the aim of discovering and evaluating better isotopes for brain-scanning purposes and the relative value of the different ones now being used. Current impressions, subject to change, are that brain scanning, as practiced here, seldom gives misleading information; on the other hand, the percentage of patients studied who have positive findings is rather low.

Linear scanner. The linear scanner installed a couple of years ago is being used regularly on almost every patient who receives a gamma-emitting isotope and is yielding useful information. The procedure is used especially on the patients who receive therapeutic doses of iodine-131 and in these patients the presence of iodine-containing fecal masses complicate the abdominal part of the scan to an annoying and often puzzling degree. The virtues and vices of this diagnostic machine are being explored further.

Scintillation crystal surgical probe. This ORNL-developed device has been valuable to the surgeons in ferreting out radioactive metastases from the thyroid gland, and it has established a place for itself in the operating room equipment. The particular device used here, which is the first ever built, has been somewhat troublesome during the past year in that it has required excessive servicing; probably an additional surgical probe, perhaps using transistorized circuitry, should be built. If this problem is to be tackled, it should be done in collaboration with Mr. C. C. Harris and his colleagues at the Oak Ridge National Laboratory.

Instrument Modifications.

2" x 2" Crystal Detectors. The Francis-Bell Medical Spectrometer uses as its standard detecting equipment a 2" x 2" cylinder of sodium iodide, and ORNL designed the so-called "flat field collimator" to fit their home-canned crystals. The home-canned crystals have caused a good deal of trouble by becoming uncanned, which causes them to lose their resolution and otherwise deteriorate. Accordingly the staff would like to switch over to commercially procurable crystals. A commercially canned 2" x 2" crystal is too large to fit into the ORNL collimating shield. The Harshaw Company has recently begun to make "integral line" assemblies in which the crystal and phototube are enclosed in the same can and external diameter is smaller than with the canned crystal alone, which has an anchoring flange. These professionally mounted, high-resolution units should be considerably more satisfactory than the earlier home-canned crystals.

3" well crystals. Two of these well crystals have been at the Medical Division for some time now, both being home-canned. One of them failed completely and the other lost its resolution, so they were sent to the Harshaw Company for evaluation. It turns out that both can be used in "integral line" (see foregoing paragraph) assemblies, and they have just recently come back. One of these well units will be

incorporated into the heavy iron well counters to be used with double-isotope studies on serum, etc., derived from the patient. The other crystal may be kept in the medical physics laboratory for special spectral studies associated with the new RIDL single-channel analyzer.

Commercial area scanner. The area scanner in routine use before ORNL lent its "brain scanner" remains available in the training unit as a spare system and also to be used in the training courses. Not long ago its detecting crystal (3" x 1" sodium iodide) disintegrated and had to be scrapped. At the suggestion of Mr. Harris of ORNL a 3" x 2" sodium iodide crystal has been procured from Harshaw and Mr. Harris has undertaken to remodel the honeycomb collimator, using the present honeycomb system but providing additional lateral shielding, which it has always needed. This should give a good quality (for moderate energies), second-string scanner for use whenever the first-line scanner gets out of order.

New spectrometer. During the past year funds became available for the purchase of a second single-channel, gamma-ray spectrometer, and this was procured from RIDL, along with two detectors using 3" x 3" sodium iodide crystals. The system uses a different preamplifier circuit from the one utilized by the older, RCL spectrometer, and so the staff have earmarked one of the two 3" detectors for the RIDL system and have modified the other for the RCL. Currently the RCL analyzer is assigned for studies on the new, cesium-137, total-body irradiation facility (TBI), for which purpose its characteristics are well suited. The newer RIDL system is currently assigned to the medical physics laboratory where it is available for the special spectral studies that are always cropping up from time to time. The RIDL system has a slower sweep mechanism and the rate meter has a long time constant, these features making it particularly adaptable to the running of spectra on fairly weak samples. In addition, the plan is to work this system into a device for counting patients who have received a fairly high dose of radioactivity; in other words, it is to become a high-level whole-body counter.

PRECLINICAL STUDIES

Introduction. (Granvil C. Kyker)

The primary objectives in preclinical research closely resemble those defined in the previous Report for 1959. Progress in these has shifted the emphasis, required much additional methodology, and led into certain experimental work on animals that parallels the increased efforts in clinical research on total-body irradiation. The rare-earth fatty liver has been a central part of the effort. This metabolic disturbance is now rather broadly characterized with respect to influencing factors. Chemical characterization at the level of primary lipid constituents has progressed measurably. Gas chromatography has extended analytical interpretation to microquantities of material. Recent reports indicate that even more delicate detection is possible by special glass-paper and thin-layer chromatographic techniques and these are in the plans for the immediate future. The scope of information on the biochemical interaction of heavy metals of central interest to nuclear medicine is limited and this has received some additional effort although now quite preliminary. The brief summaries and abstracts that follow describe the areas of most emphasis and progress during the past year. Future objectives are the completion of presently unfinished portions of these areas and the correlated evaluation, where practical, of this animal work in human patients.

Rare-earth Fatty Liver. (Fred Snyder, Granvil Kyker, Edgar Cress, Nelson Stephens, John Rafter, Paul Godfrey, and Walter Lewis)

The occurrence of an acute fatty infiltration of liver caused by certain of the stable rare earths (La - Sa) when administered intravenously to rats has been described in detail in previous reports (1-5). The fatty response, which is represented by an increase in the neutral esterified fatty acids with little change in the total phospholipids or cholesterol content, is influenced by a variety of hormonal factors. Most of our work has been done with the female rat as an experimental animal; however, the fatty infiltration also occurs in other species, such as the mouse and hamster. The reason that the fatty livers may not have been observed in some of the other species studied could be due to biochemical species differences or to differences in the time of response. The maximal increase in liver lipids occurs at 2 to 3 days after a 2 mg cerium (III) dose per kilogram; and the liver lipids return to normal

within a week after injection, even though the amount of cerium in the liver (about 70% of the dose) remains unchanged. The cerium-injected rats eat less than 2 grams of food daily during the second and third day after injection, and for this reason both the experimental and the control animals were fasted 24 hours before sacrifice in all experiments. It is worth while to note that the higher valent state of cerium (IV) cannot cause the fatty infiltration. The fatty liver cycle can be repeated by giving cerium at weekly intervals for as long as six weeks. After 75 repeated doses (five weekly) the total liver lipids were at the same level as those of the controls, and there was no evidence of cirrhosis or increased radiopacity of any organ in these animals.

The significance of poor mitochondrial oxidation of fatty acids and an elevated plasma free fatty acid level in cerium-treated rats has served as a basis for proposing a general theory on the nature of this lipid response and in setting up experiments to test its validity. We feel that the evidence supports the following idea: The injected cerium (III) combines in the blood with a critical substance capable of triggering the events that show up as eventual fatty liver. Substances that prevent the formation of this complex also prevent the fatty liver. It appears that this complex exerts its action at least at two primary sites: 1) the liver, causing an inhibition of fatty acid oxidizing enzymes and perhaps a blockage of triglyceride secretion into the plasma, and 2) an endocrine gland or glands, which results in a hormonal imbalance eliciting the release of fatty acids from adipose sites and the transport of these acids to the liver, where they are esterified with glycerol rather than used for energy purposes.

Experimental Studies Concerning the Rare-earth Fatty Liver, 1960.

Effect of different modes of cerium administration on fatty infiltration. The administration of large oral doses of cerium (as high as 4 g/kg) has demonstrated that an insufficient amount of cerium is absorbed to cause fatty infiltration of the liver. The percentage of cerium absorbed is less than 0.2 as measured with cerium-144. Cerium (0.5 mg/kg intravenously) if given in four doses 2 minutes apart or 1 hour apart will still produce the typical fatty liver cycle; normally no fatty liver will occur if just a single intravenous injection of 0.5 mg cerium/kg is given. Whether a continuous infusion of much lower cerium concentrations can affect lipid transport is unknown.

Fatty acids of liver and adipose lipids. Because the iodine number of total, neutral, or phospholipid fractions from adipose and liver lipids was not a good index for determining the degree of lipid transport from adipose sites to the liver under the influence of cerium, gas chromatographic techniques were used. The specific fatty acid composition of adipose tissue, of liver and, in one case, of the phospho-and neutral lipid fractions of the liver was compared for control and cerium-injected rats maintained on a Dietrich and Gambrill stock diet. Methyl esters were prepared with dimethoxypropane in methanol-H_2SO_4 and

separated on ethylene glycol succinate by gas chromatography. The adipose fat of rats on the stock diet was very high in oleic and low in stearic acid, whereas the opposite was found in the liver. The fat coming into the liver of the cerium-exposed rats caused this ratio to be inverted, and it was concluded that adipose fat was mobilized to the liver, exerting a diluent effect in the neutral lipid fraction. This was borne out by the distribution of the other fatty acids. A similar change was observed in the ethionine fatty liver. These changes can be observed 6 hours after the injection of cerium, whereas a significant increase in total liver lipids cannot be seen until 48 hours after intravenous cerium.

Liver phospholipid fractions. Rat liver phospholipids were fractionated by means of silicic acid chromatography. In preliminary studies, the fraction corresponding to a polyglycerolphosphatide appeared to increase in amount and to undergo qualitative changes during fatty-liver disturbances. This effect was observed both in rats that had been injected with cerous chloride and in those that had developed fatty livers during choline deficiency. Other phospholipid fractions, e.g., lecithin, cephalins, etc., appeared to change very little during the fatty infiltration of liver. The fatty acids associated with the polyglycerolphosphatide fraction will be investigated to determine the involvement of this structure in lipid mobilization.

Oxidative systems. A general observation characterizing the liver lesion at the peak of fatty infiltration is a depressed oxygen uptake for several systems studied: choline oxidase, octanoic acid, oxidase, and liver slices. Similar results have been observed in the intact rat. Both the oxygen consumption and the carbon dioxide production are depressed and the respiratory quotients of cerium-treated rats (24, 48, and 72 hours after cerium) are similar to those of their 24-hour fasted controls.

In addition, studies of fatty acid oxidation have also continued during the past year. The oxidation of 1-C^{14}-palmitate and 1-C^{14}-oleic acids is markedly depressed in liver slices at 24 and 48 hours after an intravenous cerium injection. The inhibition does not appear to be caused by the action of the fat as a diluent; this agrees with the results obtained last year with the mitochondrial octanoate system. The oxidation rates for palmitic acid of control to cerium-treated rats when expressed as a ratio showed values of 0.9, 1.3, 1.8, and 2.5 at 4, 16, 24, and 48 hours after the cerium administration. The ratio for oleic acid was 1.81 for the 24-hour postcerium rats.

Chemical-physical studies of cerium isolated from rat liver. The chemicophysical state of cerium in the liver during the various stages of fatty infiltration has been investigated with centrifugal techniques. Livers were homogenized in 0.25 M sucrose and four fractions were prepared by means of high-speed centrifugation: (a) 700 g, (b) 5000 g, (c) 31,000 g, and (d) soluble. Cerium-144 was used as the tracer in

these experiments. No sharp correlation between the amount of cerium in any one fraction and the onset or recovery phases of the fatty liver exists; however, it does appear that the cerium in the liver begins to solubilize with time. None of the cerium from any of the fractions is dialyzable. Chemical and biological studies of these fractions are planned.

Conditions that protect against fatty infiltration. Conditions that prevent the fatty liver caused by cerium also prevent the free fatty acid and fatty liver responses caused by cerium; e.g., use of male or hypophysectomized rats or Versene and hydroxide administered with cerium. All substances that either bound with cerium (Versene, plasma proteins) or produced particles (phosphate, hydroxide, ATP) also prevented the usual increase in liver lipid of animals killed 48 hours after an intravenous cerium dose. The cerium must react with the substance in question before injection, since the fatty liver and FFA response will not be prevented if Versene, for example, is administered immediately before or after the cerium injection.

Thyroidectomized and alloxan-diabetic rats behaved like the hypophysectomized animals and did not develop the fatty livers associated with a single intravenous cerium injection. Replacement therapy with pituitary growth hormone given to hypophysectomized rats did not change their response to cerium.

Involvement of carbohydrate metabolism. A decrease in the serum glucose occurs as early as four hours after the administration of cerium. The fact that the decrease in serum glucose occurs at a time when the plasma free fatty acid level in cerium-injected rats is in the same range as controls indicates that carbohydrate metabolism is interrupted before the lipid disorder occurs. Moreover, the alloxandiabetic rat, like the hypophysectomized animal, will not develop the cerium-induced fatty liver.

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Subcellular Distribution of Cerium-144 in Rat Liver. (Edgar Cress and Fred Snyder)

The intracellular localization of cerium in liver cells was studied in an effort to interpret some of the changes associated with fatty liver degeneration produced by rare earths. The livers were homogenized in cold 0.25 M sucrose and precipitated fractions were collected at 700 g (nuclear), 5000 g (mitochondrial), and 31,000 g; the cerium-144 content of these fractions and of the remaining soluble fraction was determined with a Nuclear-Chicago medical spectrometer.

Addition of Cerium in vitro. In the carrier-free cerium-144 livers, 70% of the activity was found in the soluble fraction, whereas the addition of carrier caused a marked increase of radioactivity in the 700 g and 31,000 g fractions.

Studies in vivo. The carrier-free rat livers again had the highest cerium-144 activity in the soluble fraction. The livers from rats injected intravenously with cerium-144 plus carrier showed an increase in the radioactivity of the 700 g and 5000 g fractions on the second and third day after injection.

Collecting $C^{14}O_2$ in a Warburg Flask for Subsequent Scintillation Counting. (Fred Snyder and Paul Godfrey)

This report* describes an improved technique for the collection of $C^{14}O_2$ in a Warburg flask and for the subsequent transfer of the radioactive material for liquid scintillation assay. The CO_2 absorbent used in this work was Hyamine (trade name for 1 molar p-diisobutyl-cresoxyethoxyethyl dimethylbenzylammonium hydroxide in methanol). Passman et al. (Int. J. Appl. Radiat. 7, 38, 1959) were the first to use this substance in collecting $C^{14}O_2$ for liquid scintillation counting, and their article describes some of the important characteristics of Hyamine such as its CO_2 absorbing capacity and its effect on counting efficiency.

At the end of a typical Warburg experiment the Hyamine is injected through a rubber cap covering the Warburg vessel into a small glass tube, which has been positioned on top of the center well. Sulfuric acid is then poured from a side arm into the main compartment of the Warburg flask in order to release the carbon dioxide from the medium. The transfer of CO_2 to the Hyamine is about 97% within two to three hours after the addition of hydrogen ion. The tube containing the Hyamine is then transferred in toto to a glass counting vial containing 0.4% PPO and the radioactivity is measured by means of an automatic Packard Tri-Carb Spectrometer.

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* Snyder, F. and Godfrey, P. Collecting $C^{14}O_2$ in a Warburg Flask for Subsequent Scintillation Counting. J. Lipid Res. 2, 195, 1961.

Effect of X Irradiation on Composition and Turnover of Fatty Acids of Nervous Tissue. (Fred Snyder and E. A. Cress)

Radiation effects on the central nervous system have been widely studied, but surprisingly few biochemical investigations in this area can be found in the literature. A recent, rather complete review on this subject has been made by N. N. Livshits, Moscow (In Advances in Biological and Medical Physics, vol. 7, N.Y., Academic Press, 1960, pp. 173-248). Since nervous tissue is characterized by its high lipid content, our initial studies are planned with this class of compounds. Paoletti et al. (Arch. Int. Physiol. 67, 651-660, 1959) have demonstrated that X irradiation has an inhibitory effect on brain-lipid biosynthesis and that this may be related to the well-known effects of X irradiation on $DPNH_2$ and TPN-dependent enzyme systems. Our studies in progress have shown that the major fatty acids of the female rat brain are palmitic, stearic, oleic, and arachidonic acids. The effect of 840 r given to rats (70 r/min) has shown no qualitative changes in the fatty acids from controls (4 and 24 hours after irradiation); the quantitative aspects of this study are not yet complete. Similar experiments in mice (and the analysis of brain tissue obtained at autopsy from patients treated with total-body irradiation) are a part of this project.

Effect of Total-Body Irradiation on Central Nervous System and Blood Lipids of Rats. (Fred Snyder and Edgar A. Cress)

Small animals exposed to total-body gamma irradiation (TBI) show an elevated level of neutral lipids in the blood and a decrease in the biosynthesis of lipids in brain slices. We have begun preliminary studies to interpret the hyperlipemic response and to evaluate the extent of metabolic changes in vivo of brain fatty acids of animals given TBI exposures. In these experiments rats were given a total gamma dose of 840 r (70 r/min from cesium-137) and killed 24 hours later. Gas chromatography was used to analyze the fatty acids of brain, plasma, liver, and adipose tissue of control and irradiated Carworth Farms-Nelson rats.

Thyroid Function and Hepatic Injury by Cerium. (Granvil C. Kyker, John Rafter, and Fred Snyder)

We have described an acute but transient hepatic injury caused by cerium and other lanthanons. Specific metabolic knowledge is meager for these and other nuclear minerals that offer both potential medical application and hazard. Fatty infiltration prominently reflects the metabolic upset that we have observed and a broad scope of factors modifies or prevents it. Among these, endocrine disturbances are especially noticeable including orchectomy, hypophysectomy, adrenalectomy, and alloxan diabetes. We are now reporting the effect of thyroid function on liver lipids after intravenous cerium (2 mg Ce/kg, as chloride) in rats (young adult, CFN, female). Normal and thyroidectomized rats, prepared surgically and by radioiodine, were compared. Basal metabolic rate and external scanning served to evaluate the prepared animals. Loss of

thyroid function prevented the increase in liver lipids caused by cerium. Related considerations of energy metabolism prompted measurements that suggest a large intake of carbohydrate also to be protective. This additional scope to factors that modify or prevent rare-earth fatty liver extends the metabolic involvement of nuclear minerals.

Oxidative Alterations in the Rare-Earth Fatty Liver. (Fred Snyder and Edgar Cress) (Presented at the Fifth International Congress on Nutrition, Washington, D.C., September 1960).

Some of the rare-earth elements (atomic numbers 57 to 62), prominent as fission products, are capable of causing an unusual fatty-liver cycle when the stable form of these metals is administered intravenously to rats and certain other species. An increased level of plasma free fatty acid precedes the glyceride infiltration of the liver. The hepatic lipids reach a maximum on the second and third day and return to normal approximately a week after the injection of cerium (0.014 millimoles per kilogram). A general observation characterizing this lesion at the peak of fatty infiltration is a depressed oxygen uptake for several systems studied: choline oxidase, octanoic acid oxidase, liver slices, and the intact rat. The oxygen alteration is correlated to the onset and recovery phases of the fatty-liver cycle. At 24 and 48 hours the respiratory quotients of cerium-treated rats are similar to those of their 24-hour fasted controls.

Release of Liver Triglycerides during the Rare-Earth Fatty-Liver Cycle. (Fred Snyder, E. A. Cress, and G. C. Kyker)

Gas-liquid chromatography has demonstrated that fatty acids of adipose tissue are the source of the elevated liver glycerides that occur within 48 hours after the intravenous administration of cerium. The fatty accumulation is partially due to poor mitochondrial oxidation of fatty acids; however, the results reported here show that inhibition of the release of triglycerides from liver is also an important factor. In another experiment, rats with fatty livers induced by ethionine showed a similar reduction in the discharge of liver triglyceride to the plasma.

Triton (p-iso-octyl polyoxyethylene phenol polymer) administered intravenously to rats prevents the exit of triglycerides from plasma, and under these conditions the liver is the major source of the plasma triglycerides (Byers, S.O. and Freidman, M., Am. J. Physiol. 198, 629, 1960). In this report we have demonstrated that cerium (24 and 48 hours after intravenous Ce^{+++}) does inhibit the response normally elicited by Triton injected by about 60%. The effect of cerium is no longer apparent during the recovery phase (day 5).

Internal Behavior and Hepatic Response to Rare Earths. (Granvil C. Kyker, John Rafter, and Nelson Stephens) (Presented at the Fifth International Congress of Nutrition, Washington, D.C., September 1960)

The internal behavior of rare earths comprises a prominent part of the metabolism of mixed fission products. In our measurement of distribution under various conditions, small increments of chemical dose have changed sharply or masked completely certain elemental differences that may appear with carrier-free radioisotopic tracers. The low toxicity of rare earths, by all routes except intravenous, reflects their poor mobility. Evidence suggests that aggregation and complex formation represent two major equilibria that promptly affect an intravenous dose. Distribution studies at intervals during repeated treatments show that the recently described acute fatty liver does not depend on the amount of rare earth localized in liver. The in vivo capacity of rats to withstand repeated hepatotoxic doses is shown by their resiliency to 75 treatments within 105 days without grossly apparent ill effects. Combined treatment with rare earth and Versene at various intervals indicates the acute hepatic disturbance to depend upon a reaction that occurs immediately after intravenous injection.

Cerium in Rare-Earth Fatty Livers. (Granvil C. Kyker, John Rafter, Nelson Stephens, Edgar Cress, and Fred Snyder)

Acute transient fatty infiltration of liver occurs after intravenous administration of cerium to rats. Liver lipids increase threefold within 48 to 72 hours and then return to normal almost as rapidly. The mechanism remains unexplained. We have followed the injected cerium with tracer cerium-144 to correlate hepatic localization, extrahepatic distribution, and excretion with this metabolic disorder. Twenty-one groups of four rats represented several experimental periods and conditons that affect the fatty infiltration, including sex, strain, hypophysectomy, and the chemical nature and dose of injected cerium. Ten organs and tissues, separate excreta, and the residual carcass were radioassayed. Nuclear, mitochondrial, submicroscopic, and soluble fractions of liver were also examined. Under all conditions the pharmacologic dose (2 mg/kg) as cerous chloride localized rapidly in liver; this approached 60% in four hours and varied from 70 to 75% between 12 hours and 12 days. Subsequent decrease of hepatic cerium during one year showed three apparent slopes with biological half times estimated at 35, 130, and 1200 days. Observations on the subcellular fractions were particularly sensitive to procedural conditions; cerium appeared largely in the nuclear and mitochondrial fractions and was nondialyzable in all fractions. The results consistently show a lack of correlation between the factors evaluated and fatty infiltration caused by cerium.

A Comparative Study of Intravenous Cerium, Lutetium, and Yttrium in Cortisone-Treated Rats. (Ernest A. Daigneault and Granvil C. Kyker)

Several hormones affect the acute fatty infiltration that occurs in the liver of rats treated with certain lanthanons. No explanation for these hormonal effects is known. We compared the effect of cortisone on the distribution and excretion of cerium, lutetium, and yttrium. This selection was an attempt to represent the lanthanons in groups according to atomic number and metabolic effect. Cerium represents the first five elements, each of which causes fatty infiltration; others in the series above samarium do not elevate total liver lipids. Lutetium represents the upper end of the series and yttrium closely resembles certain intermediate lanthanide elements. The properties and availability of cerium-144, lutetium-177, and yttrium-90 as radioisotopic tracers also influenced the choice of elements. Two levels of dose were used. The lower dose of each element depended on the specific activity of the tracers and approximated 10^{-9}, 10^{-8}, and 10^{-12} moles of cerium, lutetium, and yttrium, respectively, per kilogram. The higher dose was adjusted to 10^{-5} moles per kilogram by additional carrier. The chlorides of each element adjusted to pH 4 were injected intravenously. Cortisone was given intramuscularly as the acetate derivative (25 mg/kg). Fasting was imposed throughout the 24-hour experimental period. Cortisone altered the distribution to some extent. This was more noticeable at the higher level of dose, and also for cerium. The effect on excretion was more variable and showed no consistent trend. In a few experiments we also compared the elements during treatment with corticosterone, insulin, dinitrophenol, and EDTA. Again no striking effects were observed, except the increased excretion during EDTA treatment. The EDTA was given subsequent to the metal and the enhanced excretion was accordingly less than that observed by others when a lanthanon is administered as its EDTA-complex.

Certain Exzymic and Metabolic Effects of Rare Earths. (I. H. Miller, Jr. and Granvil C. Kyker)

In vitro studies. An attempt was made to further define the effects of certain lanthanons on critical enzyme systems. The fact that the intravenous injection of female Carworth Farms-Nelson rats with cerium causes a dramatic fatty infiltration of the liver, but the administration of dysprosium does not, prompted comparative studies of some enzymic responses to these two lanthanons. The activity of urease was used as the basis for comparison and three parameters were considered: 1) the relative effects of equimolar concentrations of the lanthanon; 2) the effect of a chelating agent (Versene) upon lanthanon activity; and 3) the effect of preincubation of the lanthanon with the enzyme upon subsequent activity.

In a typical experiment 0.08 M dysprosium was more effective in inhibiting the activity of urease than an equivalent concentration of cerium was; the initial velocity of the test system was inhibited only 15% by cerium, but it was inhibited 37% by dysprosium. The inhibition

is probably nonspecific and if certain functional groups are affected, they are probably ones that are general to all proteins, and not associated with a particular active site. The preincubation of the enzyme with the lanthanon strongly enhances the inhibitory effect. The response to preincubation showed a rough linearity with respect to the length of the preincubation period. The inhibition of urease by cerium and dysprosium was partially reversed by the addition of Versene to the system, but complete reversal was not accomplished. The study of other enzyme systems continues.

In vivo studies. Experience has shown that when injected with cerium intravenously, female Carworth Farms-Nelson rats exhibit a markedly restricted food intake. This observation led to an attempt to evaluate some of the factors that might influence the food consumption of these animals and the influences of food intake on the induction of fatty livers.

To study the effect of the trauma of injection, animals were injected with saline without prior anesthesia. For the study of the effect of anesthesia alone, animals were anesthetized (without injection) in the manner used when intravenous injection is given. In each of these experiments half the animals were offered food ad libitum and the other half were offered 10 g of food each per day, before and during the experimental period. To study the influence of feeding practices on food consumption and liver glycogen, animals were offered 10 g of food per day for two days before injection with cerium and then offered food ad libitum for 48 hours. In still another experiment one group of animals was fed ad libitum while another group received 10 g per day during the entire preparatory and experimental period. This regimen was followed for several days postinjection. Here, as in all experiments referred to, appropriate controls were used.

Neither the injection of saline without anesthesia nor the administration of anesthesia alone caused an appreciable or consistent change in the consumption of food by the rats, regardless of how they were fed. Animals offered only 10 g of food per day before injection with cerium, continued to eat well during the first day postinjection, dropping off sharply on the second day, whereas those that had been fed ad libitum, and were presumably in good nutritional state, exhibited a sharply restricted food intake even on the first day postinjection. At autopsy the animals that failed to eat during the first 24-hour period postinjection exhibited marked fatty infiltration of the liver and the nearly complete absence of liver glycogen; those that had continued to eat for at least a part of the immediate postinjection period had retained an appreciable amount of the normal component of liver glycogen, and did not suffer so dramatic an infiltration of the liver by fat.

The observations suggest that the nutritional state of the animal at the time it is injected with cerium has a definite bearing on the extent to which it will or will not eat after the injection. This finding suggests the wisdom of following food consumption in future experiments

involving the cerium fatty liver. The level of food intake and the storage and breakdown of liver glycogen probably influence significantly the fatty infiltration that is associated with the injection of cerium. It seems reasonable to project that cerium may have some rather specific hepatotoxic properties, not shared by some of the other lanthanons, which favor glycogen depletion and hence fatty infiltration.

Cerium Uptake by Baker's Yeast. (G. T. Johnson and G. C. Kyker)

Four species of yeast have been shown to accumulate relatively large amounts of cerium from a nutrient medium during growth (Johnson and Kyker, ORINS-34, 42-43, 1960). Recent experiments have been designed to give information on the nature and mechanism of the uptake. With resting cells of baker's yeast (Saccharomyces cerevisiae) prominent uptakes of cerium occur in very short periods (15 minutes or less). In a phosphate buffer containing citrate, the uptake is correlated with pH; the optimum is in acidic environments (pH 2.5 to 4.0), and the uptakes decrease with increasing pH (very notably above pH 6.0). Living cells take up somewhat more cerium than dead cells, but the amount bound by dead cells depends on the killing agent used (e.g., cells killed by mercuric chloride take up more cerium than cells killed by heat). The strength and nature of the binding is not yet determined; there is little leaching of radiocerium from labeled cells in water or dilute acids, but complexing agents (citrate, Versene) will remove most of this cerium, and the radiocerium from labeled cells will exchange with any stable cerium in the medium. Under appropriate conditions the amount of cerium bound by labeled cells is proportional to the dry weight of the cells. Cerium uptake by such cells is independent of temperature over a range from 3 to 38° C, is as high under anaerobic as under aerobic conditions, and is not affected by the common inhibitors for respiratory and glycolytic pathways (azide, cyanide, dinitrophenol, fluoride, iodoacetate). Among many divalent and trivalent cations tested (calcium, cobaltous, magnesium, manganous, tin, strontium, uranyl, aluminum, chromic, and ferric) as possible competing cations, only the uranyl, aluminum, and ferric ions proved effective in reducing cerium uptake by yeast. The question of possible specificity for trivalent cation binding sites in yeast cells is under investigation.

Biochemical Reactivity of Certain Lanthanons. (Granvil C. Kyker, Lois Ann Youngblood, and Raymond L. Hayes)

General patterns of distribution of the lanthanons are described by various radioisotopic tracer studies. These patterns will depend on their reactivity with chemical constituents of the internal environment. Very little specific information on the interaction of the lanthanons and biochemicals is available. Certain ionic constituents of tissue fluids are among the few efficient precipitants of the lanthanons, and repeated experimental measurements confirm their prominent tendency to combine with circulating proteins. Attempts to design routine procedures to survey the precipitating or solubilizing action of biochemical reactants were only partially successful. The procedures, however,

yielded certain results that conflict with some of the limited published information. Spectrophotometry overcame some of these difficulties in previous procedures. Neodymium and erbium were selected because of convenient spectral properties. More than 50 biochemicals were examined by this method. The spectral effects on certain peaks in the visible region included changes in wave length, optical density, and splitting into multiple peaks. These effects were seen for both the elements. No general correlation with chemical structure of the reagents was evident. Interpretation of the spectral changes as evidence of chemical interaction confirms and extends the conclusions reached by the previous procedures.

Studies with Tritium-Labeled Thymidine. (F. Comas)

In a preliminary study to determine the uptake of tritium-labeled thymidine into the cells of the bone marrow and an implanted tumor of the rat, animals weighing between 150 and 200 g were injected with 50 microcuries of the thymidine-H^3 and killed at 5, 10, 15, 20, 30, and 60 minutes after injection. A rapid uptake of the tritium by both tissues was noted and the incorporation at 5 minutes was almost the same as at 15 minutes. Subsequently, however, the uptake was noted to increase slightly up to 1 hour. In another preliminary study rats were injected with 50 microcuries of tritium-labeled thymidine after release

Internal Radiation Dose. (R. L. Hayes)

Unconfined radioactive materials constitute a potential internal as well as external radiation hazard. Depending on the conditions of exposure, the internal dose may well be the greater potential hazard. Dangerous amounts of radioactivity may be accidentally carried directly into the intestinal tract through the oral ingestion of contaminated food and water, or indirectly through inhalation and subsequent swallowing. Certain of the radionuclides will be partially or completely absorbed from the intestinal tract and distributed throughout the body in a pattern dependent on the chemical nature of the individual radioisotope. Many others, such as the rare-earth radionuclides, will be slightly absorbed, if at all, so that their radiation dose will be delivered mainly to the intestinal tract. A large fraction of by-product radioactivity is of this type.

The dose received at sites along the intestinal tract as the result of the oral ingestion of a particular radionuclide will depend on the amount ingested, the form, the half life, the decay scheme, and the fraction absorbed from the tract during passage. In addition, the change of radionuclide concentration with time, other than through radionuclide absorption, will drastically affect the dose received. In general, except for very short-lived radionuclides or those that are largely absorbed from the tract, the lower large intestine will receive the highest dose and thus will be the critical part of the tract.

Of the factors that control the dose to the intestinal tract the change of radionuclide concentration with time is undoubtedly the most variable from individual to individual. In arriving at recommendations for permissible concentrations of various radionuclides the International Commission on Radiological Protection has necessarily had to resort to the assumption of an "average behavior" for this important factor. The actual intestinal tract dose that a particular individual would receive from the ingestion of a given radionuclide would thus depend mainly on how far his behavior varied from this assumed average behavior. The extent of this variation in humans is the subject of a study currently being made at the Medical Division. Previous *in vivo* dose studies in experimental animals have shown drastic differences among individual animals and this has prompted the present investigation in humans.

A tracer technique using a nonabsorbed radionuclide makes it possible to estimate the dose that a human subject's lower large intestine would have received from the oral ingestion of known amounts of beta-emitting radionuclide. Comparison of this estimate with the dose computed from average behavior assumption provides a measure of the subject's degree of conformity to this average. Although the method is reasonably valid only for beta emitters, the data obtained could be used to roughly approximate gamma doses as well.

This study is presently being carried out on patients whose ailments do not involve nor appear to affect their gastrointestinal tracts. Too few subjects have been tested to make generalizations as yet but certain trends are now apparent. Although the average of the estimated doses for the series appears to be in good agreement with assumed average behavior, there has been a noticeable variation among individual subjects. The highest estimated dose to date for long-lived radionuclides has been a factor of seven times greater than that to be expected from assumed average behavior, while the lowest estimated dose has been five times lower, the ratio of highest to lowest dose being 35. With radionuclides of decreasing half life the ratio of the estimated dose to the average behavior dose in general tends to decrease.

A Method for Collection and Radioassay of Human Stool Specimens. (R. L. Hayes)

The collection and handling of stools is usually a troublesome as well as disagreeable task. The procedure is made more difficult when the total fecal excretion of a particular material is to be determined -- for example, in the determination of the intestinal absorption of iron using Fe^{59}. In such collections it is of paramount importance that there be as much assurance as possible that the collection was complete during the test period. If a loss does occur, the results of the test can be completely misleading.

A current study at this laboratory involves the tracer measurement of fecal excretion rate of nonabsorbed materials in humans. Early in the course of this study it became apparent that in many subjects the stool collection was far from being complete, since the recovery of radioactivity was well below that administered. Complete or near complete recovery of activity is required in these measurements, and thus the data from these subjects was worthless.

The subjects being used in this study are patients from the clinical section. It appeared that one of the main reasons for this poor cooperation was the fact that the subjects were being requested to perform a somewhat cumbersome task. The initial procedure was to have the patients make the collection in hand-held pasteboard cartons. This method is in common use in most hospitals. To obtain better cooperation from the subjects being studied, a new procedure has been adopted in an effort to make the task an easier and less distasteful one. The new method has proved to be highly satisfactory judging by the increase in the amount of usable data that have been obtained. There has been much favorable comment on the technique by visitors to the Medical Division, particularly by those in the field of pathology who in turn are frequently confronted by the same collection problem. Although there was originally no intention to publicize the collection method, the interest that has been shown by others appears to warrant a brief description of it.

The device we use is shown in Figure 1. The spring fits into the well of the carton holder shown on the right side of the illustration. It is slightly oversized at the base so that it will be held in place by tension when the device is assembled. Carton holders are constructed of stainless steel to reduce corrosion and permit autoclaving. The carton shown in Figure 1 is a commercially available one-quart container of the indicated diameter.

In use, the arms of the assembled carton holder are placed on the sides of the toilet bowl and the toilet seat is then lowered over the holder. An uncapped carton is then placed on the spring in the holder well. The dimensions of the holder are such that the device can be readily positioned under the seat to the convenience and comfort of the subject using it. The use of the spring provides a pressure guide for positioning as well as a means of accommodating subjects of varying size and proportions. With women subjects urine contamination of stools does not occur if a reasonable amount of care is used by the subject in positioning the collection device.

Our method of radioassay is to compress the stool in the carton by forcing in the top of the carton with the device shown in Figure 2. The weight of the stool is obtained by difference in weight before and after collection and an appropriate geometry factor is then applied to the activity measurement to obtain a corrected count.

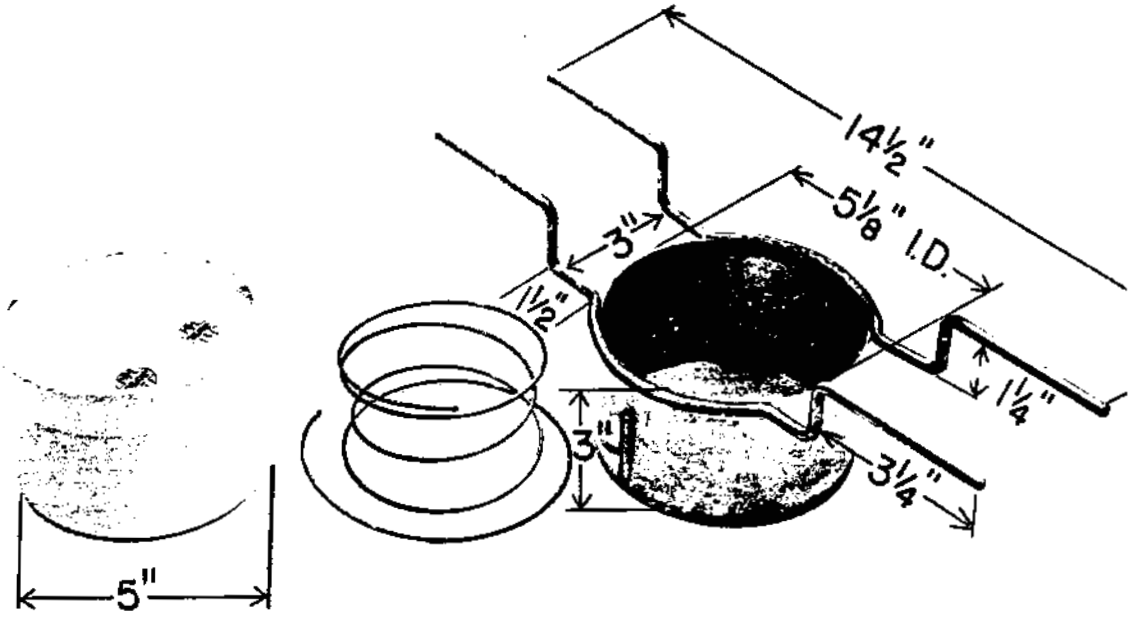

Figure 1. Device for collecting stools.

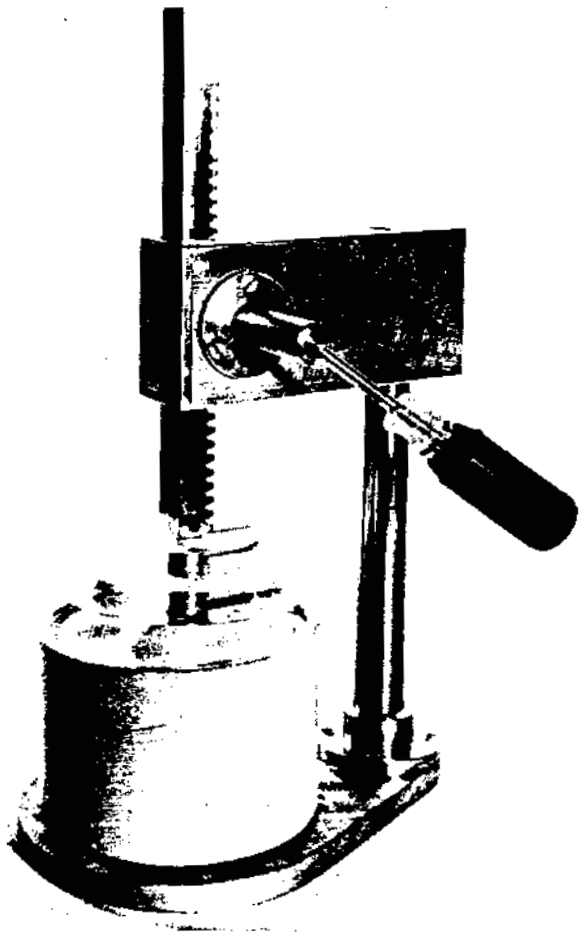

Figure 2. Device for compressing stool specimens.

Summary. The collection of stool specimens from patients is often incomplete because of the cumbersome and distasteful nature of the task the subject is asked to perform. A simple container holder designed to fit most toilets has been used to good advantage in overcoming this difficulty.

TRAINING

Introduction. (R. M. Kniseley)

Training activities continue to be an essential Medical Division activity. These may be classified in three groupings, 1) resident and fellowship training; 2) qualification courses and special seminars; 3) miscellaneous.

All are cardinal in the mission of the Medical Division to develop, collect, and disseminate information on the medical uses of radioisotopes.

Resident and Fellowship training. Ten short-term residents from several institutions averaged three to four months in training. Also two third-year medical students were here under the auspices of the Abbott Medical Student Fellowship in Nuclear Medicine. One resident in pathology was here for six months as part of the combined program with St. Mary's Hospital in Knoxville. Three one-year trainees from this country and two physicians from foreign countries were also here for one-year fellowships.

The program for these groups varied, depending on the particular training mission, but was built around the clinical program with participation in formal ward rounds, in the study and treatment of patients, in evaluating diagnostic radioisotope tests and the management of patients containing radioisotopes. In addition they were oriented to the major current clinical program on the total-body irradiation studies, marrow-graft attempts, and the study of the effects of radiation. Each of these trainees was also scheduled for the ORINS Special Training Division's basic course in radioisotope techniques and to the shorter instructional seminars of the Medical Division. Training aids are available for manipulation and study, and residents participated in the various conferences. In addition to the three half days per week of formal ward-round sessions, a weekly seminar in hematology was presented on Tuesday afternoons. A Friday noon work conference was also initiated to which residents were invited. Physicians here for longer than four months are expected to participate in one of the research projects of the Division. As before, this has proved to be valuable to the program and has given the trainee experiance in clinical basic investigative work.

During the year descriptive announcements of the fellowships were sent to various professional journals, chairmen of the departments of internal medicine of the American medical schools, and the deans of the medical schools.

A laudatory letter was received from the secretary of the Residency Review Committee on Internal Medicine of the American Medical Association, expressing admiration for the high quality of the program carried on by the Institute. Noting its highly specialized senior-level program, which goes beyond the concept of a medical residency, the committee recommended that the training offered should be labeled as a fellowship rather than as an internal medicine residency. It was felt that the program does not come under the Committee's province as a general medical residency.

Research participant program. This continuing program has been a distinct asset to the goals of the Medical Division. During the summer of 1960 there were five visitors representing medical-school or undergraduate faculties in biochemistry, pharmacology, microbiology, and chemistry. From each one, a productive piece of work resulted. The participants added the skills of their own disciplines to the Medical Division program and in return received training both in details of the research project and special help in radioisotope methodology; for example, chromatography or liquid beta scintillation counting.

In support of this research participant program, two college students and one graduate student also joined the program, two of them under the Oak Ridge Student Trainee Program. These "apprenticeship" relationships were highly successful in providing the additional man power for the experiments undertaken and also gave to these students a worth-while experience in a research setting.

Qualification courses and special seminars. The qualification courses given in separate one-week sessions as Preclinical I, Preclinical II, and Pathology were continued. Some changes in the approach to certain topics and experiments have been necessary, but major alterations have not been required. As before, Preclinical I introduces language and tools, principal topics presented in half-day or one-day sessions. A combination of films, lectures, laboratory demonstration, and informal exchange emphasizes basic physics, statistics, decay and interaction, electronics, autoradiography, and biological applications. Preclinical II introduces application of the principles presented in Preclinical I, using the same approaches to acquaint the students with instrumentation and measurement, and concepts they use in clinical methods. The topics include beta counting, sample counting of gamma emitters, survey and monitor instruments, external counting, field of vision, scanning, biological procedures and measurements, radiological safety, and autopsy technique. The third week is devoted to clinical diagnostic procedures that are performed by the students to meet AEC license requirements. Thyroid function tests, red cell labeling and survival techniques, blood volume procedures, fat absorption studies,

vitamin B_{12} excretion, iron kinetics, and scanning were done. During this week valuable assistance has been obtained from guest lecturers who come to Oak Ridge on letter contracts, presenting some of their original clinical investigations and helping with the laboratory sessions.

The special short course for 1960 was in autoradiography, and was limited to eight participants and three auditors. It was a fairly intensive and detailed course emphasizing laboratory participation. A review of basic physics was presented through films. Lectures on photographic theory, quantitation, artifacts, densitometry, and surveys of various techniques were interspersed among the laboratory sessions. Laboratories were devoted to preparation of gross bone and gross soft-tissue autoradiograms, preparation of wet mounts and stripping film methods for microscopic autoradiography. Sessions on interpretation of autoradiograms were also presented.

It was necessary to cancel the special advanced seminar scheduled for October on the topic of total-body irradiation and whole-body counting.

Miscellaneous training activities. Revision has been started of the 37 midget exhibits presenting minimum basic data that a physician must know to use radioisotopes. In addition, plans for an open-ended series of exhibits on applied techniques are under way and some of these have been prepared. Selected groups of these midget exhibits have been presented at national meetings. One group was displayed at the 1960 meeting of the Society of Nuclear Medicine at Estes Park. Selected midget exhibits were presented under the title "What the Physician Should Know about Radioisotopes" at the 1960 meeting of the American Society of Clinical Pathologists.

Members of the staff presented a symposium entitled "Training Aids" to the spring meeting of the Southeastern Chapter of the Society of Nuclear Medicine. They also were invited to present the annual evening symposium at the 1960 annual meeting of the Society of Nuclear Medicine. The topic was "Tools of the Trade." To a very large audience, the staff demonstrated and described the devices proven successful in the ORINS training of physicians in radioisotopes.

Staff members have filled a large number of requests for lectures elsewhere during the year. Lectures have been presented to the basic course of the Special Training Division of ORINS, in the Oak Ridge Traveling Lecture Program, to the U. S. Naval Medical School course in radioisotopes, the Armed Forces Medical Symposium at Sandia Base, Albuquerque, New Mexico, Medical Education for National Defense (MEND) program, and also to various other meetings and seminars. Although this has been a real contribution to other institutions and educational programs, the interchange and orientation afforded by visits of our staff to these various places has tangible benefits here.

Requests for thyroid uptake manikins continued to be received and these kits have been shipped to widely divergent geographical locations - Atlanta, Georgia; Decatur, Illinois; Los Angeles, California; Boston, Massachusetts; Sydney, Australia; Bonn, Germany.

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1960

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MEDICAL DIVISION RESEARCH REPORT FOR 1962

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ORINS

LEGAL NOTICE

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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

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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.

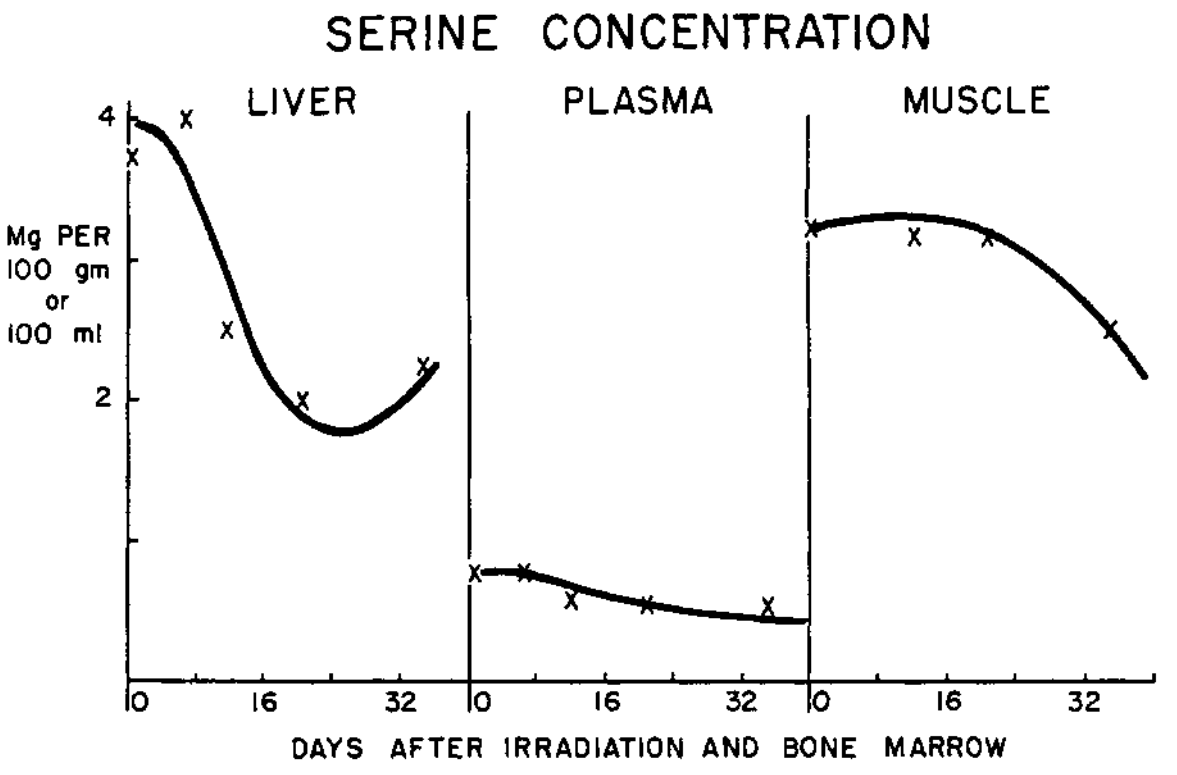

Fig. 1 Changes in serine concentration based upon an arbitrary theoretical model of serine metabolism. The solid lines are taken from the recorded output of an analog computer. The X's are taken from experimental data. The chief point of interest to come out of this particular solution was that a rapidly 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.

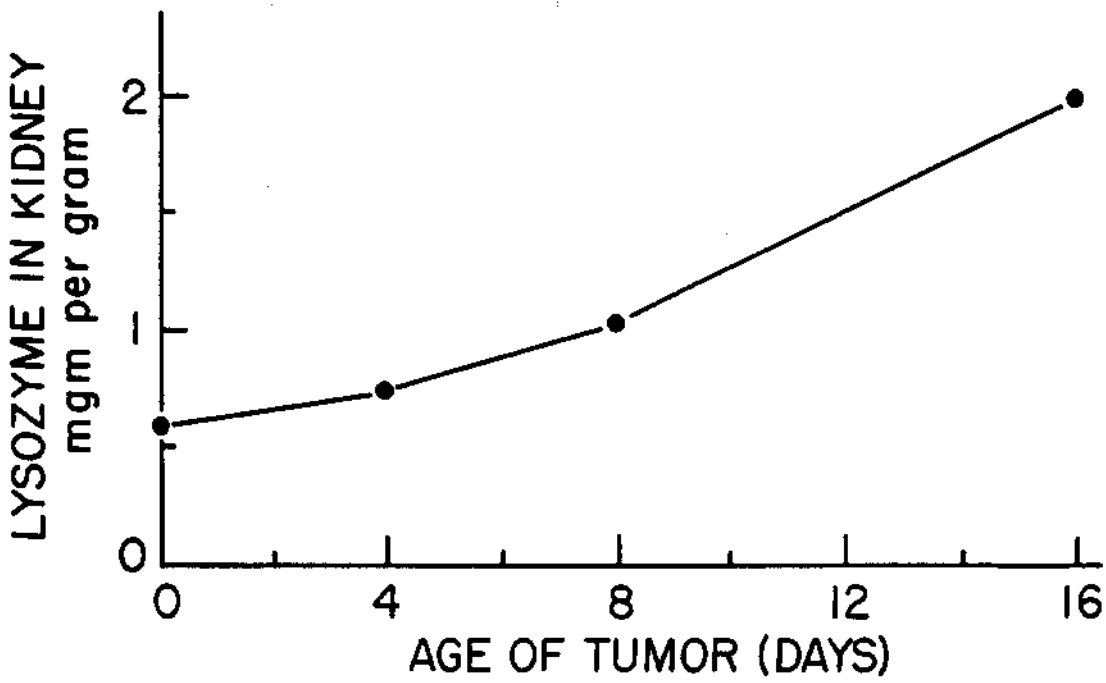

Fig. 1 Lysozyme in kidney of rats with Walker Carcinoma S-256.

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.

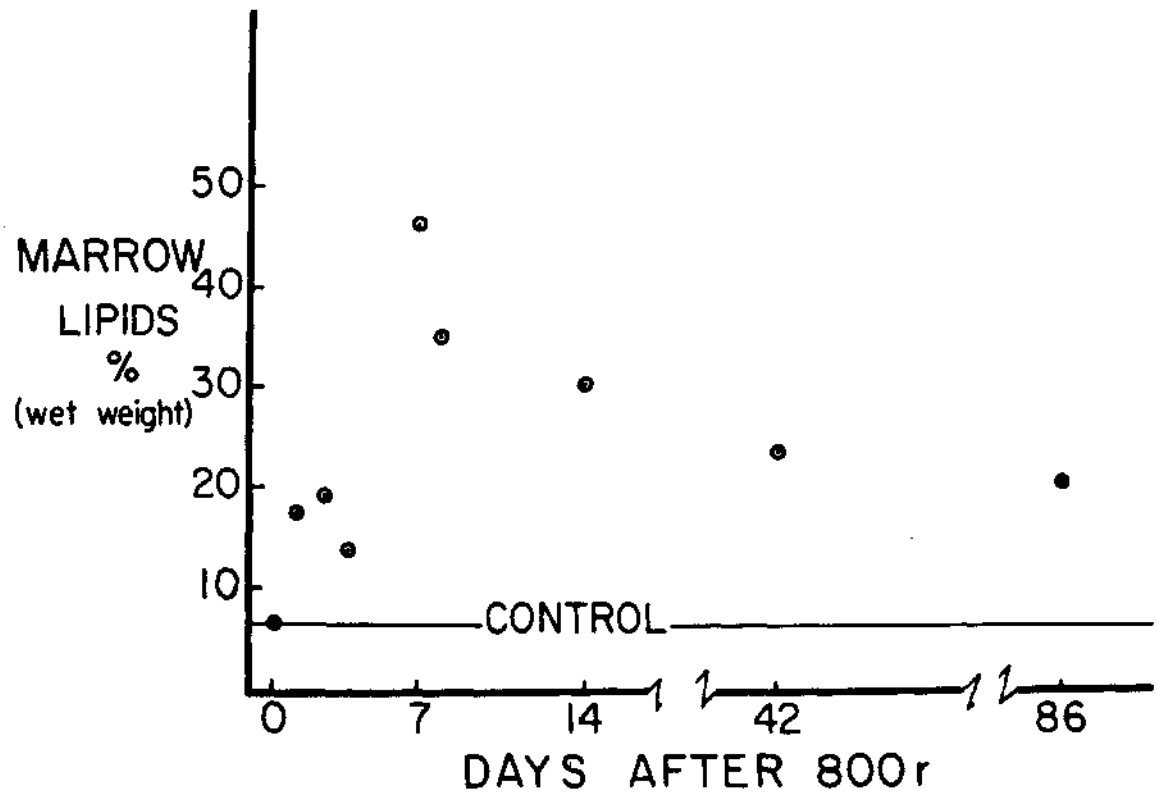

Fig. 1 Total lipids of bone marrow (wet weight) after 800 r total-body irradiation. Each value represents pooled samples from the following number of rats: 0 days = 182 rats (range 5.5 - 9.3% for 7 pooled groups); 1 day = 4 rats; 2 days = 8 rats; 3 days = 8 rats; 7 days = 8 rats; 8 days = 65 rats (range 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.

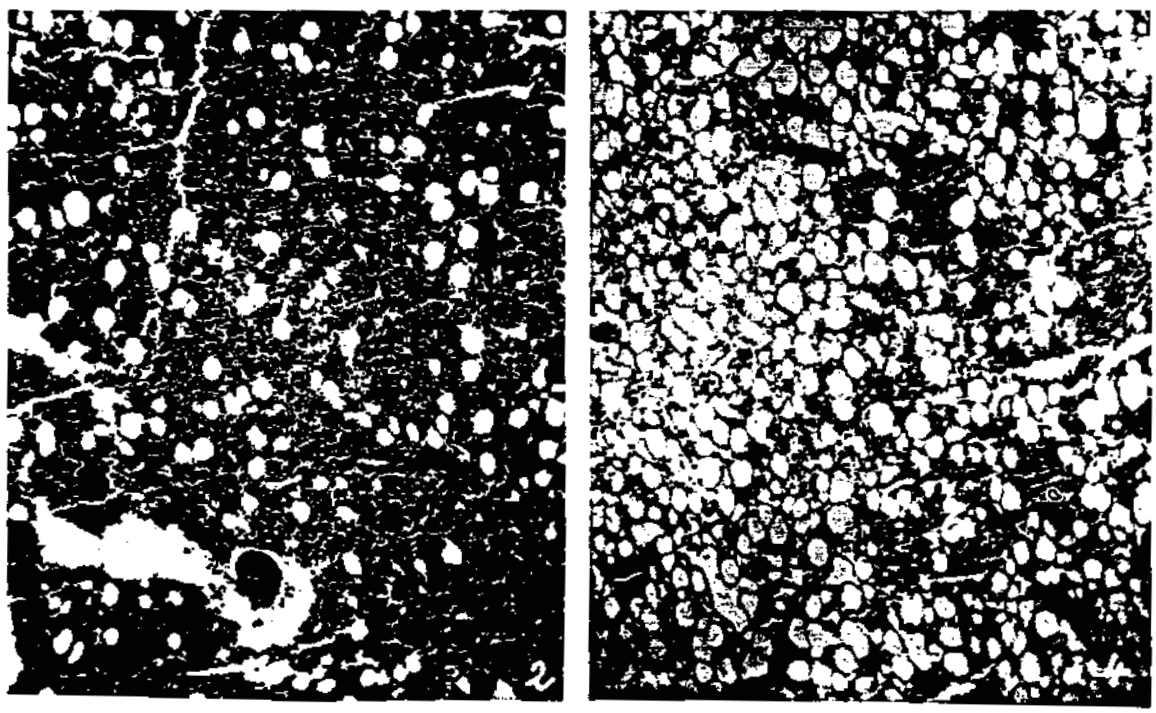

Fig. 2 Histologic sections of femur marrow from control (left) and irradiated (8 days after 800 r) rats (right). The sections were stained with hematoxylin and eosin.

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.

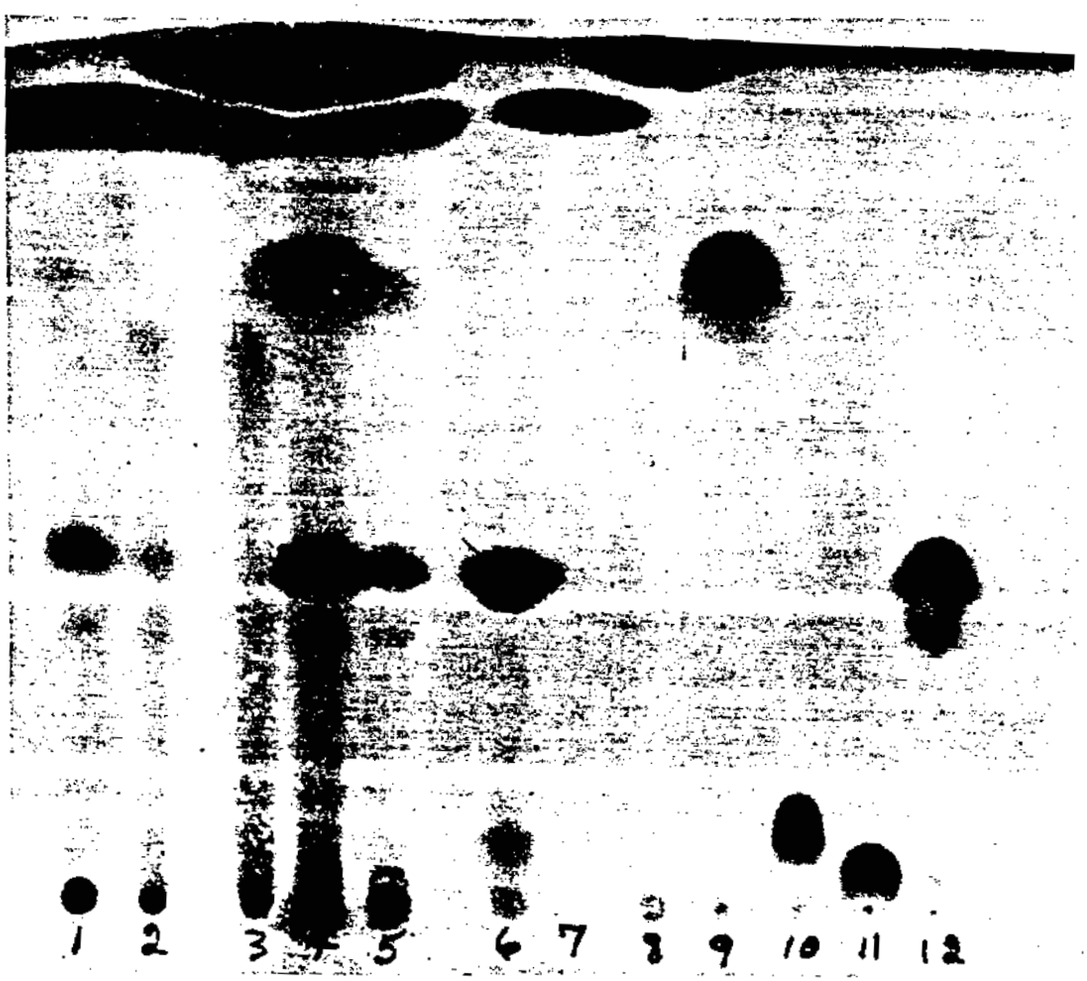

Fig. 3 Thin-layer chromatogram of bone marrow, adipose tissue, plasma and liver lipids: 1) control marrow, 2) 800-r marrow, 3) perirenal fat, 4) plasma lipids, 5) liver lipids, 6) cholesterol, 7) triolein, 8) cholesterol stearate, 9) oleic acid, 10) batyl alcohol, 11) monopalmitin, 12) 1,3-dipalmitin. 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.

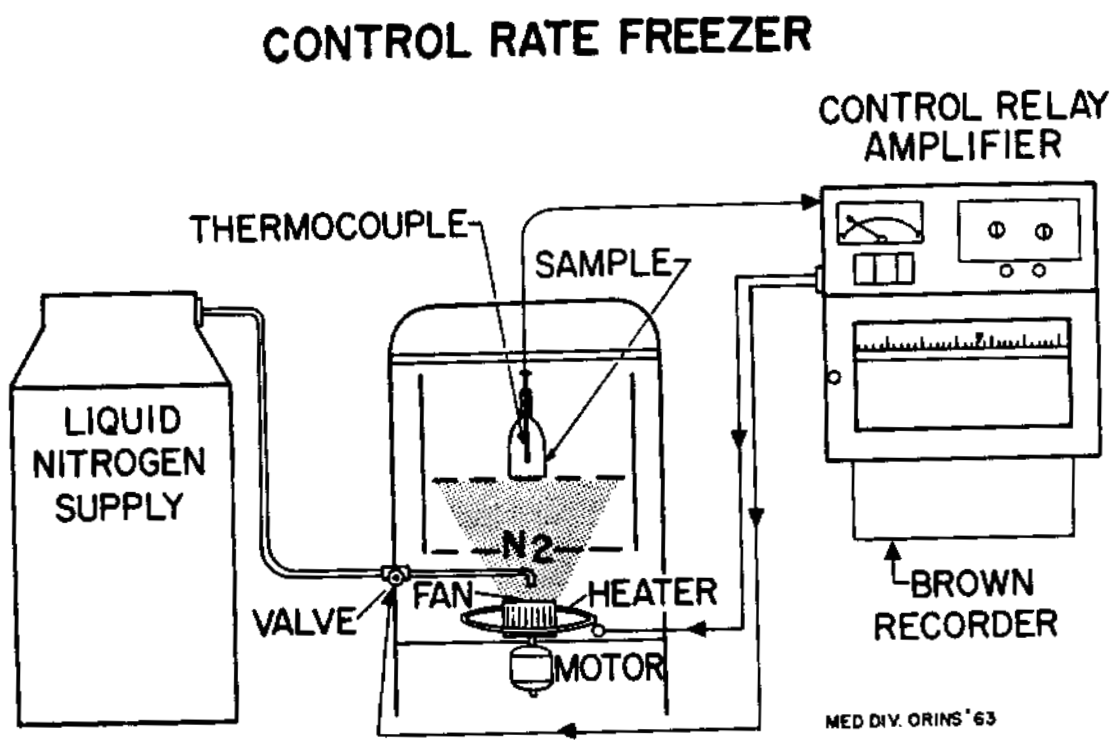

Fig. 1 Diagram of apparatus for controlled freezing and preservation of human and animal cells.

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.

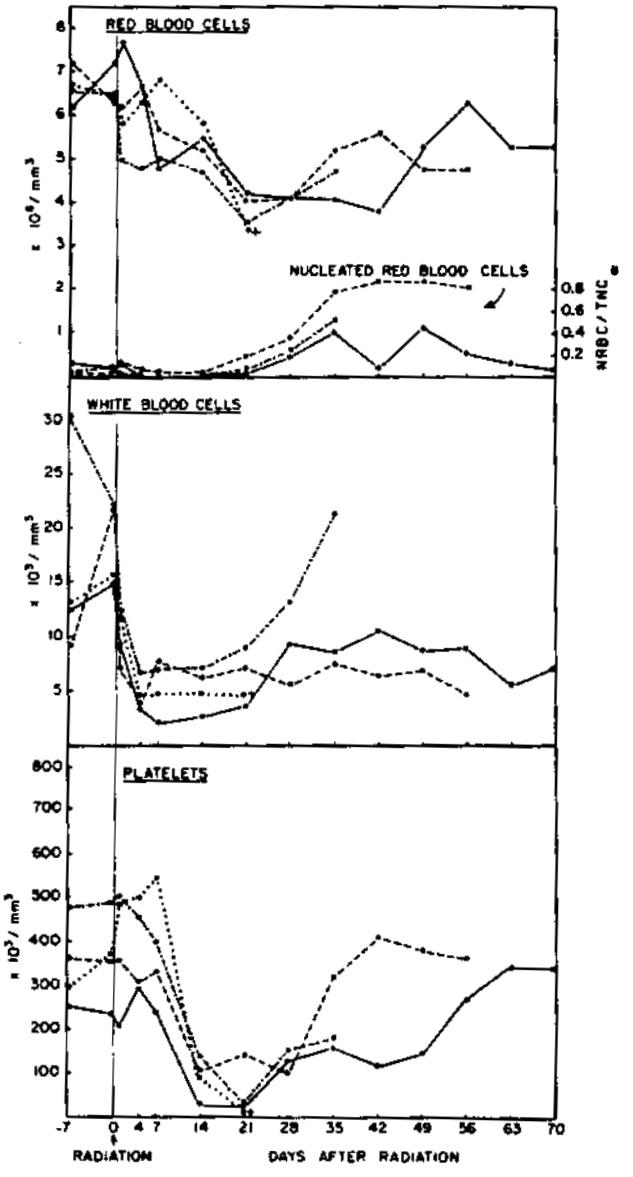

Fig. 1 Hematologic changes in tamarins exposed to 100 r total-body gamma irradiation. *NRBC/TNC indicates the number of nucleated red blood cells per total number of nucleated cells counted in differential analysis of 200 white blood cells.

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.

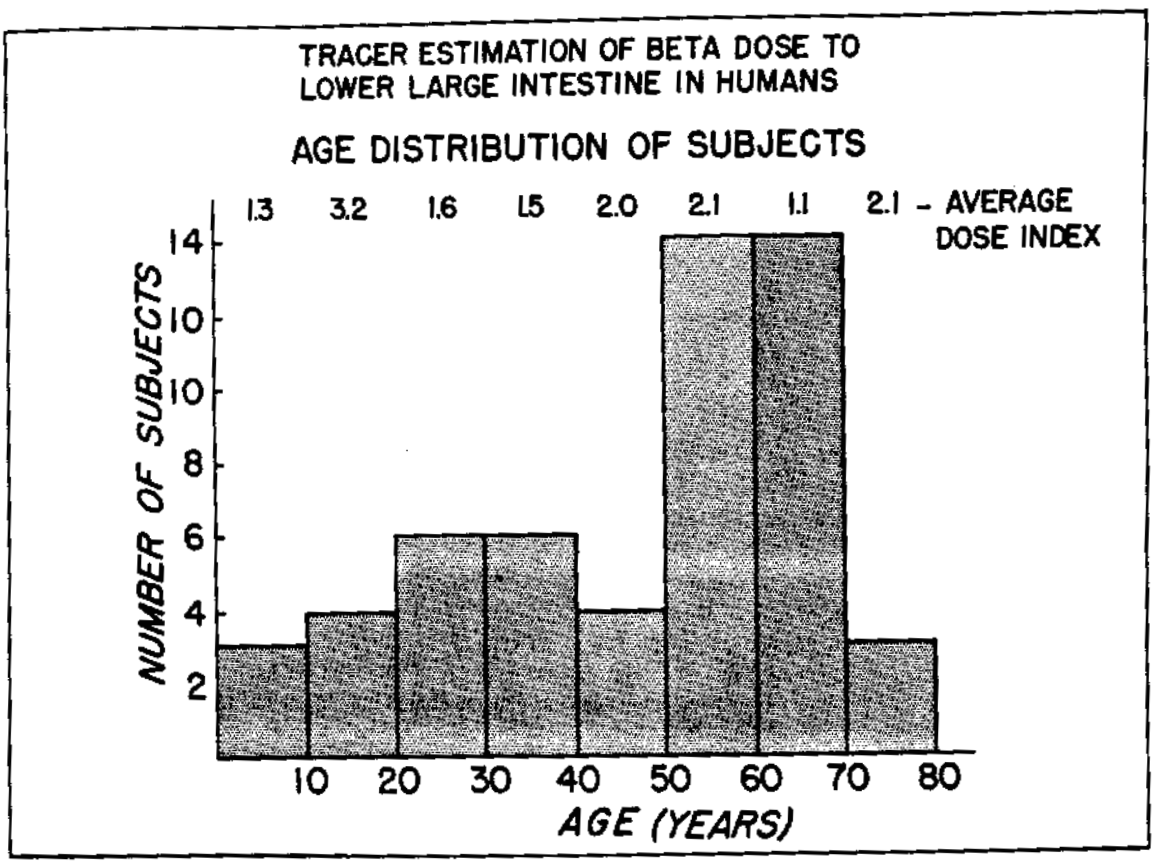

Fig. 1 Age distribution of subjects and dose index by age groups.

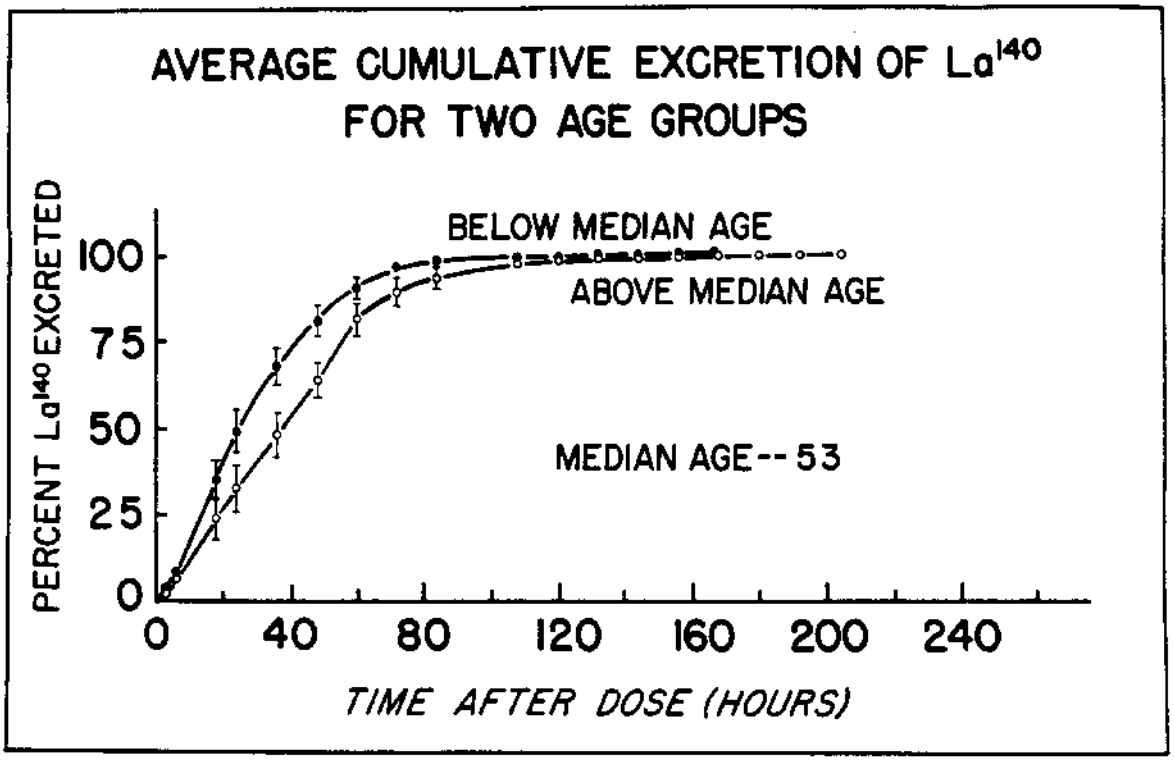

Fig. 2 Average cumulative excretion of La^{140} for a division of the subjects about the median age. Limits on points indicate the standard deviation of the mean.

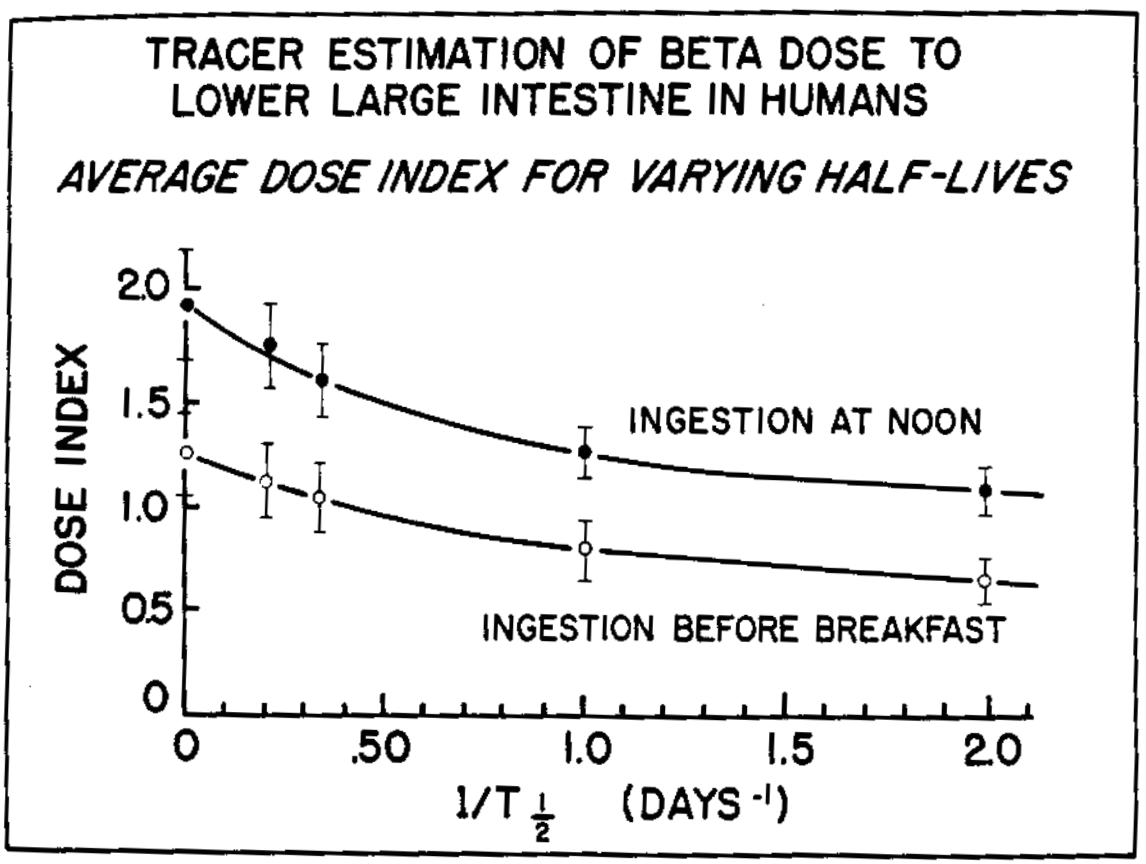

Fig. 3 Average dose index as a function of the reciprocal of the half-life for groups ingesting activity with the noon meal and two hours before breakfast. Limits on points indicate the standard deviation of the mean.

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).

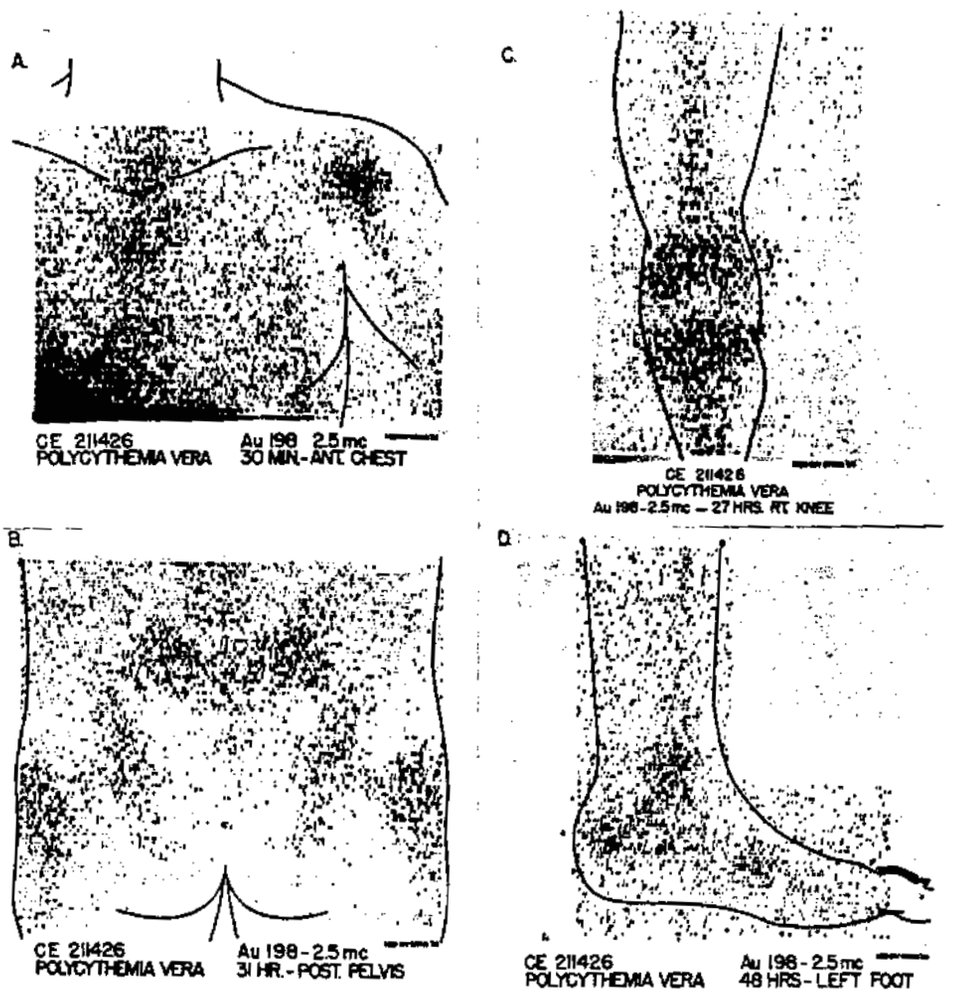

Fig. 1 a,b,c,d Bone-marrow scan showing the expanded marrow organ in a patient with polycythemia vera.

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.

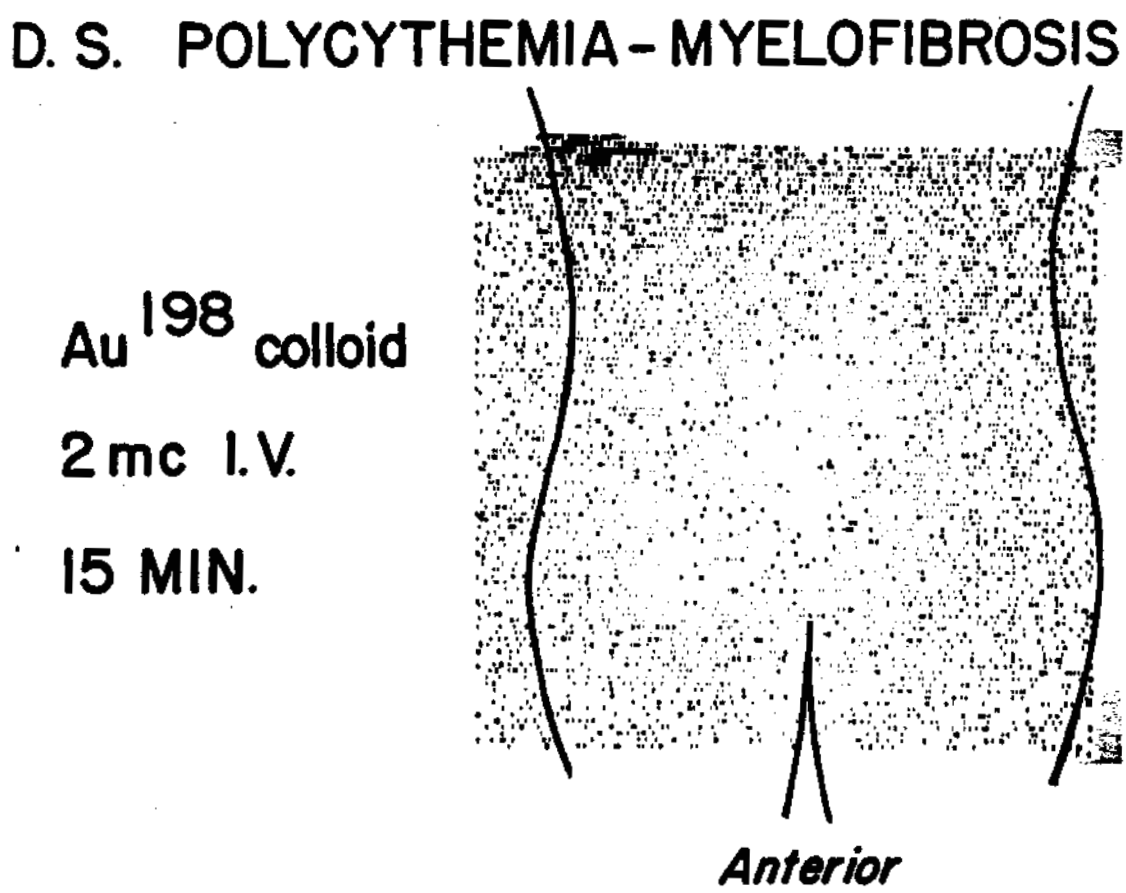

Fig. 2 Bone-marrow scan: myelofibrosis following polycythemia.

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).

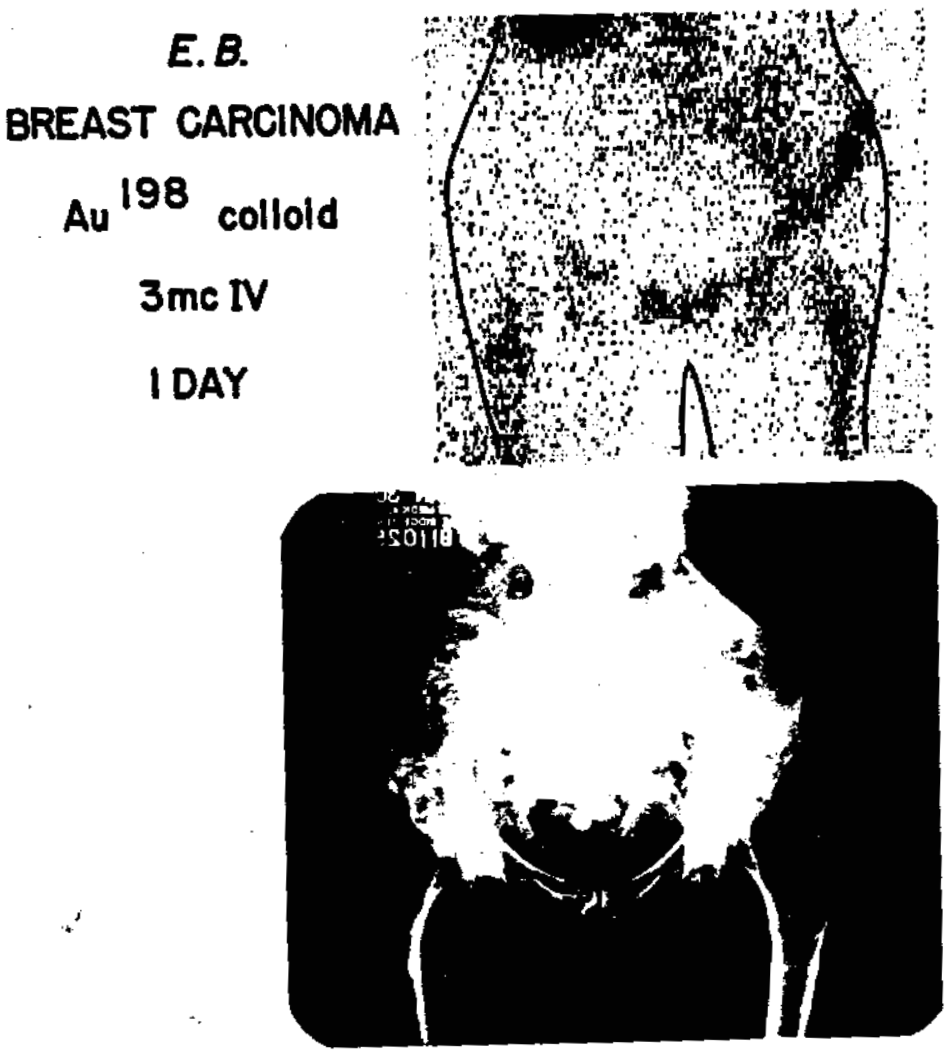

Fig. 3 Bone-marrow scan: local marrow lesion at the site of metastases and radiotherapy.

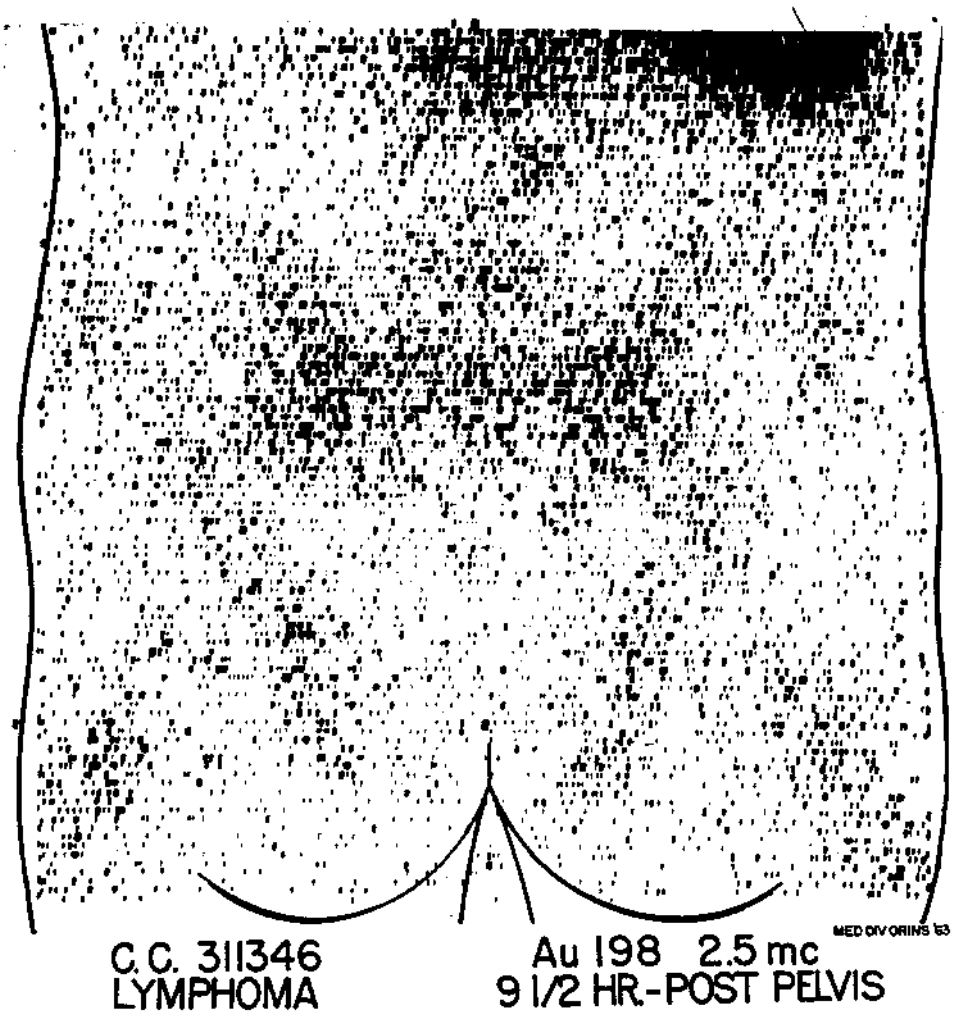

Fig. 4 Bone-marrow scan in a patient with lymphoma. Note the defect in fourth lumbar vertebra.

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.

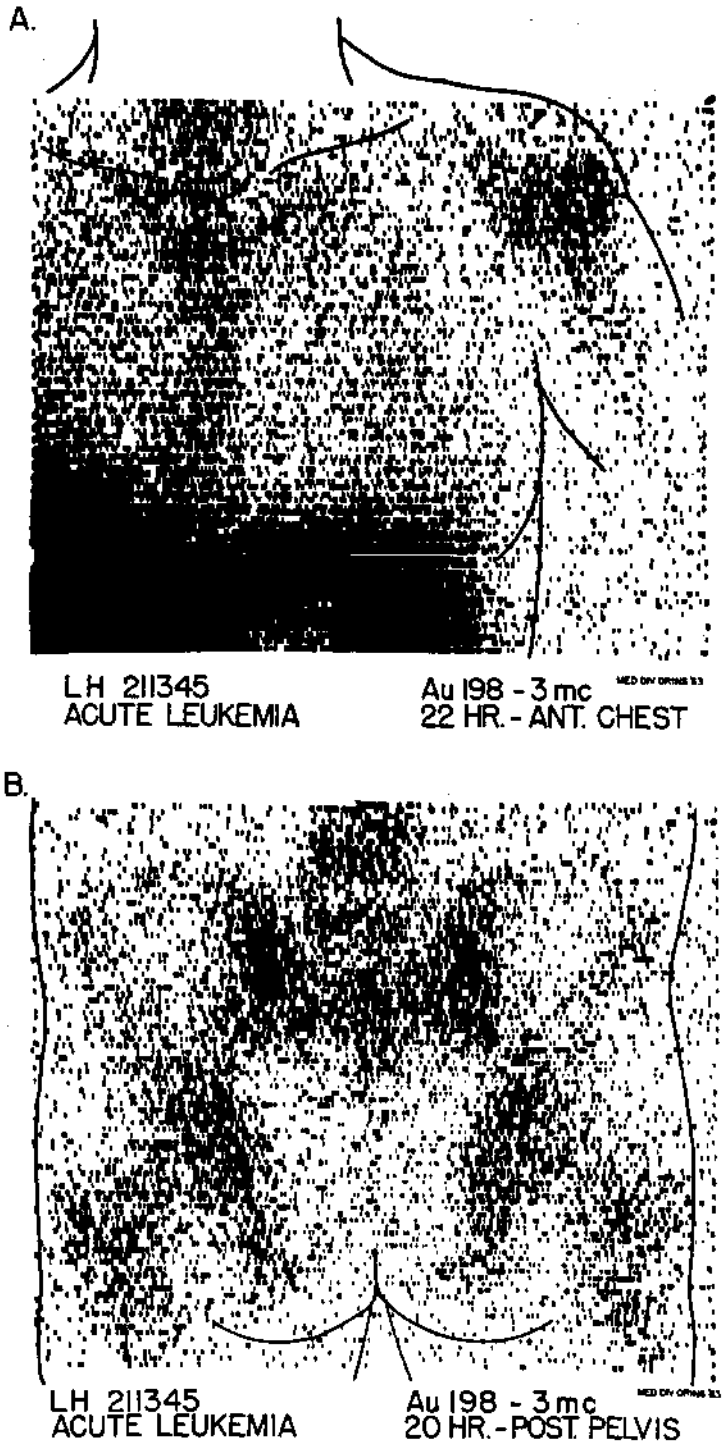

Fig. 5 a, b Bone-marrow scan in a patient with acute leukemia in partial control, showing normal or increased size of the marrow organ.

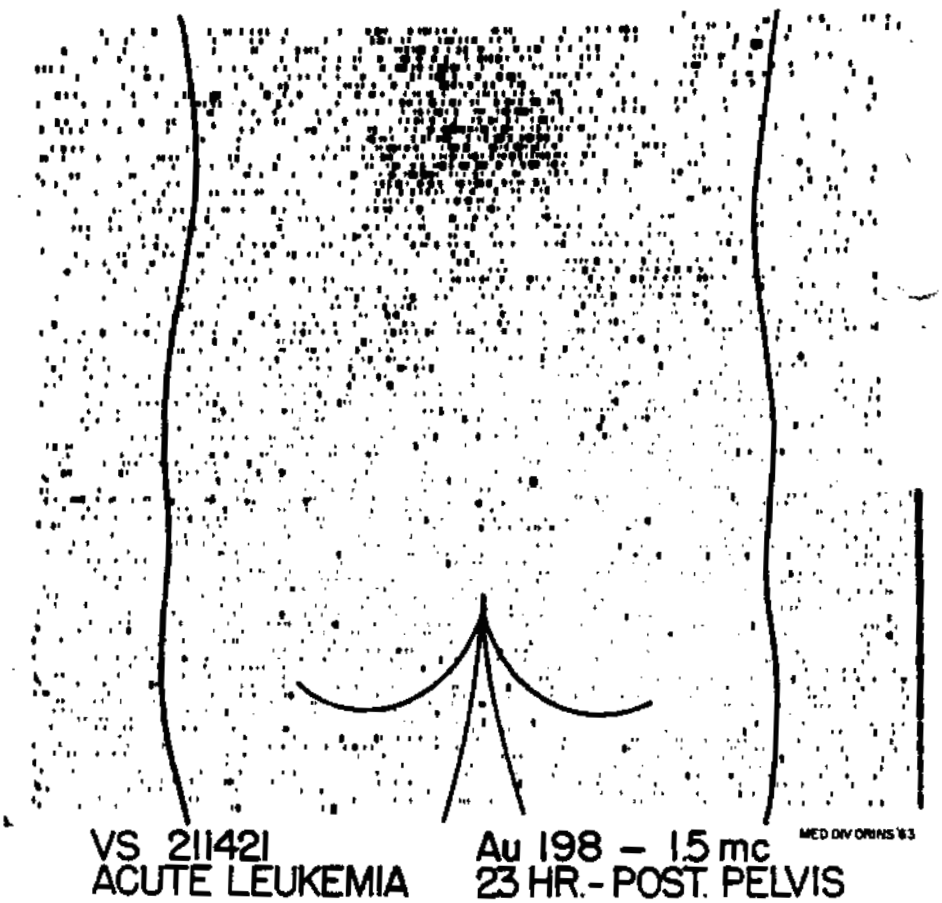

Fig. 6 Bone-marrow scan in a patient with acute leukemia, showing little uptake in the pelvis.

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.

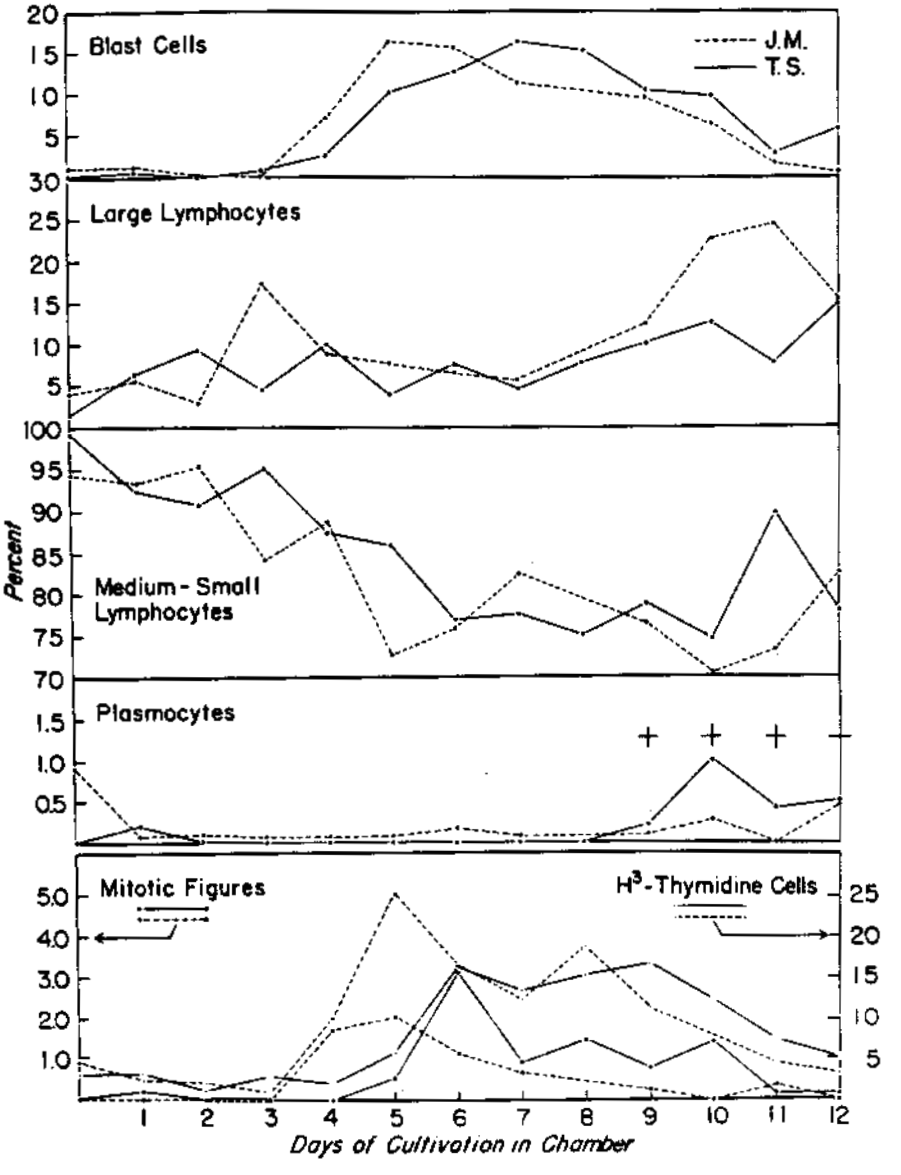

Fig. 1 Diffusion chamber; cultivation of two different human lymph node tissues.

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.

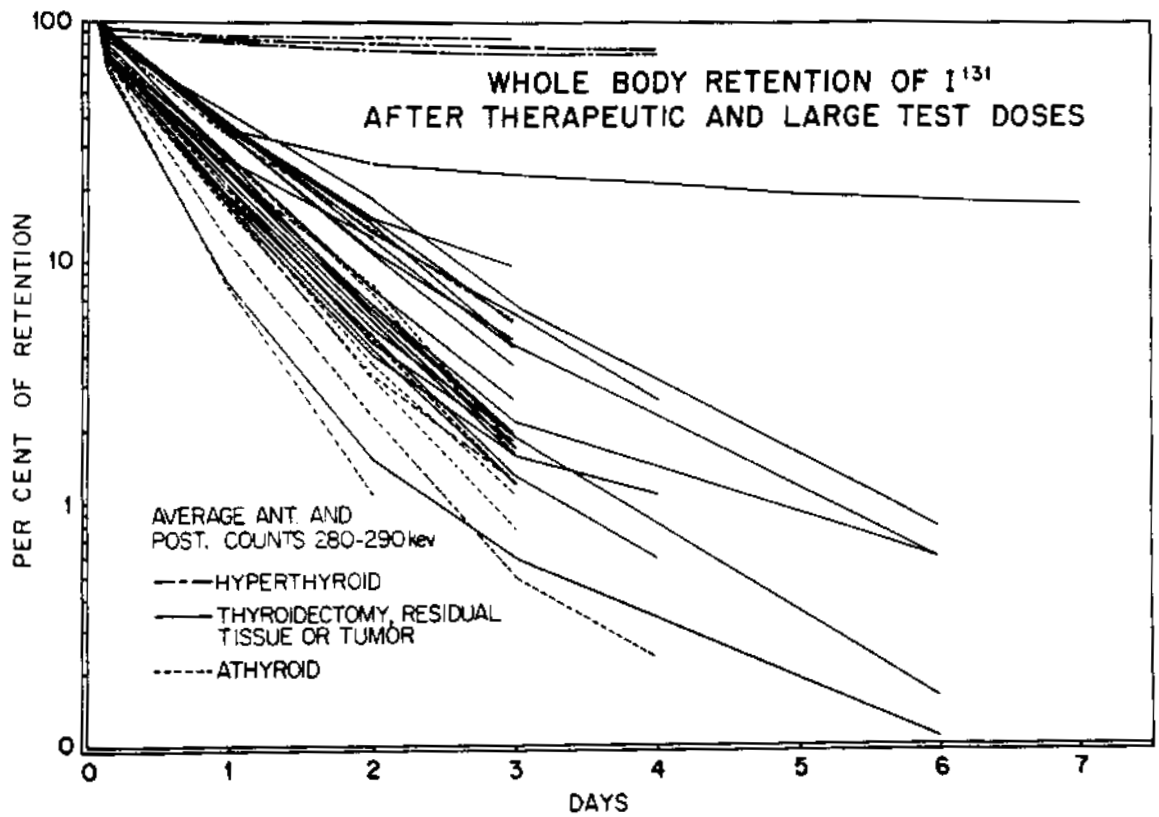

Fig. 1 **Retention of I^{131} in 33 patients for periods up to six days. All patients received either 5 millicuries or 100 millicuries.**

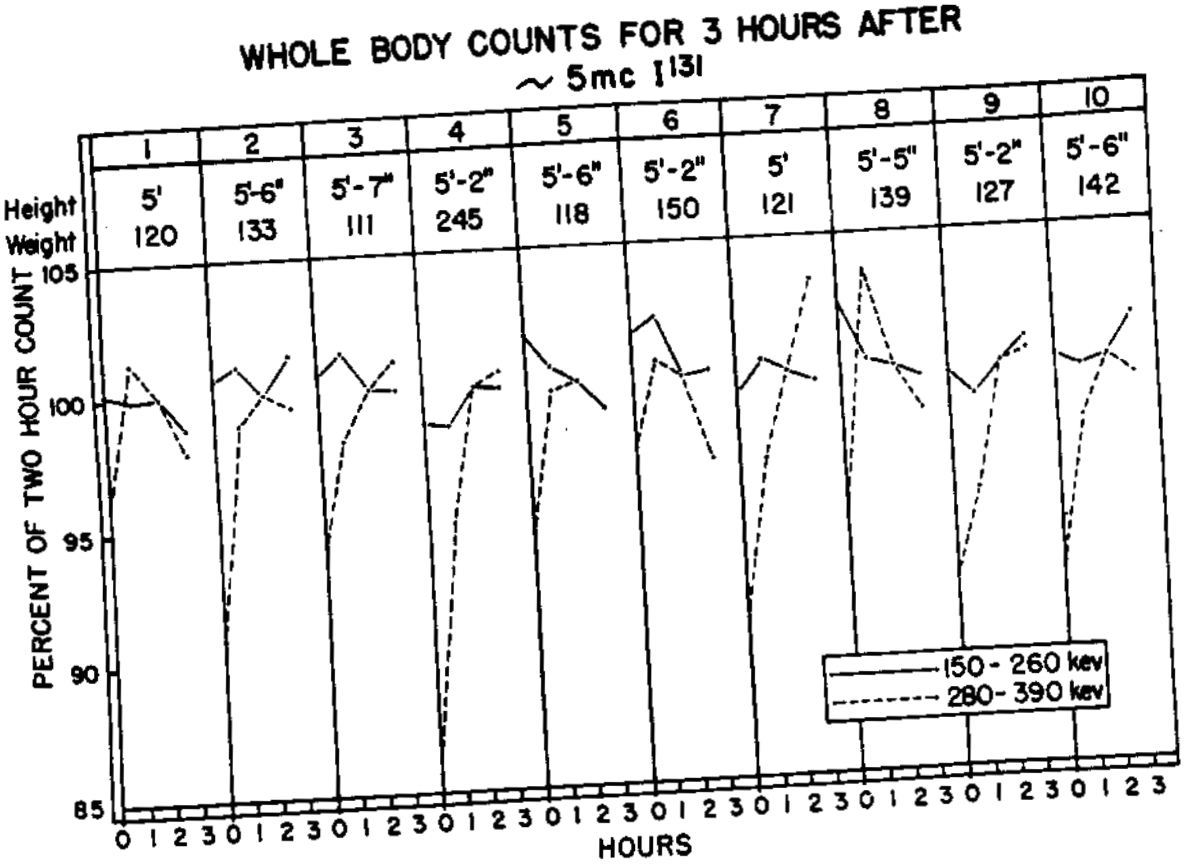

Fig. 2 Results obtained in 10 patients during the first three hours after a dose of 5 millicuries of I^{131} when the analyzer "sees" the 364 kev peak distribution is reflected by large variation in result. When only "scatter" is measured, distribution does not change the result to a large extent.

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

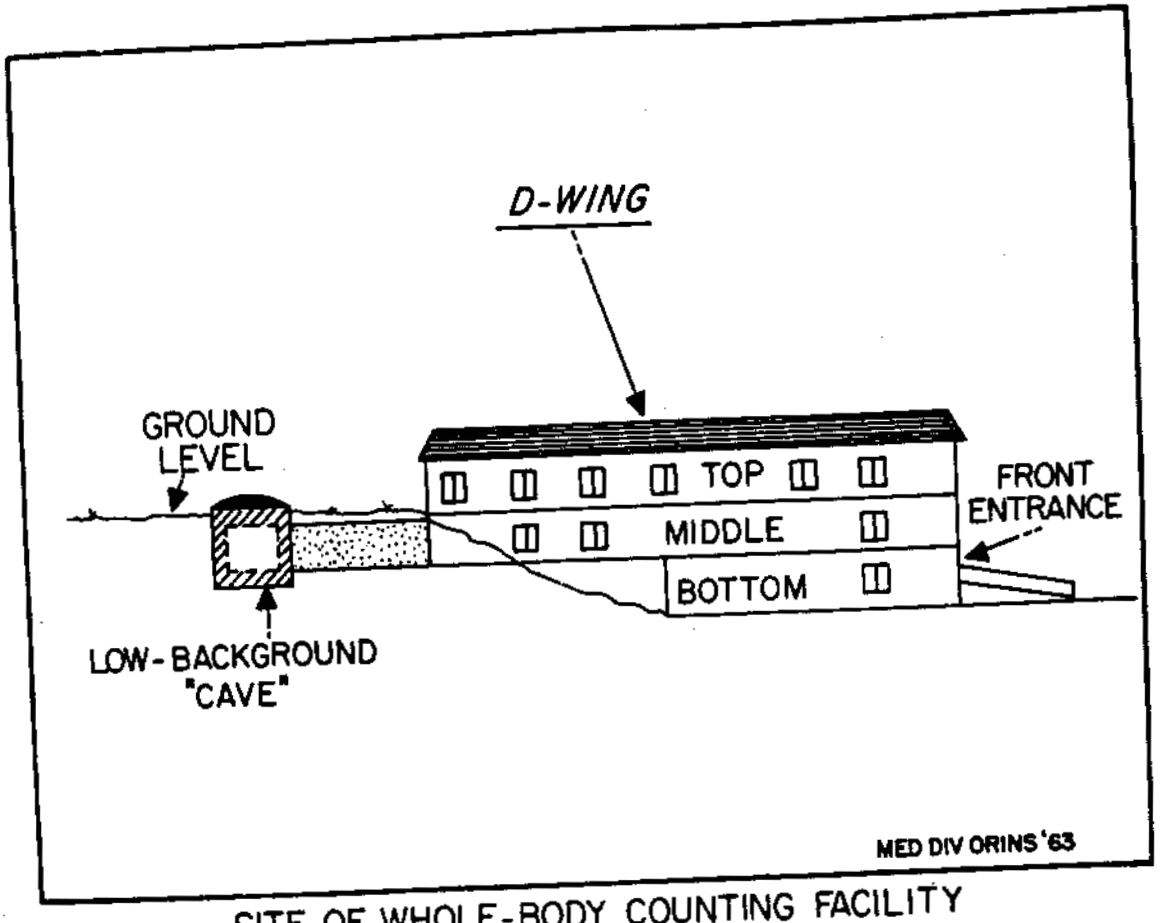

Fig. 1 Proposed location of patient-counting facility, at north end of ORINS hospital.

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.

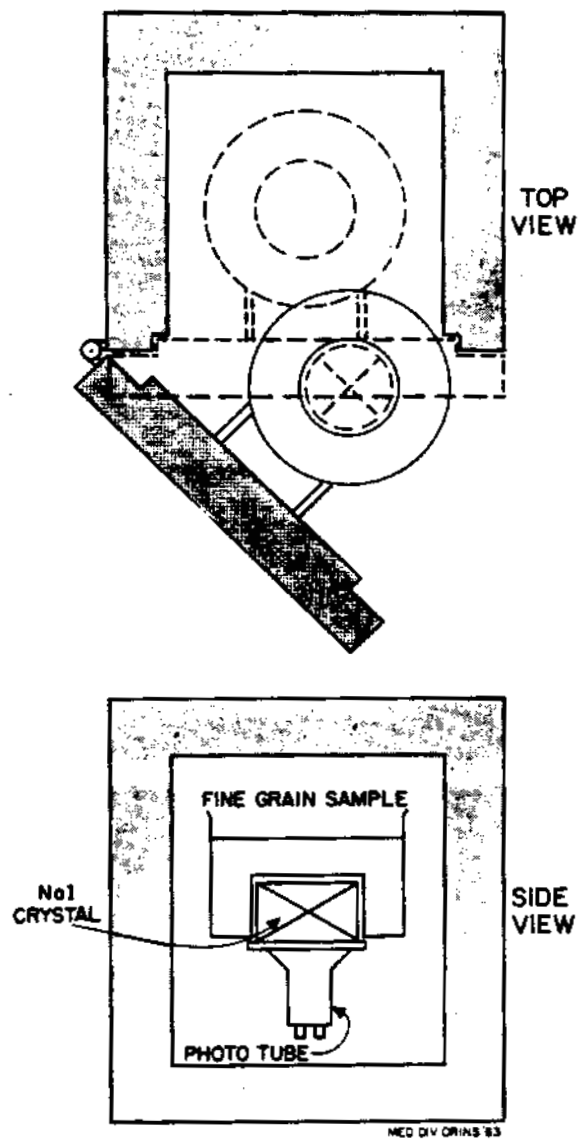

Fig. 2 High-sensitivity counter for large samples, showing the heavy shield and the detector arrangement. The material, about 3 in. thick, surrounds the crystal laterally, and covers its top.

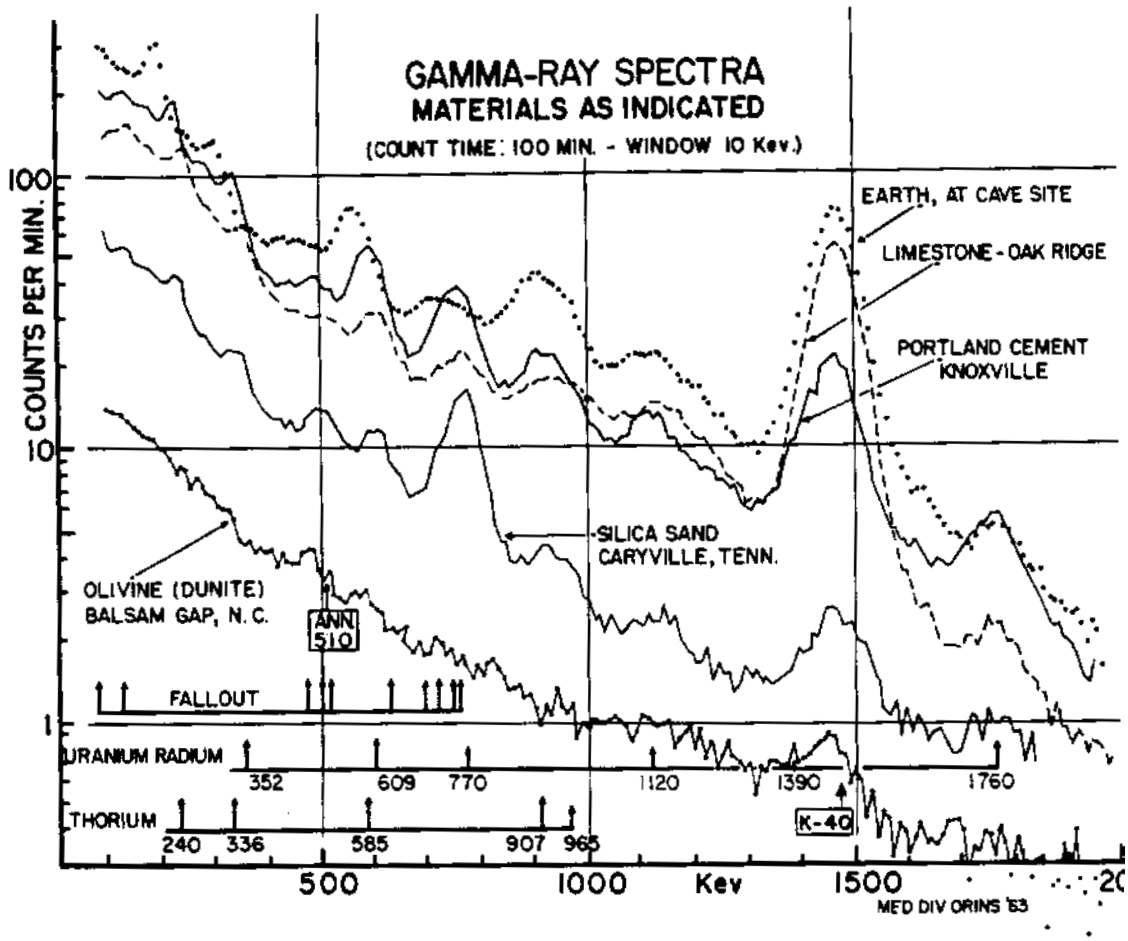

Fig. 3 Gamma-ray spectra for several materials, ranging from the "hot" earth at the construction site to the "cold" North Carolina olivine. The arrows near the bottom show the peak locations for a number of radionuclides of interest.

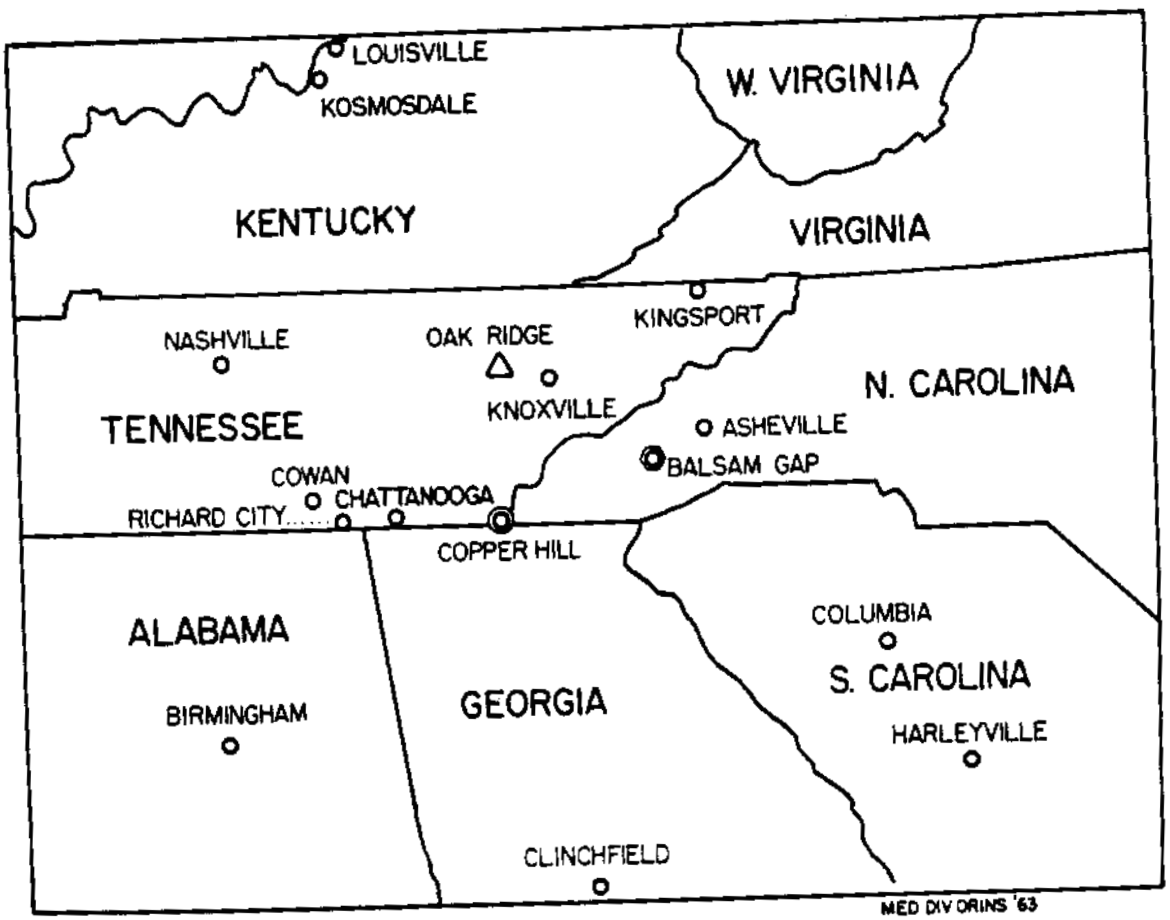

Fig. 4 Map showing locations where materials were procured.

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."

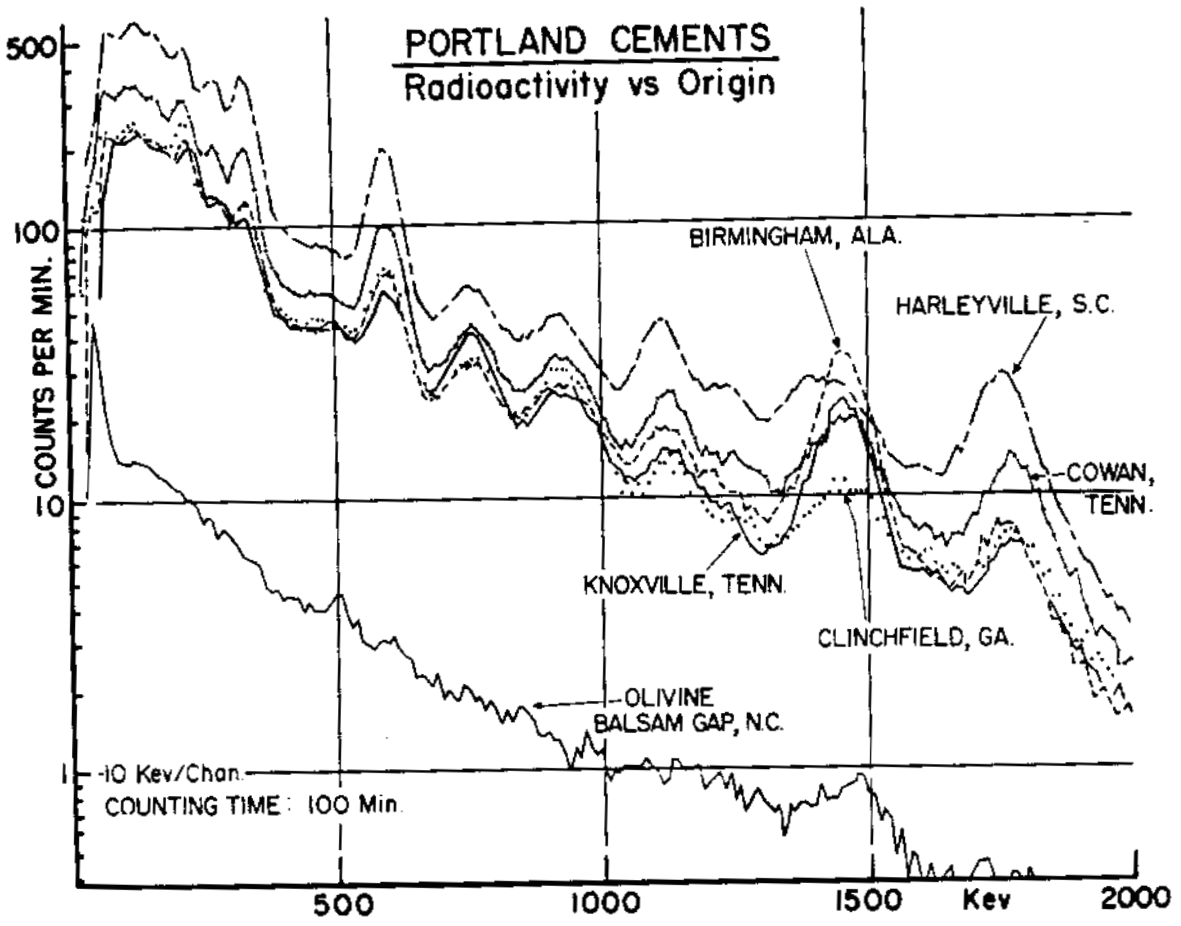

Fig. 5 Portland cements. To identify contaminants, see arrows at bottom of Fig. 3.

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."

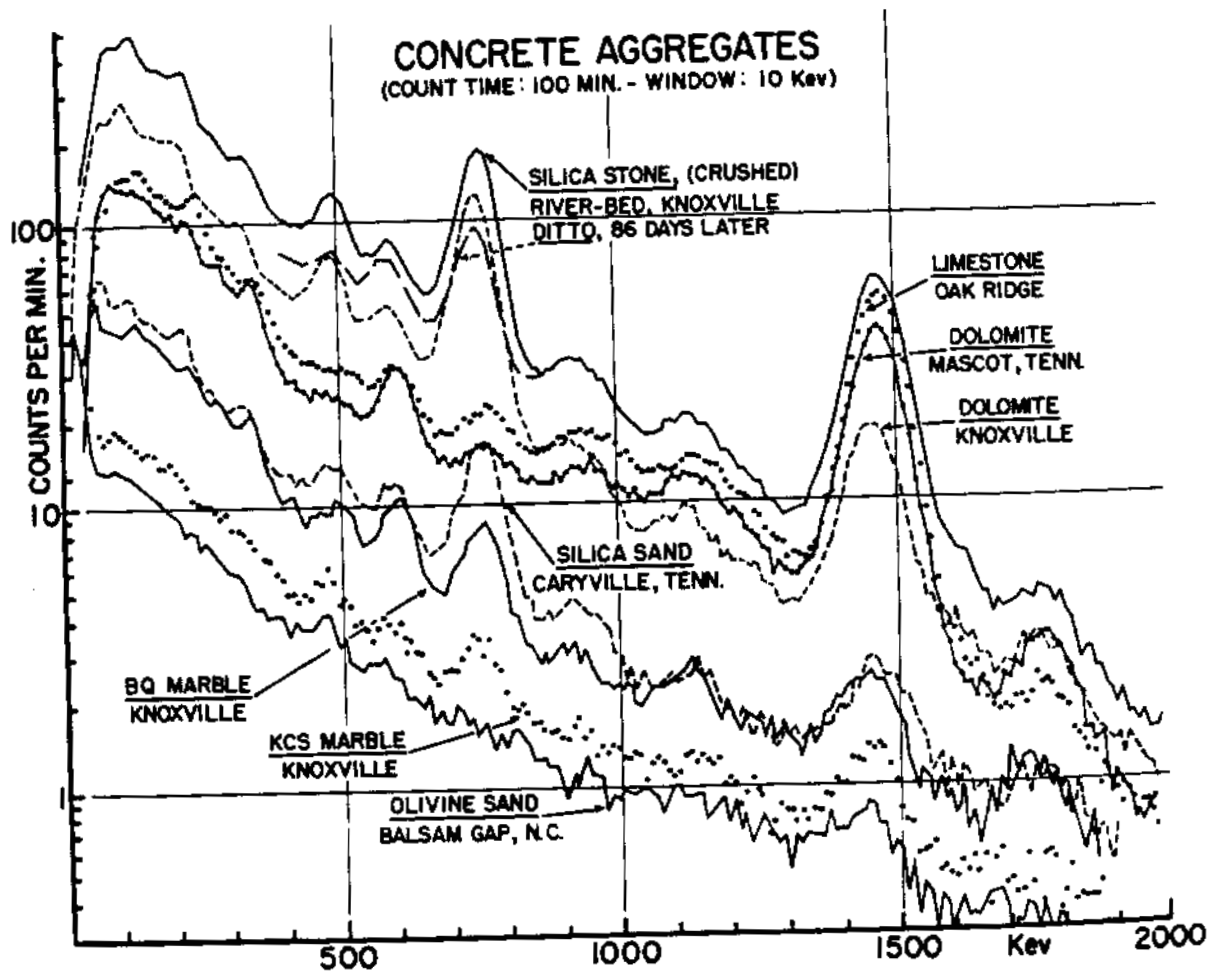

Fig. 6 Concrete aggregates. (See arrows at bottom of Fig. 3.)

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 Knoxvil

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

5) olivine (a solid solution of iron-magnesium orthosilicat 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 are

Figures 6, 7, and 8 show the spectra obtained from the 10-lit 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 amount 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 $2.5 a ton, looks a little better than the Caryville sand, and the marble can easily be crushed, washed, screened, and delivered in a ready-mad

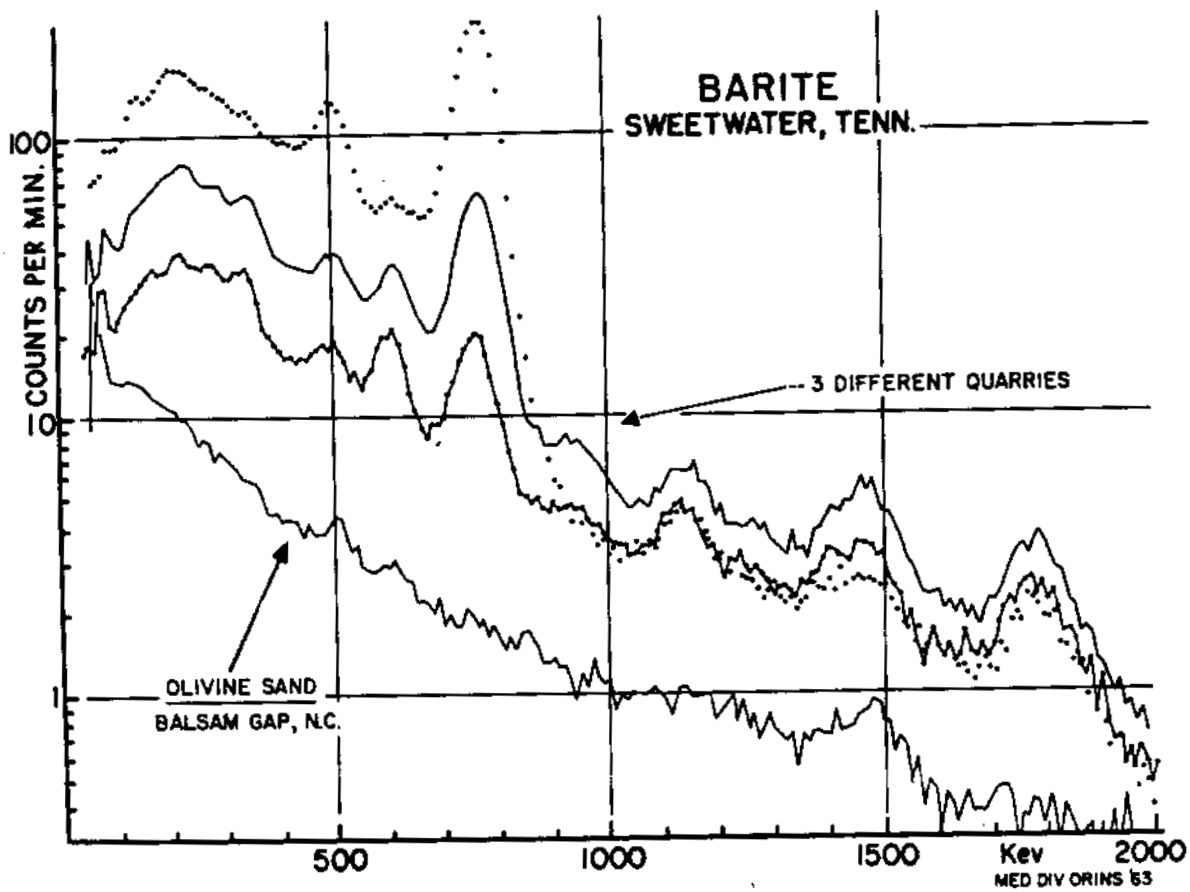

Fig. 7 Barite, from three different quarries near Sweetwater, Tennessee. (See arrows at bottom of Fig. 3.)

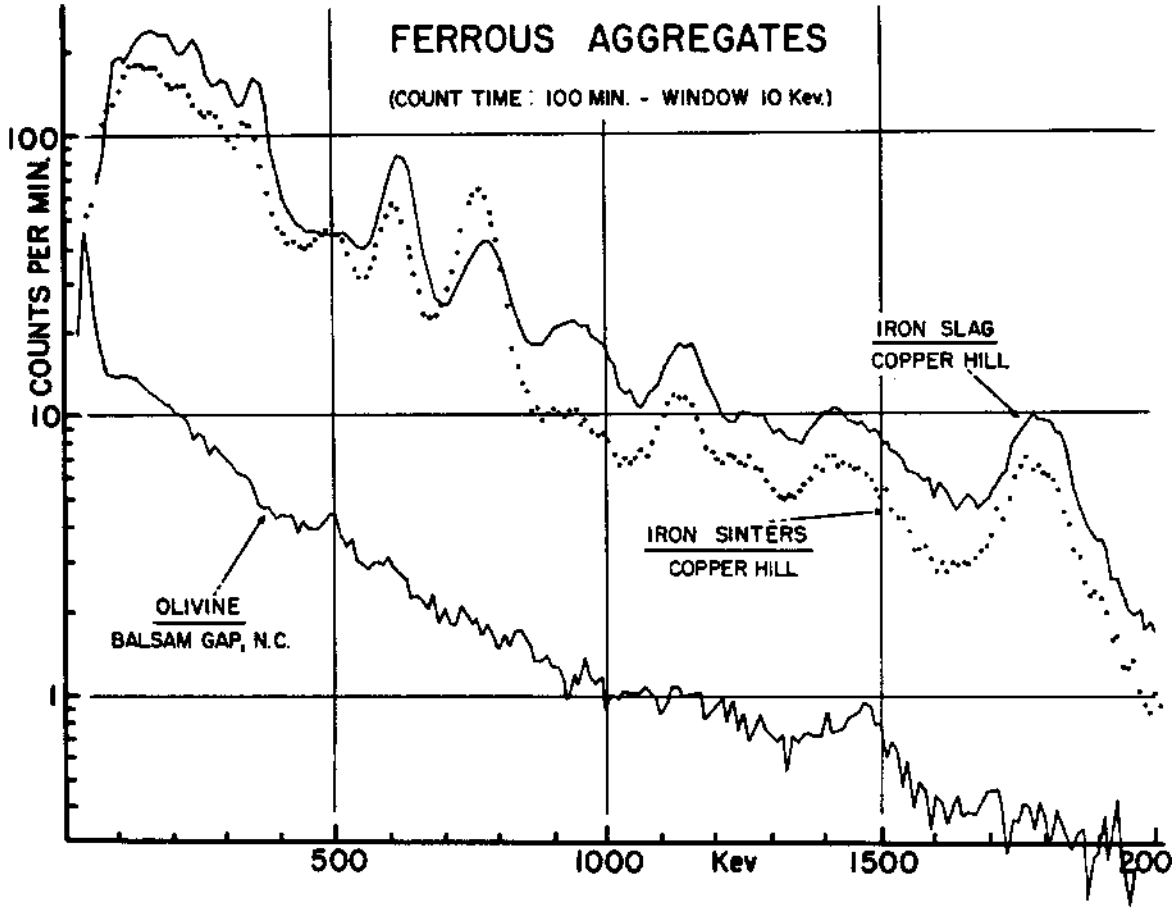

Fig. 8 Iron-rich by-products from a copper-smelting plant. (See arrows at bottom of Fig. 3.)

mixture of coarse and fine, needing only to be mixed with cement and water. A higher grade of Knoxville marble ("KGS", 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

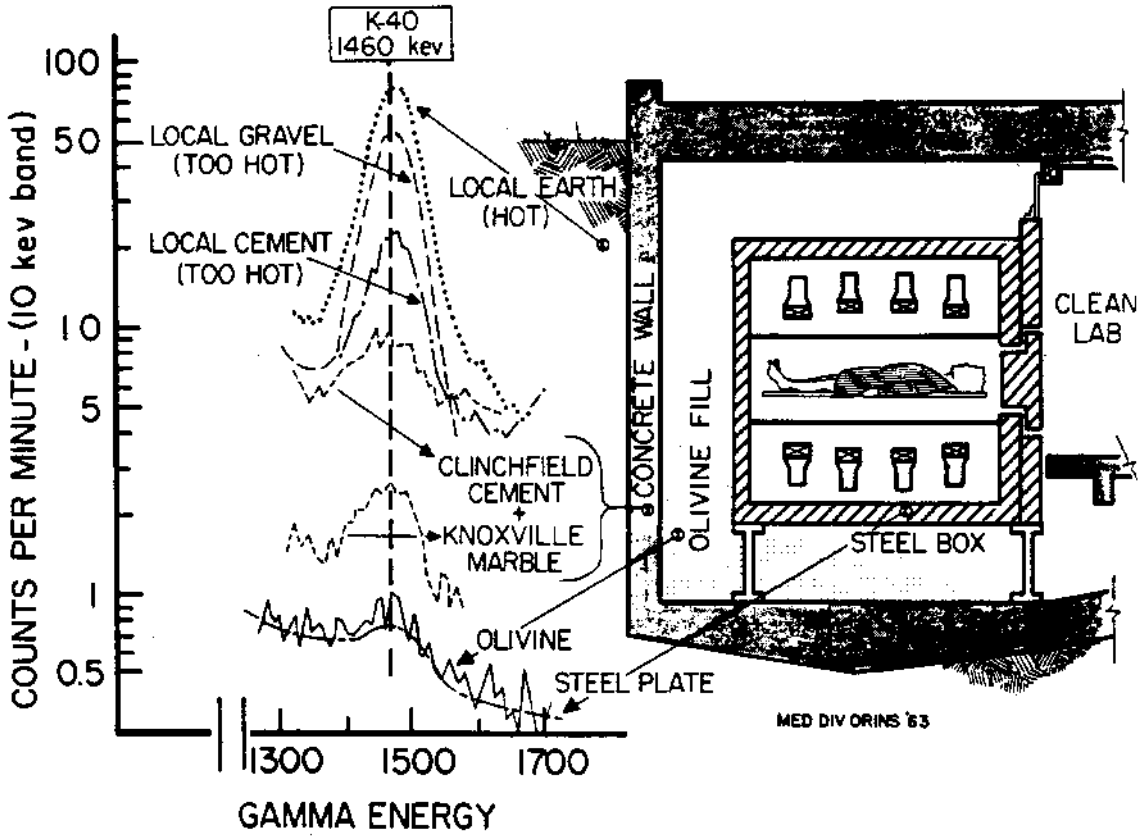

Fig. 9 Vertical section (schematic) through the patient-counting chamber, showing the wall structure. The K^{40} regions of the spectra are shown at the left, to indicate how the inherent radioactivity decreases progressively from the "hot" earth to the "cold" steel box.

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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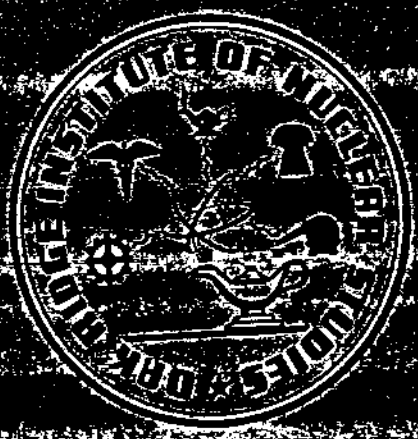
MEDICAL DIVISION RESEARCH REPORT FOR 1963



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FOREWORD

The Scientific Research Report for 1963 reflects a phase of expansion and some changes in emphasis of the Medical Division program.

Doctor C. C. Lushbaugh, formerly of the Los Alamos Scientific Laboratory, joined the Senior Staff as Chief of Applied Radiobiology. He brings a broad experience in pathologic effects of radiation, in clinical and animal whole-body counting techniques, and in experimental pathology. Mrs. Gretchen Humason, Assistant Scientist in histology, also formerly of Los Alamos, has joined his group.

Doctor J. N. Bollinger, on completion of his Ph.D work at Texas A&M University, joined Doctor Snyder's group working on biochemistry of bone-marrow lipids and the changes induced by irradiation.

The Clinical Staff was augmented by the return of Doctor Ryosaku N. Tanida, who was formerly a trainee at ORINS and more recently Clinician at St. Luke's Hospital, Tokyo.

The Board of Directors authorized the rental of a TR-48 analog computer (E.A.I.) in support of Doctor Kretchmar's program. This instrument has been applied to compartmental models related to a variety of experiments in the Division program.

A laboratory was designed, constructed, and equipped for Cytogenetics. Mr. Paul Eide and Miss Margo Steinman, both research assistants, have gotten the program under way. During this developmental phase, the program is under the supervision of Doctor Gengozian.

The staff is indebted to Elizabeth Anderson, Technical Editor; John Flora, Illustrator, and Rush King, ORINS Photographer for their excellent contribution to this report.

Gould A. Andrews, M.D.
Chairman

STUDIES OF RADIATION EFFECTS

This broad category includes much of the clinical part of the Medical Division programs and some studies in preclinical areas. Certain other studies could be included here but have been set aside as special categories; i.e., those dealing with lipids, amino acids, and the immunology program.

Some of the clinical activities of the Division are long-range investigations not covered by abstracts in the present summary. The most important of these is a study of the diagnosis and treatment of cancer of the thyroid. The staff has also a special interest in hematologic diseases and in studies of bone-marrow distribution and function. Efforts to achieve homologous bone-marrow grafts in patients have not been made recently, but marrow-storage techniques have been set up and further efforts are to be made in this area.

Single Doses of 50 and 100 r Total-Body Irradiation for Leukemia and Lymphoma (D. A. White, R. M. Kniseley, F. Comas, B. W. Sitterson, and G. A. Andrews)

A group of patients was given 50 and 100 r of total-body irradiation. Since the 1962 Research Report a few additional patients have been treated and further analysis has been made of the results. They were treated in the ORINS cesium radiation facility at a dose rate of 0.74 r per min. Treatment was given in a single dose; momentary interruptions were necessary in a few cases. The doses listed are measured in air and apply to all the area occupied by the body. The actual absorbed doses at the deepest part of the body in an adult fall to as low as 47% of the stated dose.

To date 50 treatments have been given to patients with leukemia or lymphoma. None were receiving or had recently received therapy that might alter the clinical or hematologic findings. Most were newly diagnosed patients with leukemia or lymphoma. Each patient had hematologic studies on the following days relative to therapy: -3, 0, 1, 2, 4, 7, 14, 21, 28, 35, and 42.

Three groups of patients include large enough numbers for evaluation at this time:

50 r in Chronic Lymphocytic Leukemia

Eleven treatments were given to this group. Average total white-count values (chiefly lymphocytes) fell during the first week to 68% of the pretreatment level. A more gradual decrease in the next two weeks led to a minimum value at three weeks that was 56% of the original value. This level was maintained through the next three weeks of follow-up. Average absolute granulocyte values, which averaged about 3000 per cu mm at the outset, showed a slight rise at two weeks and a fall to 2200 at the forty-second day. Platelet values showed a slight decrease at the third and fourth weeks, with subsequent recovery by the sixth week. Average hemoglobin values showed no important changes.

50 r in Chronic Myelocytic Leukemia

Only 5 patients with this disease have been treated so far. All showed a gradual fall in total leukocyte count during the first three weeks, reaching a level that averaged 46% of the initial level. Platelet values showed wide variability; three patients with high initial counts had some further elevation during the first two weeks and then a fall to somewhat below original levels during the fifth and sixth weeks. Two patients with normal platelet levels at the outset showed less consistent changes. All five patients had a slight fall in hemoglobin values during the first week and a gradual rise during the next five weeks to levels averaging about 1 g higher than the initial level.

100 r in Lymphosarcoma

Fifteen treatments were administered to patients with this disease. There was a distinct fall in leukocyte count during the first week, down to an average of 68% of the initial value, with a slight further decrease during the latter half of the 6-week period of study. Platelet values did not show a consistent early rise, but there was a distinct fall during the second, third, and fourth week to an average level 31% of the initial level. There was some suggestion of a return upward during the sixth week. Hemoglobin values on the average fell about 0.5 g during the six weeks after treatment.

Serum Uric Acid Values

This determination showed considerable variability. In the lymphosarcoma (100 r) and chronic lymphocytic (50 r) groups, a large proportion of the patients showed a rise in uric acid during the first week after exposure and a gradual fall during the next three weeks.

Clinical Effects

The overall results of low-dosage total-body irradiation given to patients with chronic leukemias and lymphosarcoma have been gratifying. As might be expected, most patients with chronic lymphocytic leukemia had significant hematologic and clinical improvement after 50 r. All patients with chronic myelocytic leukemia given 50 r had hematologic and subjective improvement. One of these patients remained in excellent control for a year without additional therapy. The majority of the patients with lymphosarcoma had marked regression of enlarged peripheral nodes and relief of symptoms. Our findings suggest that the therapeutic benefits from 50 r or 100 r total-body irradiation in patients with chronic leukemia or lymphosarcoma are entirely comparable to those obtained from more conventional forms of treatment.

Fourteen graphs are available showing hematologic and uric acid data. A typical example is shown in Fig. 1.

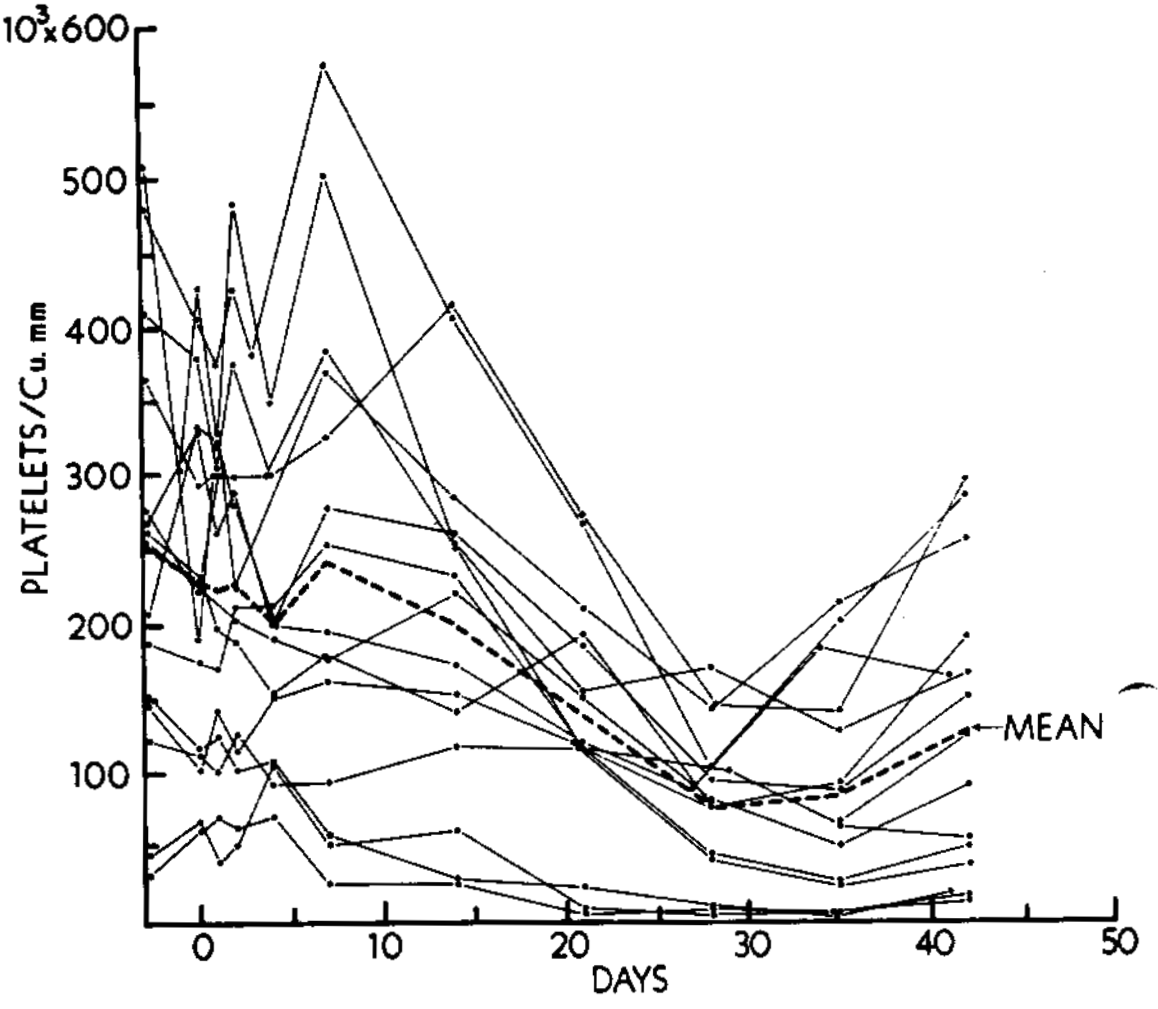

Fig. 1. Platelet values after 100 r total-body irradiation for lymphosarcoma.

Storage and Viability Studies on Frozen Human Bone Marrow
(Karl F. Hübner)

In storage of human bone marrow for infusion, one needs a container of a useful size made of material that is safe for personnel handling the frozen samples and that allows storage of cells in a state suitable for administration. Ideally the sample to be transfused should be collected and stored in a single container to avoid unnecessary handling of the cells, and to avoid possible bacterial contamination. To meet the physical conditions necessary for a desired freezing rate for all the cells (1° C/min), the container should not exceed a thickness of approximately 11 mm. Leakage must be minimized because of the danger of explosion during thawing if liquid nitrogen has entered the container through any minute holes.

Containers tested were flat aluminum ones (85 ml); plastic Fenwal transfer bags (150 ml, 300 ml); 1-ml, 5-ml, and 10-ml silica glass, machine-made ampules; and 55-ml hand-made glass ampules. The aluminum containers supplied by the Linde Company proved to be safe as far as breaking and leakage are concerned. On testing plastic Fenwal bags, the total loss was 50% during freezing or thawing. Approximately 204 glass ampules have been used for liquid-nitrogen freezing and storage of biological material to date. Eight ampules exploded in thawing owing to improper heat sealing. There was no breakage during the process of freezing.

The 55-ml ampules, made by hand, were unsafe. Five of seven broke and were lost on freezing. The small-size machine-made ampule is a workable vessel. Human bone marrow has been preserved at -196.8°C after slow controlled freezing under protection with dimethyl sulfoxide (10% final concentration) in 1- to 5-ml glass ampules on eight different marrow samples. Cell counts were performed before freezing and immediately after rapid thawing in a 40°C water bath.

The viability of these cells after storage for one, two, and three months has been tested by incorporation of tritiated thymidine and tritiated cytidine. The numbers of labeled cells per 1000 nucleated cells were compared. The average loss of nucleated cells per cubic millimeter was about 30% and the average loss of labeled cells ranged between 50 and 25%. The morphology of many cells was found to be altered. It is felt that the loss of cells is due to mechanical stress (centrifugation, washing, etc.), and the freezing and thawing. The duration of storage does not seem to alter these figures greatly.

Changes in the Frequency Distribution of RBC Volumes in Disease
(C. C. Lushbaugh)

The development of the Coulter Counter for determining by electron means the number of suspended particles led to development of methods for counting red blood cells (RBC) and white blood cells (WBC) much more accu ately than was previously possible. Recently, taking advantage of the propor tionality of the height of pulses to volume of the cells, Brecher and others have developed methods for sizing RBC. Frequency distribution curves (FD of RBC volumes resembling Price-Jones curves of RBC diameters are ob therby quite rapidly. A commercially available 25-window pulse-height ana- lyzer attachment for the Coulter Counter greatly facilitates such studies. Th adaptation of multichannel (100 to 400) pulse-height analyzers to the Coulter Counter was found feasible and was installed recently here. Studies with this device show that the resolution of the spectrum-like curves is greatly in- creased by use of 100 narrow instead of 25 relatively wide windows as in the Coulter analyzer. Figure 1 shows FDC obtained at ORINS for normal and microcytic human RBC at the same electronic particle-counter setting.

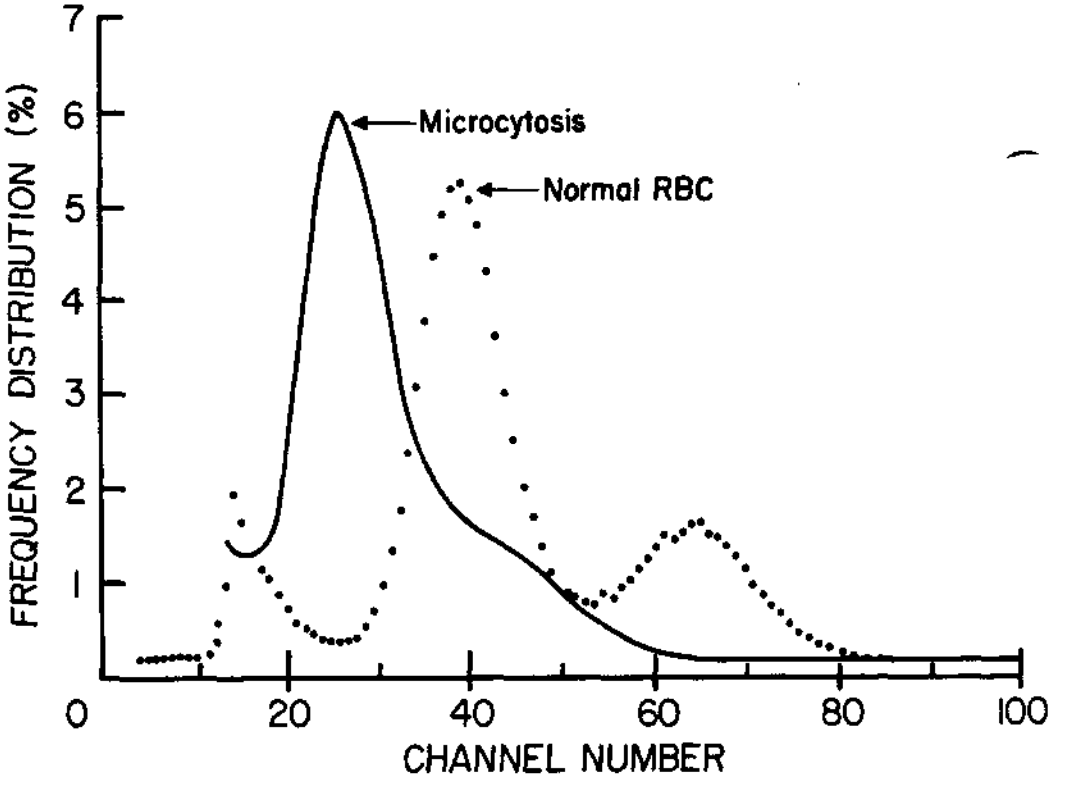

Fig. 1. Frequency distribution profiles of RBC volumes of normal man (MCV 97) and microcytosis in polycythemic patient (solid line).

The 100,000-cell aliquot in both studies appears to be composed of two subpopulations with different modal frequencies. By using Fe^{59}, we have shown in rabbits that the smaller group of large cells (on the right) consists of the most recently produced RBC. Experiments to be reported reveal that the cells composing the left-hand face of the FDC are the oldest cells and most sensitive to hemolysins and destruction by physical and chemical means. Investigations in progress indicate that electronic determination of the FDC is a clinically reliable means for determining microcytosis or macrocytosis directly, and for following changes in RBC size and age distribution resulting from disease or therapy. Studies in progress with fetal and neonatal mice and genetically anemic mice, with and without transplanted normocytic marrows, have suggested that the difference in modal size of the two subpopulations (young and old) is constant in the steady state of health in adult animals. The time required for a cell to change from the large to the smaller modal size (maturation time) appears relatively short compared to the amount of time spent in adult life around the smaller modal size. Experiments with phenylhydrazine in rabbits indicate that maturation time for rabbit RBC after their delivery into the peripheral blood is less than 10 days but greater than 7 days. An investigation is in progress to determine whether this time can be determined more precisely in animals and man after total-body irradiation has curtailed RBC production. Other studies being conducted with this apparatus are directed toward determining variation in size of platelets and WBC in blood dyscrasias.

Breast Cancer and Cytologic Dysplasia in Many Organs After Therapy with Busulfan (Myleran) (Bill M. Nelson and G. A. Andrews)

At autopsy a woman who developed breast cancer while being treated with busulfan for chronic granulocytic leukemia was found to have large bizarre cells in diverse epithelial tissues. These cytologic changes, similar in many respects to those seen after irradiation, have recently been described in a few other case reports. In our patient and in two previously reported cases the changes in the epithelium of the cervix uteri, as shown in Papanicolaou smears, were regarded as indicative of malignancy by competent pathologists. Similar problems in cytologic diagnosis might arise from the examination of sputum or bronchial washings because the abnormal giant cells were found in the pulmonary alveoli. Other tissues involved multicentrically included urinary bladder, pancreas, liver, adrenals, kidneys, esophagus, pituitary, skin, and breasts. The relation of these cytologic changes to the development of the breast carcinoma in our patient remains obscure, but the possibility of a carcinogenic effect of busulfan is raised. Even if no such potential were present, the observation of the enlarged abnormal cells has clinical significance and should be kept in mind when diagnostic cytologic studies are done on patients treated with busulfan.

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

International (ICRP) recommendations for maximum permissible concentration of various radionuclides in water and air are based on a Standard-Man model of average behavior. Previous work with animals has shown a high degree of variation among subjects where the intestinal tract was the critical organ. Estimations of dose to the lower large intestine were made on human subjects by the use of a tracer technique.

A paper giving details of the study on 54 clinical subjects has recently been published (Health Physics 9, 915-920, 1963). To date a total of 78 subjects has been studied. Arrangements are being made to continue the study using normal (nonclinical) subjects.

The results to date lend themselves to certain tentative generalizations. The following points appear to be of importance:

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

2) A sizable 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 expe ence a dose three times that of the Standard Man and 6% as much as five times that of the Standard Man.

3) As expected, the dose experience of the population studied showed a wide variation. The average dose was, however, only 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 meal time) or through water (between meals) does seem to grossly affect the dose received.

If the results with this population sample are borne out in further studies, possibly some adjustments in the assumptions for the Standard Man are in order. If, for example, in the Standard-Man assumptions for the lower large intestine, the entrance time into the lower large intestine is changed from 13 to 18 hr and the in-residence time from 18 to 31 hr, the average dose index will be essentially independent of half-life.

Irradiation Under Anoxia (Frank V. Comas)

This is a continuing project and the Research Report for 1962 (USAEC Report ORINS-42, pp. 2,3) presented results that have received further analysis and an interpretation since that report.

In this experiment the degree of radiation effect on a transplantable rat tumor and on femoral bone-marrow cells in vivo was compared when irradiation was given under normal oxygenation and under anoxia. Anoxia was induced by temporarily occluding the blood supply to the left leg. Radiation effect was gauged by determining the duration of depression of DNA synthesis as measured by means of thymidine-H^3 incorporation into DNA of tumor and bone-marrow cells. A plot of radiation dose versus the log of duration of DNA synthesis depression gives reasonably straight lines. On comparing the slopes of oxygenated with anoxia bone-marrow lines, it is found that vascular anoxia "protected" bone marrow by a factor of 2.0 (Fig. 1). The protective effect of anoxia on the tumor was less: 1.6 (Fig. 2). These results indicate that, in the system tested, the net effect of irradiating the tumor under anoxia is to increase its radiosensitivity by 2.0/1.6= 25%.

The lines relating radiation dose to duration of depression of DNA synthesis in bone marrow intersect the time axis at 12 hr for zero radiation dose. A tentative interpretation is that this time corresponds to the duration of mitosis and the G-1 period of these cells; it is predicated on the assumption that (1) surviving irradiated cells are blocked in the G-2 period for a time that is proportional to radiation dose; (2) when the block in G-2 is removed, the G-1 period has the same duration as that of nonirradiated cells.

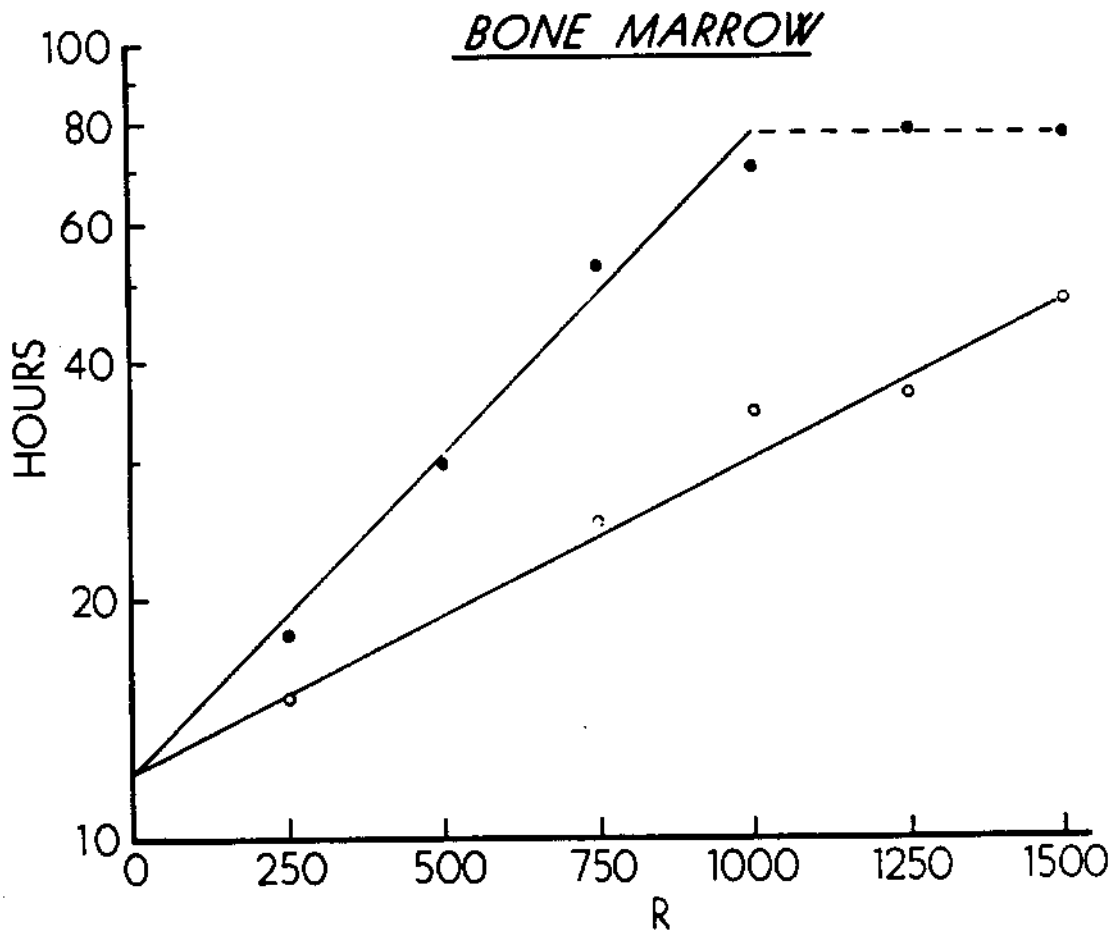

Fig. 1. Plot of duration of DNA synthesis depression for oxygenated (closed circles) and anoxic (open circles) bone marrow, versus radiation dose. The ratio of the slopes of the two lines is 2.03.

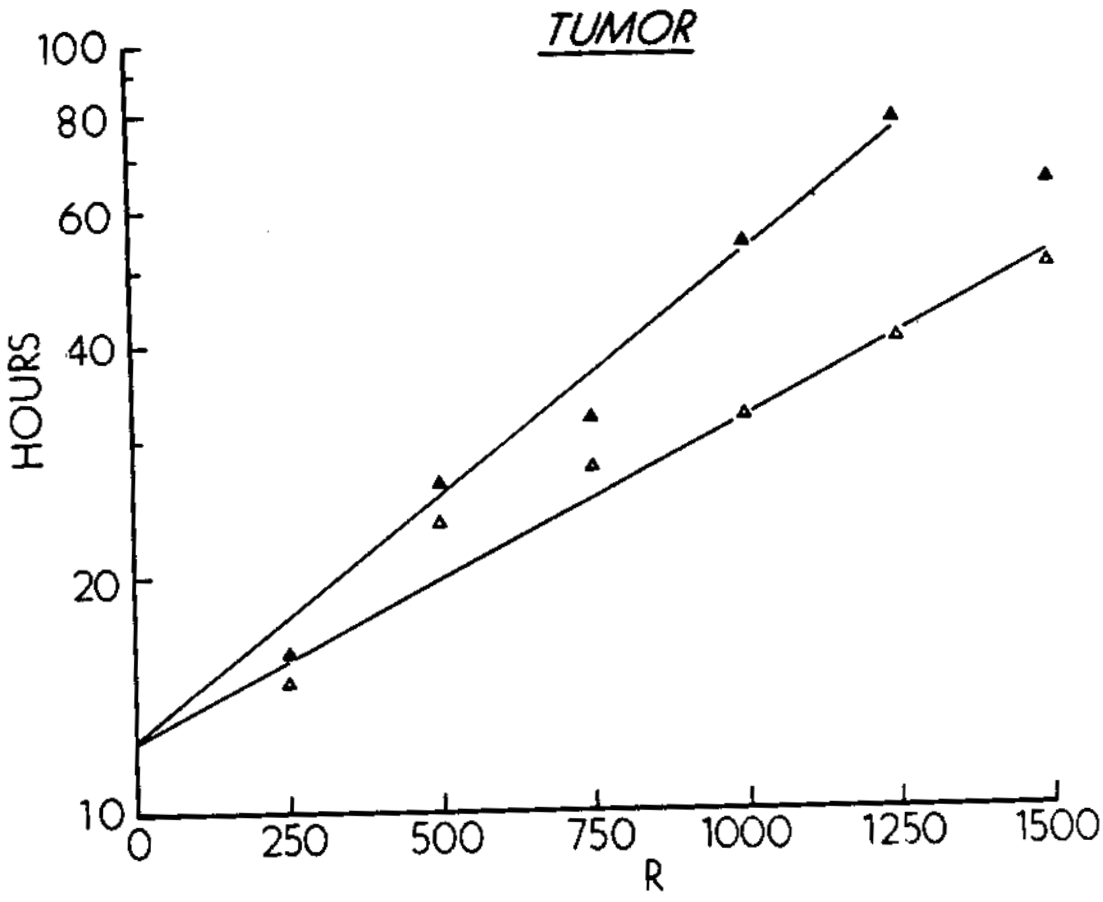

Fig. 2. Plot of duration of DNA synthesis depression for oxygenated (closed triangles) and anoxic (open triangles) tumor, versus radiation dose. The ratio of the slopes of the two lines is 1.58.

LIPID METABOLISM AND RADIATION

This program is concerned primarily with lipid metabolism in bone-marrow cells, but a long-range study involving tissue lipids available from cancer patients (especially leukemics) is also under way.

Elucidation of the mechanism of adipose-cell formation in bone marrow, especially that resulting from total-body irradiation, and the effect of these accumulated lipids on cell repopulation in host and donor marrow are the major objectives of this work. A specific facet, now being initiated by a newly appointed staff member, Dr. James Bollinger, concerns adipose-cell depletion in homologous disease. A smaller portion of our program is devoted to evaluating fats as radioprotective agents. Our concern here has mainly been the glyceryl ethers, normal constituents of hematopoietic tissue. Abstracts of some of the more significant results obtained over the past year describe our progress to date.

Bone Marrow Lipids and their Metabolism

(Fred Snyder, with the technical assistance of Edgar Cress and Nelson Stephens)

Bone-marrow lipids, even in highly active hematopoietic areas, are quite similar to adipose tissue lipids found in the genital and perirenal areas consisting primarily of triglycerides. A sudden change in bone-marrow activity brought about by total-body irradiation, in which replacement of blood-forming cells by simple adipose cells occurs, results therefore predictably in essentially the same type of lipid classes as is found in active marrow.[1] Femoral rat bone marrow has been used in these studies, since, unlike the marrow of most long bones, this marrow is a very active hematopoietic tissue and has the experimental advantage of (1) being readily accessible without causing damage to intact cells, and (2) responding to irradiation in a manner similar to human marrow cells. The dose rate used in these studies has been about 4 r/min; under these conditions the L_2D_{50} for the rat is between 1100 and 1200 r (Table 1) rather than 700 to 800 r [2] when higher dose rates are used.

Table 1 - Lethality of Total-Body Irradiation When Given at a Low Dose Rate (4 r/min) to Rats

Total-body irradiation (r)	Total number of rats in group	Days after irradiation (accumulative total number of dead rats)									
		4	8	12	16	20	24	28	32	60	120
800	60	0	0	0	0	0	0	0	0	0	0
1000	15	0	0	0	0	0	0	0	0	0	-
1100	25	0	0	0	0	0	0	0	0	-	-
1150	20	0	0	0	1	3	-	-	-	-	-
1200	30	0	5	23	24	25	25	25	25	25	
1400	15	0	5	5	13	15					
1600	20	0	11	18	20						
2000	12	2	8	*							

* The remainder of this group was killed at 8 days for another experiment.
- Not completed yet.

To better understand the variation in the chemical nature of bone-marrow lipids, marrows from a number of other species have been studied with respect to lipid class composition. Figure 1 shows a thin-layer chromatogram of a total lipid extract from bone marrow of the chimpanzee, tamarin monkey, human, guinea pig, rat, rabbit, dog, minipig, sheep, and steer. The similarity of the lipid patterns of marrow from the different species and different marrow sites is remarkable, the main component always being triglycerides. The human sample and the other primates to a lesser extent show a very prominent spot directly above the triglyceride area, which is thought to represent a diester of a glyceryl ether.

The percentage of total lipids and the fatty acid composition of the main class, triglycerides (determined by gas-liquid chromatography), from different marrow sites in the various species are reported in Table 2. Palmitic and oleic acids are the main components esterified as triglycerides in the marrow of all species, but stearic is also quite high in the triglyceride fraction isolated from the pig, sheep, and steer.

Fig. 1. Thin-layer chromatogram of bone-marrow lipids from different species. Left to right: chimpanzee femur, monkey femur, human rib, guinea pig femur, rat femur, rabbit femur, dog rib, pig rib, sheep femur, steer femur. Thin-layer chromatography separation of total marrow lipids in a 90:10:1 (hexane: diethyl ether, acetic acid) system. The major spot for each species represents the triglyceride area.

Table 2 - Fatty Acid Composition of Bone-Marrow Triglycerides in Various Species

Species	Bone marrow site	Total* lipids (%)	Percentage of fatty acids in triglyceride fraction									
			12:0	12:1	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3
Human	Vertebra	31.4	-	-	-	-	27.0	2.5	7.7	55.3	7.4	T
	Rib	27.9	-	-	-	-	22.5	3.3	8.5	58.0	7.7	T
Chimpanzee	Femur	75.8	T	T	4.8	1.1	32.0	6.3	7.8	40.1	7.9	T
Rat	Femur	19.8	T	-	3.1	T	28.7	7.0	6.6	44.0	10.5	T
Dog	(a) Humerus	79.3	1.1	T	2.4	1.8	31.8	4.6	20.3	37.7		
	Tibia											
	(b) Femur	72.4	T	-	1.2	T	19.1	6.8	5.9	55.3	11.7	T
	(c) Rib	31.0	T		1.9	-	22.5	8.0	7.1	55.6	4.9	T
Sheep	Femur	81.5	1.3	-	3.8	1.1	29.2	3.2	18.6	42.5	T	
Steer	Femur	89.2	T	-	4.2	1.9	31.7	7.2	19.4	35.6	T	
Rabbit	Femur	71.2	T	-	2.4	1.7	25.6	5.0	6.8	30.3	28.1	T
Pig	(a) Humerus	91.4	T	-	1.6	-	25.0	3.1	16.8	42.1	11.4	T
	(b) Femur	92.1	T	-	1.6	-	27.9	2.6	16.5	42.4	8.9	T
	(c) Rib	51.7	T	-	1.3	-	29.6	2.4	19.1	43.3	4.3	

* % of dry tissue

Table 3 - Fatty Acid Incorporation into Tissue Lipids After 800-r Total-Body Irradiation

A. Oral administration of K-palmitate-1-C^{14} (10 μc/100 g body weight).

Tissue	Hours after administration of radioactivity	Total lipids cpm/mg lipid		Percentage of total radioactivity as triglyceride	
		0	800 r	0	800 r
Marrow	1	45	180	40	48
	4	210	275	45	68
	6	100	690	48	75
Liver	1	765	675		
	4	1360	810		
	6	1210	495		
Serum	1	5155	4550	69	87
	4	2030	2070	29	40
	6	2100	1740	19	27

B. Intravenous administration of palmitic-1-C^{14*}, stearic-1-C^{14*}, and oleic-1-C^{14*} acids (albumin complex).

Fatty acid ⟶	Palmitic-1-C^{14*}				Stearic-1-C^{14*}	Oleic-1-C^{14**}	
Days after 800 r ⟶	1	3	4	7	4	1	4
Tissue / Dose (r)	Percentage of total radioactivity as triglyceride						
Marrow 0	26	22	30	24	26	51	40
800	38	55	48	49	62	53	54
Liver 0	72	58	56	53	8	71	75
800	67	58	52	49	5	67	73
Plasma 0	-	-	-	-	28	9	12
800	-	-	-	-	32	11	15

* Rats were killed 30 min after an intravenous injection of 20 microcuries.
** Rats were killed 60 min after an intravenous injection of 10 microcuries per 100 g body weight.

Although total lipids of marrow vary greatly with marrow site, species, and age of the animal, marrow phospholipid content is usually only a minor portion of total lipids present. Collaborative experiments with Sister Maria Benigna (St. Joseph College, Hartford, Conn.) have shown that the major components of this small phospholipid fraction in normal rat marrow are sphingomyelin, phosphatidyl ethanolamine, and several unidentified compounds of the phosphatidyl inositide or phosphatidyl serine types. Noteworthy, however, was the absence of phosphatidyl choline as a component of the normal marrow phospholipids of this species.

Total-body irradiation was found to result in the accumulation of adipose cells in otherwise active hematopoietic tissue. A significant decrease in the phospholipid phosphorus percentage of total marrow lipids occurs, owing to dilution by triglycerides. On the basis of total marrow weight, lipid phosphorus is essentially the same in the irradiated and control rat femur. The phosphatide composition of the marrow is not qualitatively altered by 800-r total-body irradiation, and the triglycerides that accumulate under these conditions have the same fatty acid composition as before irradiation.[1]

Metabolic studies have consisted primarily in studying the incorporation into lipids or the oxidation, or both, of acetate-1-C^{14}, acetate-2-C^{14}, glucose-C^{14} (u), palmitic-1-C^{14}, stearic-1-C^{14}, oleic-1-C^{14}, and P^{32} by in vivo and in vitro bone-marrow cells from rat femurs. Table 3 demonstrates that irradiation stimulated the uptake of labeled lipid by marrow cells after oral and intravenous C^{14}-labeled fatty acids. Approximately 75% of the total radioactivity in the bone marrow was present as triglyceride 6 hr after the oral administration of K-palmitate-1-C^{14} to a rat given 800-r total-body irradiation. In the palmitic and stearic fatty acid studies, irradiated marrow cells had two to three times the amount of radioactivity in triglycerides as the nonirradiated cells. The specific activity (cpm/μeq of triglyceride ester) was 387 for control and 1105 for 800-r exposed rats that had received intravenous palmitic-1-C^{14} acid, and was 256 for control and 508 for 800-r exposed rats that had received intravenous stearic-1-C^{14} acid. It appears from these studies that the major effect of irradiation on marrow lipid metabolism is to stimulate the deposition of newly formed triglycerides and to depress fatty acid oxidation.[3]

Phosphorus-32 is incorporated into only one phosphatide fraction in both control and irradiated femur marrow of rats. The Rf of this fraction suggests that the component incorporating P^{32} is phosphatidic acid; its specific activity is elevated after total-body irradiation (800 r). The possibility exists that the phosphatidic acid might be serving as the skeleton structure for the esterification of the fatty acids in the formation of the triglycerides in the marrow cells. Experiments testing this idea are in progress. Another possible explanation for the accumulation of triglyceride in irradiated marrow is the uptake of triglyceride as such.

Separation of cell types if being attempted to understand whether a former hematopoietic cell becomes fat, and, if so, by a reversible process, or whether the existing adipose cells are precursors to newly formed ones found after total-body irradiation. A promising technique for such separation to be used for metabolic and chemical analyses consists of using sucrose and albumin media in conjunction with gradient centrifugation.

Our special thanks go to Jean Vnecnchak (Mount Holyoke College), Pat Murphy (University of San Diego), Dorothy Litton, and William Fishback for their valuable contributions toward this work.

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Glyceryl Ethers and Irradiation Leukopenia (Fred Snyder and Paul Godfrey*)

Orally administered glyceryl ethers

Tha alpha glyceryl ethers can under certain conditions [1] significantly lessen the leukopenia observed in rats exposed to total-body irradiation. They are known to exist in tissues as free ethers (unesterified), fatty acid esters, and as phospholipids, but their metabolic significance in mammalian cells is unknown. The effect of glyceryl ethers on irradiation leukopenia is variable, and the interpretation of much of the data can be complicated by infection and its effect on leukocyte levels in the irradiated animal. In early work, including our own, the glyceryl ethers were given intraperitoneally, subcutaneously, and intramuscularly. Recently we have given these compounds in diets containing various levels (0.1, 1, 5, and 10%) of different glyceryl ethers to weanling rats (32 to 37 days old), and to other rats by stomach tube. We enumerated leukocytes after exposures of 150 to 200 r of total-body irradiation; marrow samples were also taken and analyzed for lipid composition. Orally administered glyceryl ethers were not very effective in preventing the leukopenic response after irradiation, except where single doses of selachyl diacetate (10 and 100 mg/day) were used; even here the effect did not approach that of the nonirradiated group. Bergström [2] has suggested that an enzymatic cleavage of the ether bond occurs in the gut, which could explain the negative results obtained.

Organic synthesis of batyl alcohol

To study the metabolic fate of the orally administered glyceryl ethers, we carried out some organic synthesis of C^{14} and tritium-labeled batyl alcohol. The lack of commercially available labeled glyceryl ethers led to this investigation of their synthesis. A C^{14}-labeled batyl alcohol having a specific activity of at least 1 microcurie/milligram was sought. Because of the difficulties in distilling quantities of one gram or less of material, the procedure of Gupta and Kummerow, [3] which involves only crystallization techniques for purification, was investigated first. The reaction sequence for this synthesis is shown in Fig. 1. An 85% radiopure product (130 mg) was obtained, with the 15% impurity being the C^{14}-octadecanol. The specific

* Summer Research Participant from Louisiana State College

activity of the final product was 0.7 microcuries/milligram. The conversion of the labeled alcohol to the sulfonate ester was the step of least dependability because of difficulties in thoroughly stirring this small quantity of a two-phase system in the absence of moisture. The preparation of the ester by this procedure thus did not give sufficiently reproducible results for adequate yields. Adaptations of Kornblum and Holmes [4] and Howe and Malkin [5] to a semimicro scale were also tested. (Dr. Godfrey is continuing with this work at his own laboratory.)

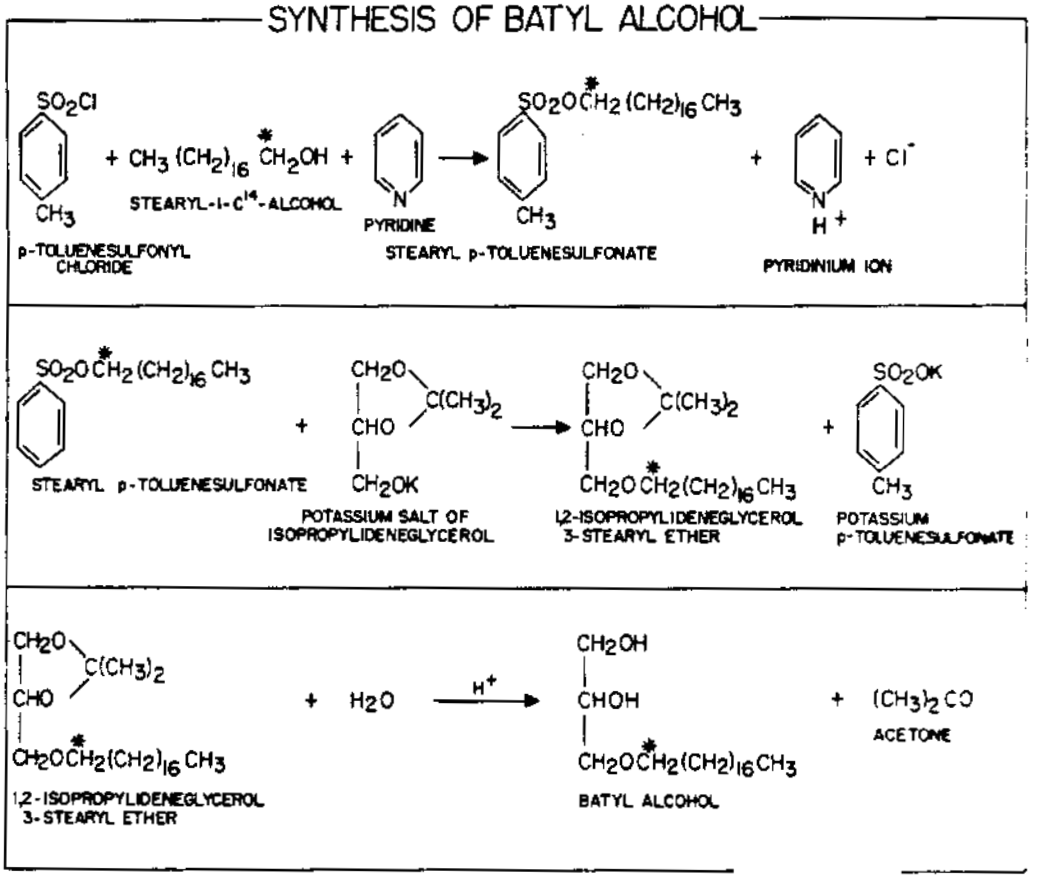

Fig. 1. Reaction sequence for the synthesis of batyl alcohol.

A simpler approach to obtaining a label in the batyl alcohol proved to be the titration of a sample of natural selachyl alcohol by exposing the alcohol to 5 curies of tritium in a Wilzbach apparatus. The labile tritium was removed by refluxing with fresh ethanol and subsequent distillation. The purified product will be used in biological experiments similar to those described.

Acknowledgements

Our appreciation goes to Dr. Claude Piantidosi (University of North Carolina) who provided us with the isopropylidiene and to Edgar Cress and Dorothy Litton for technical assistance in these experiments.

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Radioassay of Thin-layer Chromatograms (Fred Snyder)

Radioassay of thin-layer chromatograms can be accomplished by 1) external scanning [1-5]; 2) elution of radioactivity from the adsorbent followed by subsequent detection with Geiger-Müller ionization or scintillation detectors; 3) autoradiography [6, 7]; and 4) direct counting of adsorbent in liquid scintillation solutions [8, 9]. The last procedure is essential for quantitative assay of low-activity, low-energy biological samples. The radioassay of thin-layer chromatographic plates as described by us earlier [8] has been improved by designing a scraping device for rapid and quantitative transfer of small zones of adsorbent from narrow glass plates into counting vials for liquid scintillation radioassay. A scintillation solvent system that deactivates silica, thereby preventing adsorption of many polar compounds, is also described.

The scraper pictured in Fig. 1 consists of a spring-loaded fixed single-edge replaceable razor blade (A) mounted so that narrow glass plates (2 cm wide) (B) can be moved along a guide edge (C) while the adsorbent falls from the razor's edge into a counting vial. Finger holes (D) along the guide edge facilitate proper placement of the glass strip. The counting vial is inserted into a sliding spring-loaded holder (E) that maintains the vial against the edge of the glass plate. The movement of the glass strip is controlled by 3 gears

(a modified Geneva drive) attached to a drive shaft that permits 1-, 2-, and 5-mm increment scanning of the thin-layer plates. A push-button control (F) regulates the positioning of the gears so that one complete revolution of the crank (G) causes the plate to move 1 mm, 2 mm, or 5 mm. A release (H) on the drive shaft permits free movement of the plate to accommodate its removal from the device. An example of a zonal scan for an impure commercial preparation of tripalmitin-$C^{14}OOH$ that was obtained with this scraper and an autoradiogram of the same chromatostrip is shown in the insert of Fig. 1. As little as 100 dpm in a single peak can readily be detected with this technique.

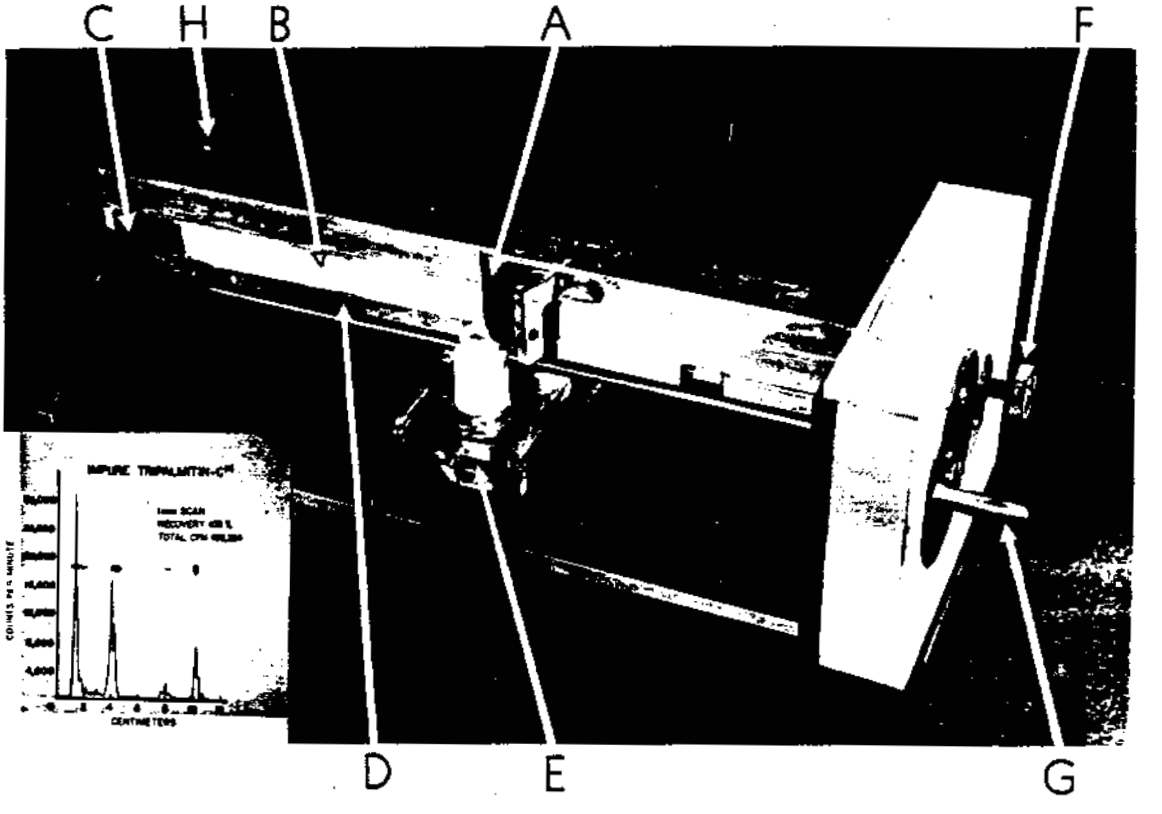

Fig. 1. Thin-layer chromatogram scraper.

Figure 2 is an example of a 2-mm zonal scan of a low activity (1216 dpm) biological sample, which shows the distribution of C^{14} in bone marrow total lipids after the oral administration of palmitic-1-C^{14} acid to an irradiated rat. Identification of the areas is accomplished by exposing the plates to iodine vapor, which visualizes the compounds, and comparing their R_f's to previous behavior obtained in a particular chromatographic solvent system. Addition of "cold" standards is made if the labeled sample does not contain a sufficient quantity of the stable compound to be visualized with iodine.

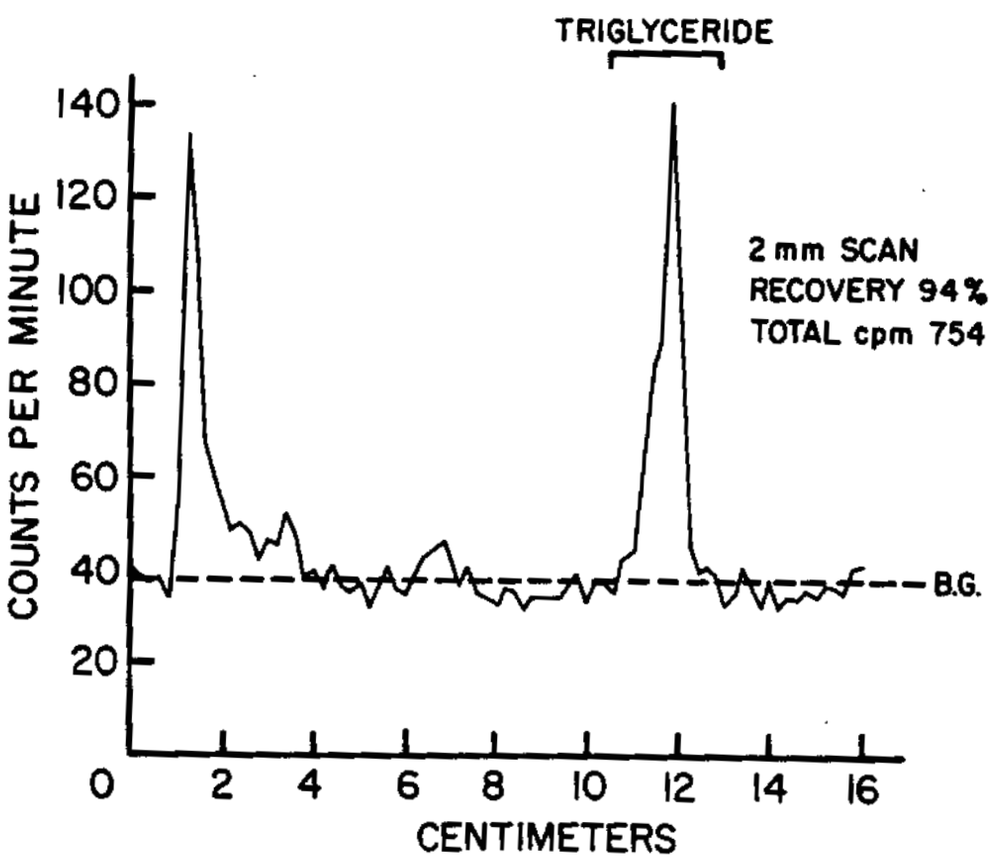

Fig. 2. Carbon-14 distribution in bone marrow lipids 6 hours after the oral administration of palmitic-1-C^{14} (in corn oil) to an irradiated rat. (4 days after 800-r total-body irradiation).

The scintillation solution of choice for counting carbon-14 (62% eff.) and tritium (12% eff.) from silica scrappings has been dioxane (1.5 liters), napthalene (150 g), water (0.3 liter), PPO (7 g) and POPOP (0.3 g), and sometimes containing Cab-O-Sil (4%). The water serves to deactivate the silica, since adsorption of radioactivity on silica particles in more nonpolar solvent systems can result in self-absorption losses ($\approx$ 10% for C^{14} and 25% for H^3) with 10 to 25 micron silica particles. The silica, iodine, dichlorofluorescein, and rhodamine-6G have no quenching properties in this system, whereas elemental carbon (H_2SO charring) causes severe quenching. Recovery of the radioactivity from the plate based on a direct pipetting into a vial serves as an internal check on the total system used in the analysis.

The high resolution (1 mm) zonal scans of thin-layer plates reveal tha caution must be applied when interpreting radioactivity data from thin-layer plates in which larger areas have been assayed. The 1-mm scans have shown as many as 3 peaks in an area as small as 2 cm wide. Autoradiograms also show overlapping areas of radioactivity, but the resolution obtained is not so clearly delineated as in our scans. External scanning equipment does not resolve the components in an area of this size. The special scraper described for carrying out the rapid quantitative removal of adsorbent into scintillation vials is extremely useful in accomplishing high resolution radioassays of thin-layer chromatographic separations made with low-activity biological samples. The complete automation of this device is under construction to expand the utility of this procedure.

Acknowledgements

The technical assistance of Nelson Stephens in this work was greatly appreciated.

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AMINO ACID METABOLISM AND IRRADIATION

Lysozyme Activity in Radiation Chimeras (Vu-Thi Suu*, C. C. Congdon*, and A. L. Kretchmar)

Interest in lysozyme stems from the problem of secondary disease (graft-against-host reaction) in homologous bone-marrow (HBM) chimeras. A "metabolic starvation" appears to follow the immune reaction. Altered metabolism in secondary disease may be the important feature in causing death after treatment with foreign bone marrow. Study of the striking changes in lysozyme activity in homologous bone-marrow chimeras might contribute either to an understanding of the biochemical role of lysozyme in tissues or to the mechanism of metabolic alterations during the foreign bone-marrow reaction.

Hybrid mice of known phenotype were used as recipients and donors of isologous or homologous bone marrow. Animals in this series were killed 3 to 322 days after irradiation and treatment with bone-marrow cells. Four groups were investigated. Group I consisted of normal mice not irradiated and not given bone marrow; group II was given 950 r total-body irradiation but not bone marrow; group III mice were similarly irradiated but given 40×10^6 isologous bone-marrow (IBM) cells; and group IV mice were irradiated and given 40×10^6 homologous marrow cells. Marrow was obtained from femurs of donor mice and suspended in Tyrode's solution. The nucleated cells were counted and the volume was adjusted with Tyrode's solution to give 40×10^6 cells/milliliter of suspension. Recipient mice were given 1 ml intravenously within 5 hr after irradiation. After X irradiation and marrow treatment, animals were kept in a cage and allowed free access to food and water.

* Oak Ridge National Laboratory

The animals were weighed and killed and the organs were removed and weighed. In the lysozyme assay of plasma, mice were anesthetized and cardiac blood was aspirated into heparinized syringes. The tissues were homogenized and then centrifuged. The sediment was discarded and the supernatant was used for the assay. In part of this study, in an attempt to obtain more complete disruption of the tissue cells, the suspensions were frozen in dry ice plus acetone and thawed at 37°C four times. Cells were rehomogenized and centrifuged at high speed.

Measurement of enzyme activity was made with a spectrophotometer with temperature controlled at 25°C. The procedure of Shugar standardized against Worthington lysozyme was used in the assay. The reagents were kept 30 min in a constant temperature bath at 25°C before measurement of enzyme activity. One-tenth milliliter of the supernatant was used for each lysozyme assay. Absorbance was read at 30-sec intervals and the course of the reaction was recorded for 2-1/2 min.

In the homologous chimeras (Fig. 1) during the first 6 days after irradiation and bone marrow treatment, the lysozyme activity was almost normal; then a pronounced increase, beginning at 7 to 9 days, rapidly decreased after the tenth day and was only slightly above normal after the third week.

Lysozyme assay of different organs (Table 1) of normal mice indicated highest values in bone marrow, small intestine, lung, kidney, spleen, and colon. Minimal activity was found in the brain and skeletal muscle. In X irradiated mice not given bone marrow, a decrease in activity was found in 7 of the 11 organs examined. In the chimeras, greatest activity was found in the bone marrow, with the HBM group higher than the IBM. The next highest activity was in kidneys of mice given HBM. Lysozyme activity in kidneys of animals given isologous marrow was not elevated.

Since lysozyme activity is known to be high in granulocytes, much greater than normal enzyme activity in the bone marrow of IBM and HBM mice 9 days after transplantation of marrow would be expected if the regenerating marrow was granulopoietic. In HBM-treated mice, hyperplasia of granulopoiesis has been reported.

The change in lysozyme activity in some of the organs is probably associated with transplantation of granulopoietic elements.

The close time relations among liver weight, kidney weight, liver aspartic acid concentration, and lysozyme activity in kidney all increased 7 to 14 days after irradiation, and treatment with homologous bone-marrow cells suggests that all are related to the same underlying metabolic alteration in the host, presumably triggered by the immunologic interaction of graft and host.

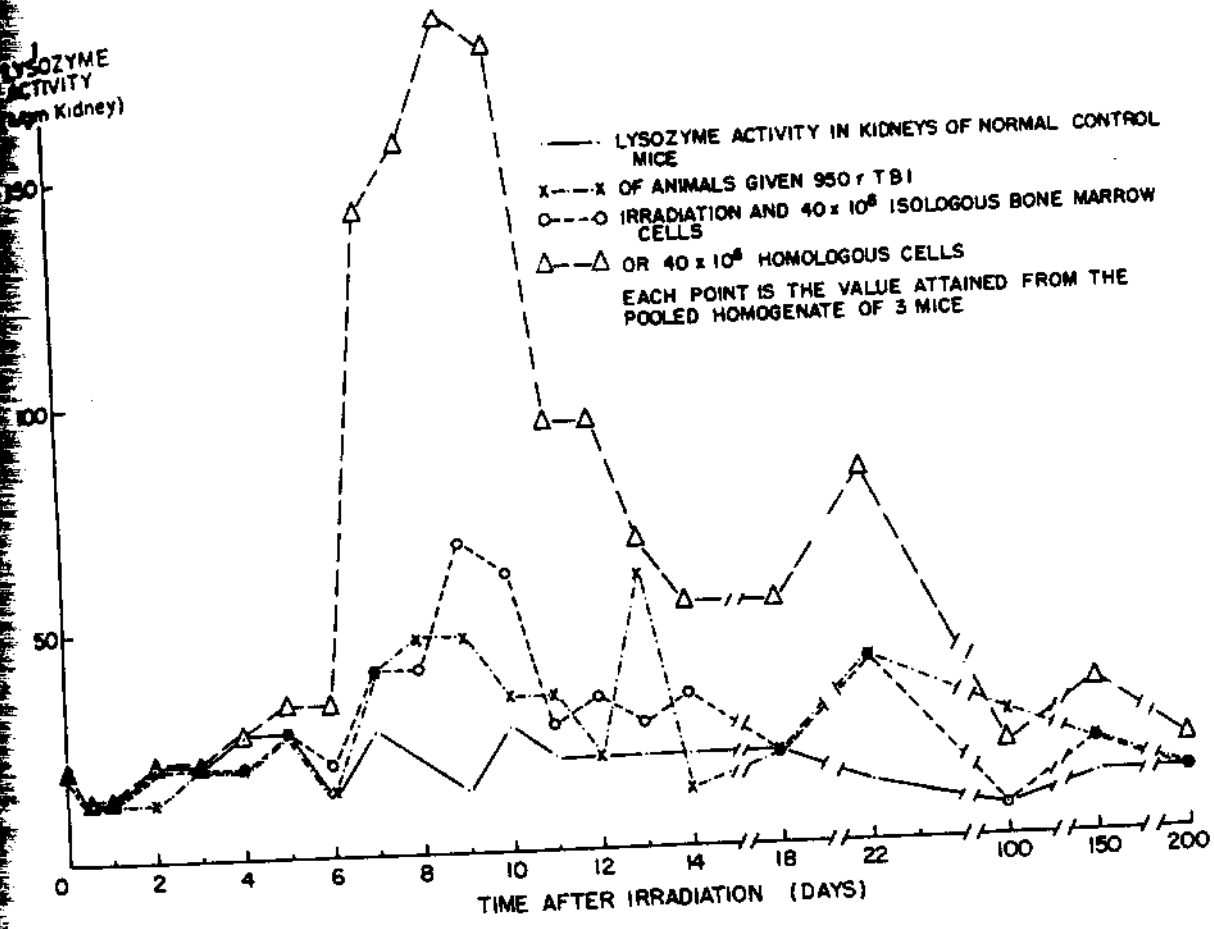

Fig. 1. Lysozyme activity in the kidneys of irradiation chimeras.

Table 1

Lysozyme Activity in Different Organs of Normal Mice, and of Mice Nine Days after Irradiation (950 r total-body irradiation), Irradiation and 40 x 10^6 Isologous Bone-Marrow Cells or 40 x 10^6 Homologous Cells. *

Tissue	Lysozyme Activity (γ/gm tissue)							
	Normal control		X-ray control		IBM		HBM	
Bone marrow	180	(11)	29	(10)	350	(13)	459	(13)
Small intestine	51	(3)	81	(3)	57	(3)	53	(3)
Lung	48	(4)	36	(3)	56	(4)	64	(3)
Kidney	28	(3)	21	(3)	42	(3)	196	(3)
Spleen	17.3	(5)	3.8	(13)	39	(4)	56	(3)
Colon	17	(3)	4	(6)	6	(3)	4	(4)
Lymph node	8	(5)	0	(11)	22	(8)	25	(3)
Submaxillary gland	4.6	(5)	5.2	(5)	2.6	(5)	2.4	(5)
Thymus	3.4	(11)	0	(34)	3.8	(22)	6.6	(23)
Liver	2.2	(3)	4.4	(3)	4.0	(3)	6.6	(3)
Plasma	1	(3)	1	(7)	0	(4)	1	(3)

* The number of mice is given in parenthesis.

A Possible Metabolic Relation Between Liver Weight and the Mass of Proliferating Cells in Animals (A. L. Kretchmar)

Three different kinds of experiments may be cited in support of this suggestion (Fig. 1).

The first is a situation where there is an immunologic reaction that is probably host antigraft.[1] The upper curve in Fig. 1 shows the enlargement of liver that occurs in rats after the transplantation of a Walker carcinoma. Similar results have been reported by others.[2] The period of maximum rate of increase in liver size could conceivably coincide with the maximum rate of proliferation of cells in the tumor; later, when the tumor is large and when the rate of proliferation may be expected to decrease because of limited blood supply or because of host reaction against it, the rate of increase in size of liver also appears to decrease.

The second is an experiment where the reaction is probably graft vs. host. The second set of curves in Fig. 1 is taken from Simonsen and Jensen[3] and shows the increase in size of liver after transplantation of adult spleen cells into young mice. After a short initial period there is apparently a rapid proliferation of grafted cells as reflected by the rapid increase in size of spleen. During this period the liver enlarges. As the proliferation of the graft ceases and the cells leave the spleen or die in situ in reaction against the host (decrease in weight of spleen) the weight of liver also decreases and returns to normal.

Third, the lower set of curves in Fig. 1 shows how the liver of irradiated animals given bone marrow cells enlarges after treatment. We have also found that the enlargement of liver is correlated with the enlargement of the spleen in these animals. In the irradiation experiments the enlargement of liver occurs in animals given isologous cells (IBM) in a strain where no difficulty is encountered with intrastrain skin grafts. Moreover, the weight of liver and spleen in these animals is highly correlated and the maximum size of liver coincides with the maximum size of spleen. From histologic studies it is known that the increase in weight of the spleen is due to a massive proliferation of blood-forming cells during the fifth to ninth or tenth day and that subsequently this activity diminishes and the spleen returns to normal size. Similarly the liver returns to normal weight.

In the irradiated mice given homologous cells (HBM) there is a more prolonged and sustained proliferation of hemopoietic cells as well as a proliferation of cells in the white pulp of the spleen that does not occur in the mice given isologous cells. Likewise there is a more prolonged and sustained increase in the weight of the liver. Again the weight of the spleen and liver is highly correlated.

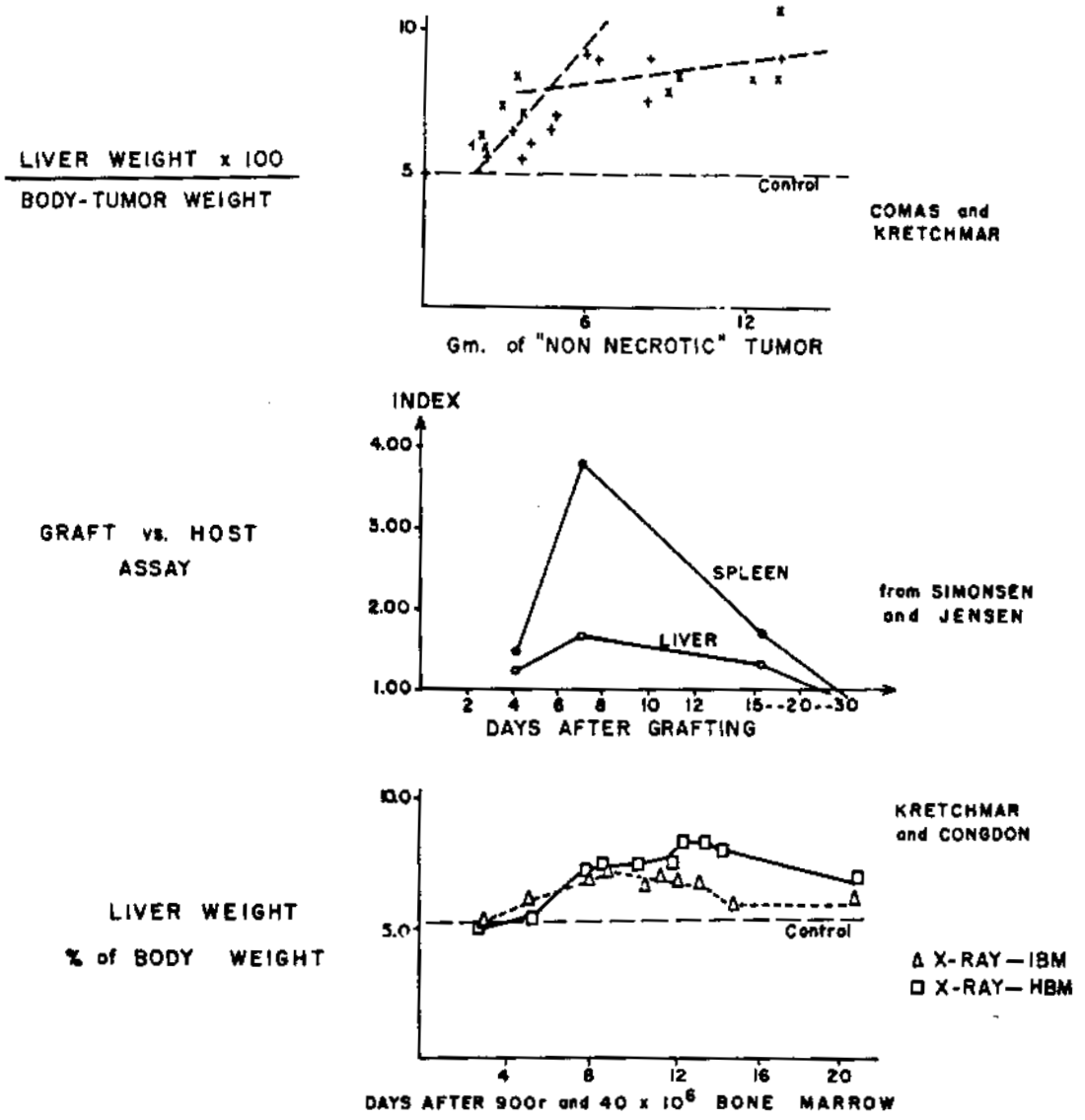

Fig. 1. Graphs showing the relation between liver weight and size of tumor or time after grafting or irradiation and injection of bone marrow cells. Upper graph shows liver weight divided by body weight minus tumor weight as a percentage; the middle graph shows liver and spleen weight as a calculated "index"; and the bottom graph shows liver weight as a percentage of body weight. Irradiated animals were given 40 x 10^6 isologous (IBM) bone-marrow cells.

In these three different kinds of experiments there is the common feature of rapid proliferation of cells. The irradiated animals given isologous cells show enlargement of liver and spleen and this could not have been a graft versus host response.

We have also found, in further experiments, that the enlargement of liver is due primarily to an increase in size of the liver cord cells. Fat does not increase and the glycogen content of the liver decreases moderately. On the other hand, nitrogen content and the phosphorus-to-nitrogen ratio increase significantly. These results are consistent with the histologic picture of hypertrophied liver parenchymal cells, which show an increased basophilic staining.

Taken with Lajtha and Vane's experiments showing the relation between liver function and nucleic acid synthesis in hematopoietic cells[4], the work just summarized suggests the hypothesis that one of the factors controlling the size of the liver in vivo is the number of proliferating cells in the animal and that this is related, in part at least, to the synthesis of precursors of nucleic acid for these cells.

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The Increased Liver Weight in Irradiation Chimeras (A. L. Kretchmar)

We have previously reported (Kretchmar and Congdon 1961, Congdon and Kretchmar 1963) that the liver weight in irradiated mice given foreign bone-marrow cells is increased, and that this reaction also occurs but to a more limited extent in similarly irradiated mice given isogenic marrow cells.

The liver of these animals does not appear to be damaged (Congdon and Kretchmar 1963) since histologic study shows an increase in size of liver parenchymal cells with increased basophilia but no evidence of parenchymal damage. Biochemical studies showed a normal nitrogen concentration, with increased nitrogen content, slightly diminished glycogen concentration, and no change in fat concentration. The phosphorus-to-nitrogen ratio was increased.

Table 1 summarizes the findings with respect to water content, RNA, and DNA concentration. These results indicate an increase in RNA concentration during the second week after irradiation and injection of bone marrow-cells.

Our results are so far consistent with the idea that proliferating hematopoietic cells require precursors of nucleic acid that are synthesized in some other organ (Totter) presumably in the liver (Lajtha and Vane, Perretta et al.).

Table 1

GROUP	WATER Percent	RNA-Phos mg/g	DNA-Phos mg/g
Normal			
7 day	68.0	0.73	0.26
9 day	67.9	0.69	0.30
14 day	67.9	0.69	0.29
X ray			
7 day	71.6	0.79	0.24
9 day	71.4	0.82	0.29
14 day	dead		
IBM			
7 day	70.1	0.74	0.23
9 day	68.3	0.69	0.25
14 day	69.2	0.72	0.28
HBM			
7 day	73.2	0.74	0.24
9 day	72.6	0.80	0.30
14 day	71.8	0.76	0.29

Values of RNA-Phos underlined are significantly higher than normal control levels of the same time group.

The Free Amino Acid Levels in Plasma, Liver, and Muscle of Irradiation Chimeras (A. L. Kretchmar)

The free amino acid levels in tissue and plasma of irradiation chimeras were determined, since the secondary-disease syndrome that develops in these animals involves muscle wasting (body-protein mobilization) and at least one rather specific block to protein synthesis (hair growth).

Tissues and plasma were extracted with 1% picric acid according to the procedure of Tallan, Moore, and Stein and the extracts were analyzed for free amino acids by the procedure of Spackman, Stein, and Moore using the automatic amino acid analyzer.

Results for liver are summarized in Fig. 1. Changes in levels of glutathione and glutamine occurred in both bone-marrow treated groups and their controls given X ray only. In mice treated with isologous marrow cells the levels returned toward normal by 35 days; in animals given homologous marrow, however, the levels of these two amino acid derivatives showed a secondary fall to low levels after partial recovery on the twelfth day. The levels of aspartic acid were markedly elevated in mice given homologous marrow, whereas this amino acid was present in normal concentration in mice given isologous cells and in controls given only X ray. In the mice given homologous bone marrow, the serine and glycine concentration was below normal after the seventh day while the isologous-cell-treated and X-ray-only groups showed normal levels of these amino acids. These changes have been reported and discussed (Kretchmar and Congdon 1961, Kretchmar and Congdon, 1963). The data, however, are so extensive that a complete analysis of the results seemed impossible without automatic data processing and computer use. A beginning in this approach has been made.

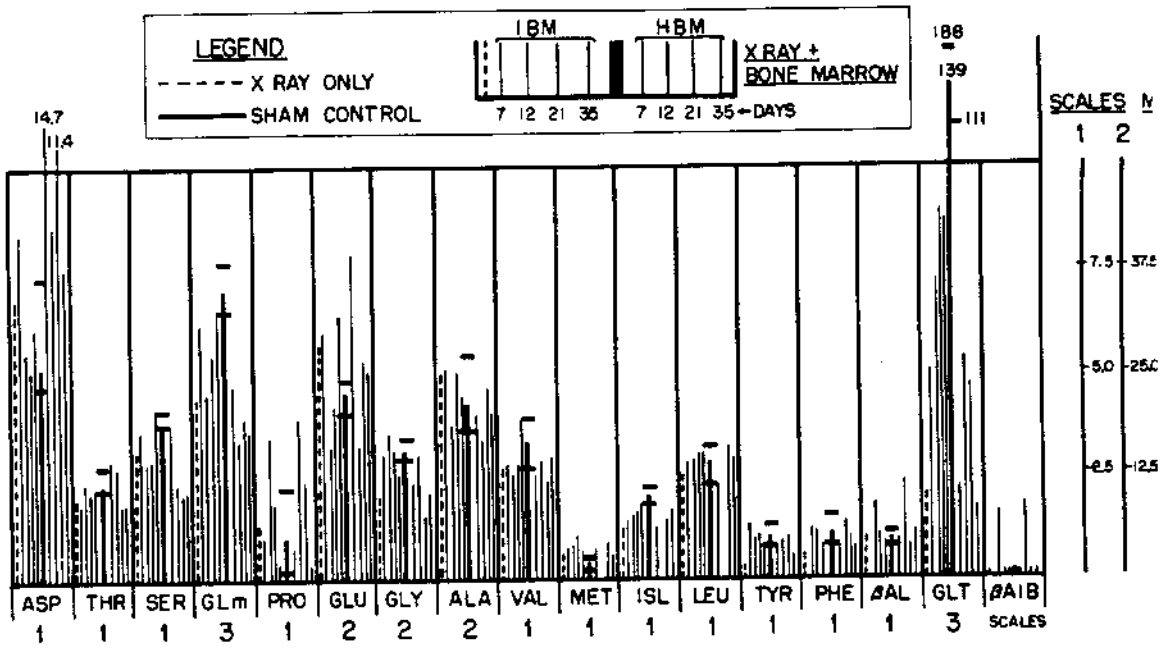

Fig. 1. Free amino acids of liver.

IMMUNOLOGY

Effect of Low-Temperature Storage on Human Antibody-Forming Cells
(N. Gengozian, J. S. Batson, B. Rabette and K. Hübner)

Previous studies in this laboratory have shown the feasibility of inducing human tissues, particularly lymph node and spleen, to form antibody when cultivated in in vivo diffusion chambers. To further enhance the use of such a system for direct experimental studies on human cells, it became imperative to have available a procedure that would provide for a continuous, constant source of cells. Preservation of tissues by a controlled-rate freezing process followed by storage at liquid-nitrogen temperatures is well-known and appeared to offer a system suitable to our needs.

Human lymph-node tissue obtained from a patient after a surgical procedure was teased apart in tissue-culture fluid. To an aliquot of the cell suspension was added a known quantity of S. typhosa organisms for antigenic stimulation of the cells. Of this mixture, 10×10^6 cells were placed into each of 20 diffusion chambers, which were then placed intraperitoneally in irradiated mice for cultivation. The remaining aliquot of teased cells was treated with dimethyl sulfoxide to a final concentration of 10% and this suspension was distributed into ten 1-ml ampules. The sealed ampules were then subjected to a controlled slow-rate freezing process, the temperature lowered at the rate of 1°C/min to -25°C after which the ampules were immersed directly in liquid nitrogen. Ampules were removed from storage at intervals of 4-1/2 and 8 months, and placed immediately into a 37°C bath for rapid thawing. After washing the cells with tissue culture fluid to remove dimethyl sulfoxide, the cells were counted and a known amount of S. typhosa organisms was added for antigenic stimulation. Portions of this mixture were then placed in diffusion chambers for cultivation similar to that done with the fresh cells before freezing.

Several cirteria were used to assess the effect of the freezing process and storage on the viability of the human cells: (a) quantitative cell recovery; (b) percentage of eosin-postive cells; (c) proliferative activity in culture as determined by mitotic index and tritiated thymidine incorporation; (d) cellular differentiation in culture; and (e) antibody and protein synthesis. Results have shown an almost complete quantitative recovery of the cells with only a slight increase in eosin-positive cells after storage. The most

significant finding has been a delay in the proliferation and cellular differentiation of the cells during cultivation, resulting also in a delay in the formation of antibody. The amount of antibody formed is also decreased as indicated by the anti-H agglutinin titers, although this is shown to be variable The protein responsible for antibody activity is the same with the fresh and frozen tissues as shown by immunoelectrophoresis.

In addition to providing a system of long-term storage of human antibody-forming cells, this study has shown that the diffusion-chamber technique can provide more meaningful criteria on the viability of the preserved cells, particularly when these are to be used for transplantation into humans.

Proliferation of Human Antibody-Forming Cells (P. Urso* and N. Gengozian

Diffusion chamber cultivation studies done in this laboratory with human lymph-node tissues have shown a high proliferative activity of the immune-cell population immediately before and during antibody production, as indicated by the incorporation of tritiated thymidine in vitro. It became of interest therefore to determine the proliferative capacity of the antibody-containing cells as compared to the cells in the same population that were not forming antibody. This was done by using both in vitro and in vivo exposures of the cultivated cells to tritiated thymidine (H^3T) and subsequent staining of the cells for antibody by immunofluorescence.

Human lymph-node cells stored under liquid-nitrogen temperatures and known to have the capacity to form antibody to S. typhosa antigen by previous tests were used for this study. Cells obtained from storage after thawing and removal of the preservative, dimethyl sulfoxide, were incubated in vitro with the S. typhi antigen and aliquots of this mixture were then placed into diffusion chambers for subsequent cultivation in irradiated mice. One group of chamber recipients was injected intraperitoneally on the sixth day after implantation with H^3T over a period of 2-1/4 days, receiving a total of 20 microcuries in four injections. A second group received no in vivo exposure to H^3T, but the cells obtained from these chambers at time of death were incubated in vitro with H^3T for 1 hr at 37°C. The animals in each group were killed at 10, 11, and 12 days postimplantation, the chamber fluid was recovered, and the cells were processed for autoradiography and staining with immunofluorescent reagent. The immunohistochemical staining was performed by the double-layer technique, using a fluorescein isothiocyanate conjugated rabbit antityphoid serum in conjunction with the flagellin protein antigen extracted from S. typhosa flagella.

*Summer participant, 1963, from Seton Hall University, South Orange, N.J.

All chambers had fluids that were positive for hemagglutinin against typhoid H antigen and yielded cells whose cytoplasm stained a brilliant green with the immunofluorescent reagent, indicating the presence of precipitating antibody against the extracted protein of typhoid H flagella antigen in the cells.

Subsequent autoradiographic analysis of the cells showed that a higher percentage of antibody-containing cells incorporated H^3T after in vitro incubation at 10 days as compared to the cells of the total population, the frequency being seven times greater in the antibody-containing cells (14.3% to 2%). At 12 days, none of the antibody-containing cells incorporated H^3T, this frequency being comparable to the amount of incorporation (0.7%) seen in cells of the total population at the same time interval. These results indicate that the competent antibody-containing cells proliferate at a greater frequency than the incompetent cells during the early phases of antibody synthesis and that the proliferative capacity is reduced to normal levels during the later phases of antibody synthesis. In addition, it was found that the frequency of H^3T labeling in the antibody-containing cells increased from 75% to 92% on days 10 to 12 after in vivo exposure to the radioactive compound and was 3 to 4 times greater than in the cells of the total population. These data suggest that the mature antibody-containing cells are derived from immature precursor cells through somatic division.

Synthesis of Two Molecular Forms of Antibody by Spleen, Lymph-Node, and Thymus Tissues of the Mouse (N. Gengozian and B. Rabette)

Reports in the literature have indicated the differential synthesis of two molecular forms of antibody depending upon the course and duration of immunization. With certain antigens, the antibody protein produced within the first and second week after injection is considered to be a macroglobulin (19S), which is then gradually replaced by a lower molecular weight antibody (7S). Also, a booster stimulus of antigen after the primary injection generally results in the production of only the smaller antibody protein. Utilizing S. typhosa vaccine for immunization of mice, studies have been undertaken in this laboratory to determine the sequential formation of these two molecular forms of antibody and determine the site of the antibody formation of tissue cells in in vivo diffusion chambers by cultivation.

Mice were given a primary injection of S. typhosa followed one month later by a series of secondary immunizations. Approximately two weeks after the booster injections, the animals were killed for immune serum; the spleen, lymph nodes, and thymus were removed for tissue cultivation. Cell suspensions of each organ were prepared separately. To one aliquot of each was added a known amount of S. typhosa antigen for restimulation. A second aliquot of each suspension received no additional antigen.

The cells were cultured in diffusion chambers implanted intraperitoneally in irradiated mice. After one week the chambers were removed and the fluid contents were titrated for H agglutinins. Aliquots of the chamber fluids and donor immune sera were also treated with 2-mercaptoethanol (2-ME). This sulfhydryl compound inactivates high molecular weight antibody by dissociating macromolecules into smaller subunits without affecting the activity of smaller, 7S antibody protein.

The immune sera obtained from the tissue donors at time of death were virtually unaffected after treatment with 2-ME, suggesting the presence predominately of a 7S antibody protein. Cultivation of the different tissues in the chambers, however, revealed significant production of a macromolecular antibody. Thus, cells not reexposed to antigen at the time of implantation produced both the small and large molecular weight antibody protein, the latter being more prominent. On the other hand, spleen cells restimulated with antigen at chamber implantation produced significant amounts of both the 7S and 19S antibody protein, while restimulated lymph-node and thymus cells produced almost exclusively the large macroglobulin antibody as revealed by susceptibility to 2-ME treatment.

The study thus far indicates that in the mouse the primary source of the 7S antibody protein is the spleen. Furthermore, although the immune serum appeared to contain predominately this low molecular weight protein, cells capable of synthesizing 19S antibody were present in both the lymph-node cells and the thymus. The data also confirm the thesis that thymus cells from preimmunized animals have antibody-forming potential, and studies in progress indicate their ability also to initiate a primary response in diffusion chambers.

Effect of Total-Body Irradiation on a Small South American Primate, Tamarinus nigricollis* (N. Gengozian and J. S. Batson)

Studies on the use of a small South American primate, Tamarinus nigricollis, for total-body irradiation and hematopoietic graft transplantation are being continued under the support of the U. S. Air Force Aerospace Medical Division. Progress during the past year has been limited by the inability to obtain sufficient animals of good quality. Additional data have been obtained, however, on the effects of total-body irradiation for a more accurate estimate of the 30-day LD_{50} for this species. Exposures were made in the ORINS Cs^{137} total-body irradiator at 4.1 r/min. As we noted previously, the tamarin appears to be quite radiosensitive as compared to other species of

* Research supported by United States Air Force Contract No. AF 41 (657)-398. Aerospace Medical Division, Air Force Systems Command, United States Air Force, Brooks Air Force Base, Texas

primates and mammals. Thus, a plot of the mortality versus radiation dose as shown in Fig. 1 indicates an LD_{50} of about 170 r, with an LD_{100} of 400 r. The 30-day mortality among the six groups (100 through 600 r) expressed as a function of time of death and radiation dose is shown in Fig. 2. All animals exposed to 400 r or more died within 13 days, the earliest death occurring in the 600-r group on day 7. With a decrease in dose, there was a greater range in time of death as shown for those given 200 r and 300 r. The single death in the 100-r group occurred on day 27 postirradiation. As shown in Fig. 2 by the curved line joining the group mean values, the mean time increased exponentially with a decrease in radiation dose. Mortality beyond the first month was observed in each of the 100-r, 200-r, and 300-r groups. Thus, the single survivor in the 300-r group died on the forty-ninth day. Deaths in the 200-r group occurred on days 53 and 253 postirradiation, with one animal still alive at 203 days. Of the nine animals surviving the 30-day period in the 100-r group, one each died on days 57, 123, 166, 314, and 357. The remaining four animals are alive at 195 (3) and 78 (1) days.

Although the effects of radiation on immunologic capabilities are well documented in the literature for a variety of species, a study of this physiologic parameter was undertaken in the tamarin for two reasons: (1) to see whether the apparent radiation sensitivity of this animal extended to its natural defense mechanisms, and (2) to provide some basis for future experiments on homografting of foreign bone marrow as a therapeutic measure. Groups of four tamarins each were exposed to 100 r, 300 r, or 400 r, and injected with 1.0 ml antigen (P. tularense) within two hours after total-body irradiation. Antibody formation by these animals relative to the normal tamarin response curve is shown in Fig. 3. The agglutinin curve of the mean titers shows an almost stepwise decrease with an increase in radiation dose. Peak titers comparable to the normal groups were attained in both the 100-r and 200-r animals, although delayed three and seven days. Of interest was the antibody formation obtained in the 300-r and 400-r animals. Although five of the eight animals in these two groups died within 14 days after radiation, antibody formation was not completely suppressed, and indeed, was formed in significant amounts. Thus, one cannot equate mortality with immunologic suppression. Preliminary attempts to transplant bone marrow in tamarins exposed to 400 r (an LD_{100}) have failed, due in part to this capacity of the animals to respond immunologically against the foreign antigens and prevent a "take" of the marrow.

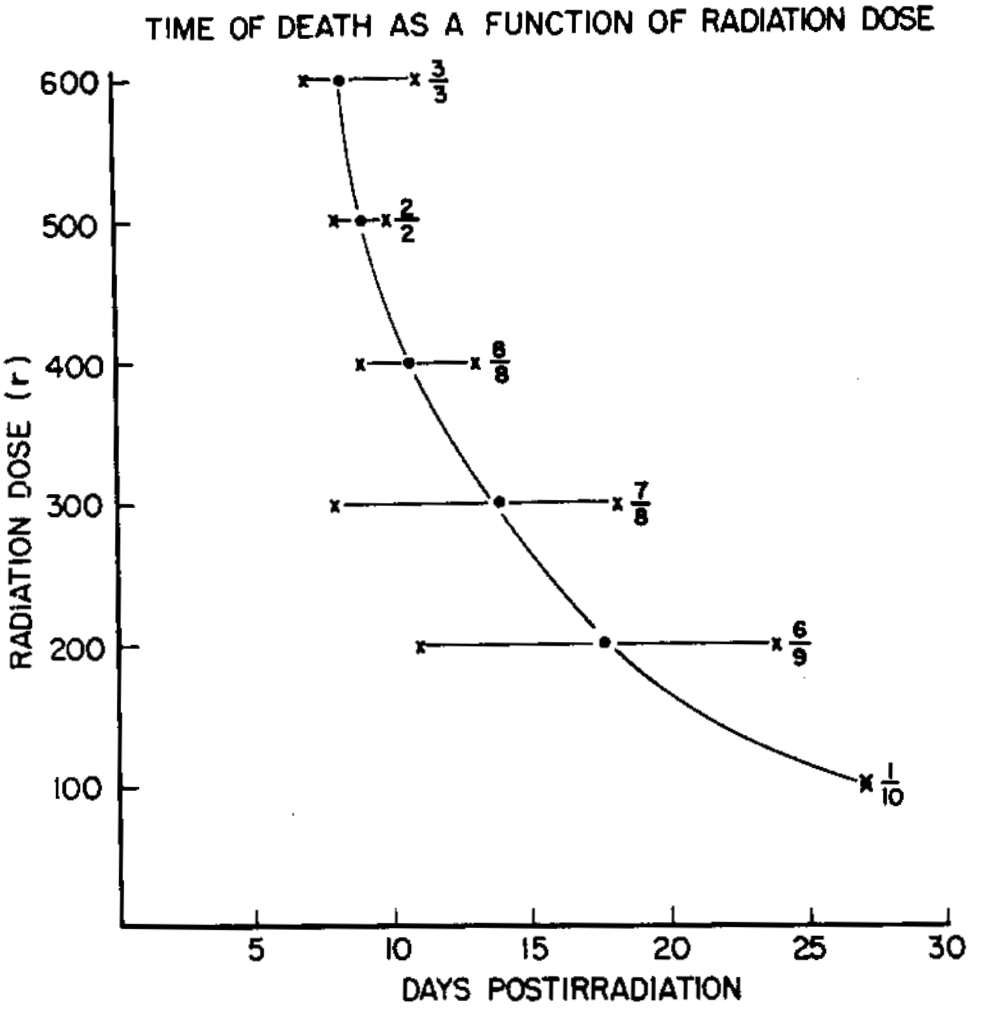

Fig. 1. Plot of mortality versus radiation dose indicating an LD_{50} of about 170 r with an LD_{100} of 400 r.

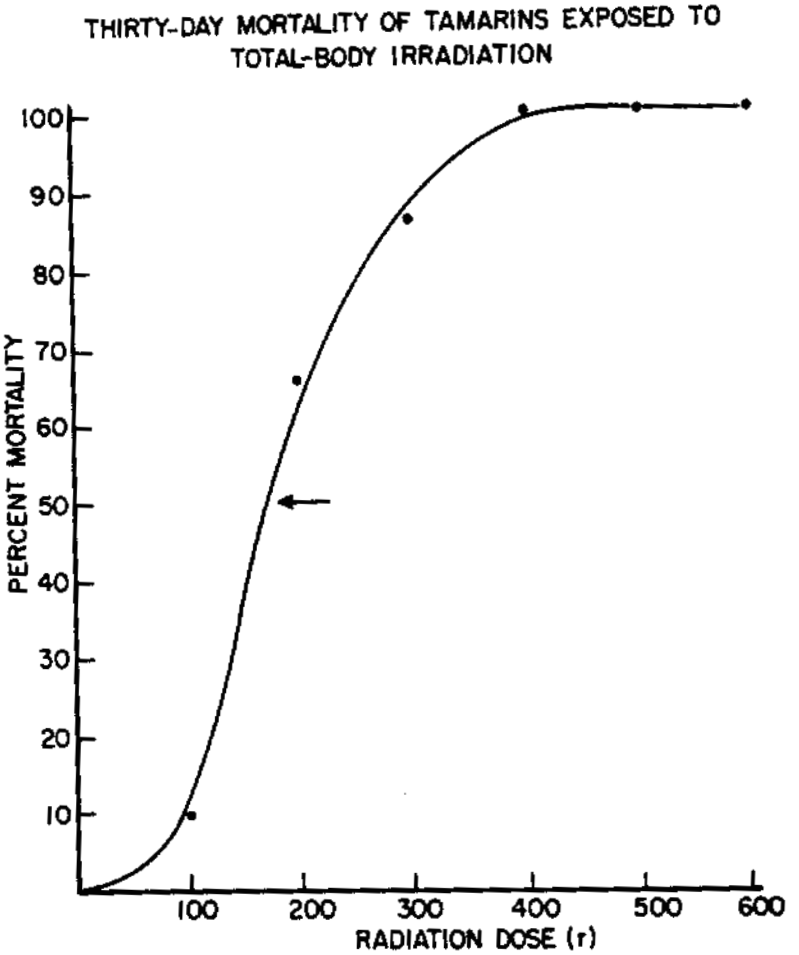

Fig. 2. The 30-day mortality among six groups (100 through 600 r) expressed as a function of time of death and radiation dose.

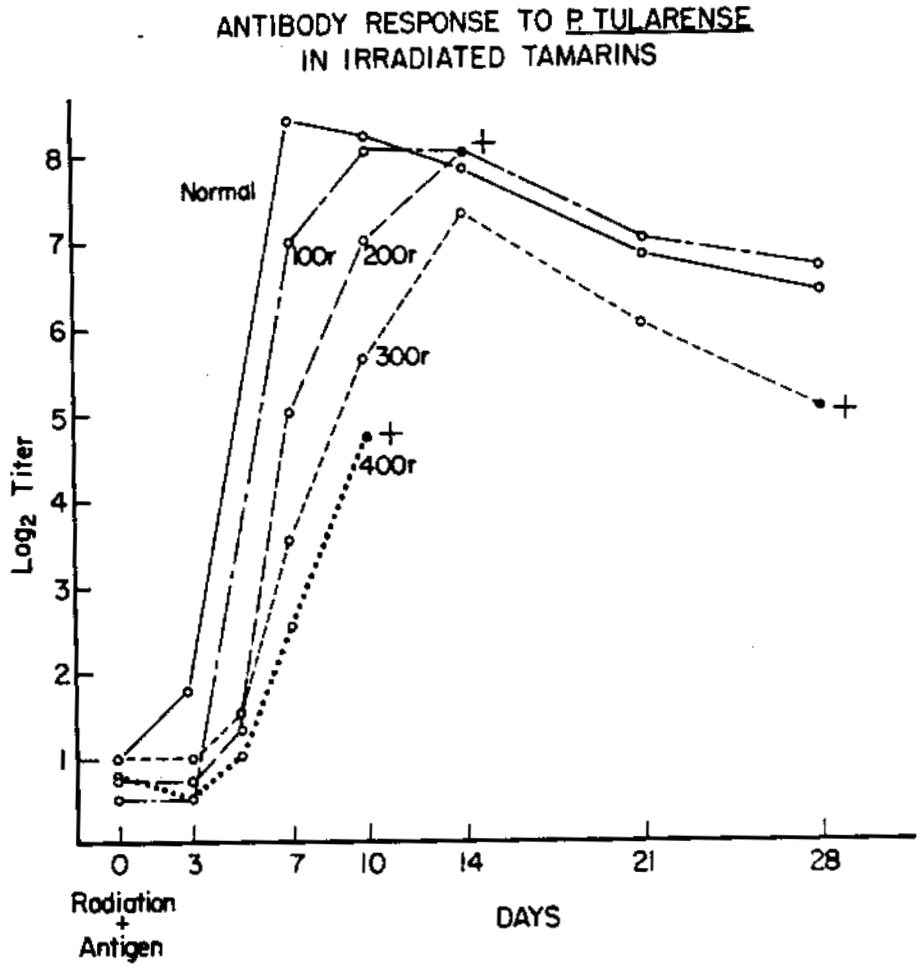

Fig. 3. Antibody formation by irradiated tamarins relative to the normal tamarin response curve.

Chimerism in Tamarinus nigricollis as Determined by Hematologic and Cytogenetic Analysis* (N. Gengozian, P. Eide, and J. S. Batson)

Projected studies on marrow transplantation in irradiated tamarins necessitated the availability of a suitable marker for demonstration of a "take" of the donar marrow elements in the host. Since we as yet do not have any reliable red-cell antigenic differences to distinguish one animal from another, it was decided to test the feasibility of using the female sex chromatin (drumstick) in the neutrophils as a marker after injection of donor female marrow into an irradiated male host. Blood smears of both male and female tamarins were analyzed to determine the frequency of neutrophilic drumsticks and their value as a transplantation marker system. Table 1 shows the result obtained upon examination of peripheral blood smears from 17 female and 16 male tamarins. On the basis of 500 neutrophils counted for each blood smear, it can be seen that the number of neutrophilic drumsticks observed ranged from 0 to 12 among the females and 0 to 7 among the males.

Because fraternal twinning among the tamarins is almost a consistent occurrence in litters of this species of primate (Wislocki, 1938), the foregoing data suggested that chimerism existed in these animals by virtue of vascular anastomosis during embryonic development. Evidence for such female-male chimerism was obtained by cytogenetic analysis for the sex chromosomes. Bone marrow from a female tamarin (Number 2 in Table 1) was suspended in tissue culture media and 10×10^6 cells were placed into several millipore diffusion chambers for cultivation in irradiated mouse recipients. On the eighth day after implantation, the recipients were injected with colchicine and the chambers were removed six hours later to collect the cells that were in mitotic metaphase arrest. These cells were then subjected to hypotonic treatment and methyl-acetic acid fixative to obtain chromosome spreads for sex chromosome determinations. Of 50 cells scored, 15 (30%) were found to have the Y chromosome, thus indicating the presence of male cells in a female tamarin. On the basis of this result, the data of Table 1 could be reexamined for possible interpretation of the varying numbers of drumstick neutrophils among the male and female blood smears. Thus, if one were to use a minimum value of 6 drumsticks per 500 neutrophils counted as indicative of a true "female," 8 of the 17 tamarins (or approximately one-half) would fall into this class. Furthermore, if we were to set the criteria that 0 to 1 drumstick counted per every 500 neutrophils was indicative of a "male," 8 of the 16 male tamarins (or one-half) would fall into this class. Those males and females then falling outside their drumstick "sex" range would then represent male-female chimeras, the twinning phenomenon resulting in a decreased frequency

* Research supported by U.S. Air Force Contract No. AF 41(657)-398. Aerospace Medical Division, Air Force Systems Command, U. S. Air Force, Brooks Air Force Base, Texas.

of the drumsticks in the females and a corresponding increase among the males. These data coincide with the 50% probability that fraternal twins will be of the opposite sex.

The results of this study have shown (1) that the use of sex drumstick as a transplantation marker among tamarins would be limited, being applicable only in those situations where the absence of such cells in potential male recipients could be shown conclusively and (2) chimerism in the tamarins. Studies are now in progress to determine whether this hematopoietic chimerism (bone marrow) extends also to cells having immunologic functions, such as the thymus and lymph-node cells. In this regard, the diffusion-chamber system for cultivation of proliferating cells offers a microtechnique to obtain cells suitable for chromosome analysis.

Table 1

Analysis of Peripheral Blood Smears of Male and Female Tamarins for the Neutrophil Sex Chromatin "Drumstick"

Animal No.	Frequency of Drumsticks/500 Neutrophils	
	Sex	
	Female	Male
1	3	0
2	1	1
3	4	4
4	3	4
5	2	4
6	9	2
7	9	1
8	2	7
9	3	0
10	9	0
11	0	1
12	9	4
13	12	5
14	5	1
15	8	1
16	8	5
17	6	-

METALS METABOLISM AND MEDICAL RADIONUCLIDES

Objectives during 1963 have dealt primarily with extending and completing certain topics introduced in the previous summary (USAEC Report ORINS-42, 1962, pp. 22-29) and with methodology selected for a further phase in the study of metals metabolism. The metals of interest are mainly elements of the lanthanide series along with certain others that offer either potential medical problems or application. This interest continues to stand (1) on their increasing importance of these elements in nuclear medical and industrial problems; (2) on the abundance of their available radioisctopes, which closely resemble each other in biochemical properties but differ widely in radiophysical properties; and (3) on the need for basic information to explain their metabolism and effects.

The current work has emphasized the use of cerium more than other elements. This is because of certain convenient radioisotopic properties and because of our considerable experience in studies to characterize the fatty infiltration of liver that occurs in rats after an intravenous dose of any element in the cerium group of lanthanons. This striking biochemical response to a heavy metal has recently found a parallel in the actinide series of elements. An intravenous dose of neptunium, which analogously in the first third of that series, causes a fatty liver with characteristics apparently similar to those for cerium (personel communication, Hanford Laboratories).

The abstracts that follow summarize (1) recently completed measurements on comparing the induction of rare-earth fatty liver by three (Ce, Nd, and Sm) similar series of chelates; (2) preliminary studies on selectively irradiating lymph nodes by the intralymphatic injection of heavy metals that show colloidal properties internally; and (3) methodology (Sephadex gel chromatography) for both in vitro and in vivo measurement of metal binding, protein fractionation, and heavy cationic transport. Studies paralleling the second and third of these, but not abstracted here, are under way using technetium-99m (140 kev gamma, no beta, half-life 6.0 hours) in a colloidal system to scan bone marrow and using polyacrylamide gel (disc) electrophoresis (20 to 25 serum protein fractions) as an analytical tool supporting preparative Sephadex fractionation.

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

The purpose of this work is to evaluate factors affecting selective localization of radioactive materials after intralymphatic injection; the ideal preparation would localize throughout successive nodes in the path of drainage without reaching the blood stream. The work reported in progress last year (USAEC Report ORINS-42, 1962, p. 25) was extended to include evaluation of several other radioisotopic preparations administered to dogs by intralymphatic injection. The tentative conclusions for the previously studied preparations were strengthened by additional animals also.

The results emphasized the importance of both the size and the chemical composition of colloidal or suspended particles in determining the lymphatic localization and circulatory distribution after lymphatic injection. The effect of size is believed to be a direct one although this does not presume simple filtration to be the full explanation. The effect of composition is indirect, at least for part of the preparations that were studied.

The additional groups, not previously summarized, include carrier-free Ce^{144}, a suspension of smaller ceramic microspheres containing C^{144} (MS-2, 0.5 to 3 μ diam.; 3 M Company), two differently tagged preparations of colloidal chromic phosphate, and certain chelates of cerium. Like carrier-free yttrium, the low chemical dose of cerium showed poor lymph nodal localization and much of the dose distributed throughout the body in a manner similar to an intravenous dose. Whereas the metals were given as soluble chlorides, their distribution patterns, known from various separate studies, is consistent with radiocolloidal properties. Those preparations along with gold colloid (15 mμ) are examples of particles too small to avoid large leakage into the circulation. In defining an upper limit, the effective size for gradual lymph nodal localization is less than 3 μ in diameter since the smallest microspheres did not move significantly past the first node in their channel of drainage. Moreover, repeated observations showed much of the dose to sludge and remain in the lymph channel between the injection site and the first node.

Two kinds of preparations showed varying degrees of intermediate behavior. Chromic phosphate (P^{32}-tagged, 0.05 to 1.0 μ, Cr^{51}-tagged, 0.03 to 1.0μ) showed a wide spread of nodal localization with little leakage into the circulation. A solution containing a chelate of cerium (Ce-HEDTA)

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midway in stability in a series from citrate to DTPA gave favorable localization in nodes from the popliteal to the mediastinal region after its intralymphatic injection in the dorsum of the foot. Tables 1 and 2 and Figs. 1 and 2 show quantitative comparisons of these various preparations.

Table 1

Intralymphatic Localization of Radioactive Materials

(7-Day Dogs)

Specimen	Percent of Dose per Total Specimen							
	Yttrium Cl_3		Au^{198}	$Cr^{51}PO_4$	$CrP^{32}O_4$	MS-2	MS-1	Chelate*
	CF	C						
Lymph Nodes[a]	2.1	14.1	50.4	90.9	72.1	41.5	38.0	82.5
Liver	12.8	9.0	30.0	6.2	1.2	0.23	0.10	11.0
Spleen	1.1	0.36	0.10	0.51	0.07	0.01	0.01	0.62
Muscle[b]	0.73	0.10	0.00	0.00	0.00	0.00	0.00	0.00
Skeleton[c]	12.0	1.3	0.10	0.10	0.10	0.00	0.00	0.10
Other tissues[d]	1.5	1.2	1.0	0.10	1.0	10.0[e]	12.5[e]	0.30[e]
Blood	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Excretion	21.4	13.8	1.0	1.3	1.3	0.10	0.10	3.5
Total analyzed	51.6	39.8	82.6	99.0	75.8	-	-	98.0
Unobserved	48.4	60.2	17.4	1.0	24.2	-	-	2.0

* - Ce-HEDTA (1:2)
a - Usually seven or eight nodes from various sites analyzed separately.
b - Skeletal muscle calculated as 45% of body weight.
c - Rib and femur analyzed; average content used to calculate amount in skeleton as 10% of body weight.
d - Includes adrenals, heart, lungs, GI tract and kidney.
e - Lymphatic vessel per gram from injected part to popliteal node.

Table 2

Differential Absorption Ratio of Nodes and Organs
(7-Day Dogs)

Specimen	Radioactive Preparation, Intralymphatic						
	Yttrium Chloride (Y*)	Yttrium Chloride (Y* + Y)	Au^{198}	$Cr^{51}PO_4$	$CrP^{32}O_4$	MS-2	Chelate (Ce* + Ce)
	Total Body = 1.00						
NODE, popliteal	161	1,250	7,020	6,000	19,000	3,400	5,800
superficial inguinal	-	41	-	32	0.3	5.1	3.0
inguinal	2.5	-	-	37	-	-	18
iliac	122	380	4,800	1,360	93	0.9	4,800
periaortal	2.9	32	88	1.3	1.3	0.3	440
mesenteric	0.6	0.5	0.5	0.04	0	0	0.4
mediastinal	2.2	55	490	32	0.9	0	420
SPLEEN	0.4	1.4	0.6	1.0	0.3	0	1.5
LIVER	2.4	2.7	9.2	1.9	0.3	0.1	3.0
	Liver = 1.00						
NODE, popliteal	67	463	763	3,160	63,670	34,000	1,930
superficial inguinal	-	15	-	17	1.0	51	1.0
inguinal	1.0	-	-	19	-	-	6.0
iliac	51	141	522	716	310	9.0	1,600
periaortal	1.2	12	10	0.7	4	3.0	145
mesenteric	0.25	0.19	0.05	0.02	0	0	0.1
mediastinal	0.91	20	53	17	3.0	0	140
SPLEEN	0.17	0.52	0.07	0.5	1.0	0	0.5

* Radioisotopes: Y^{90}, Ce^{144}

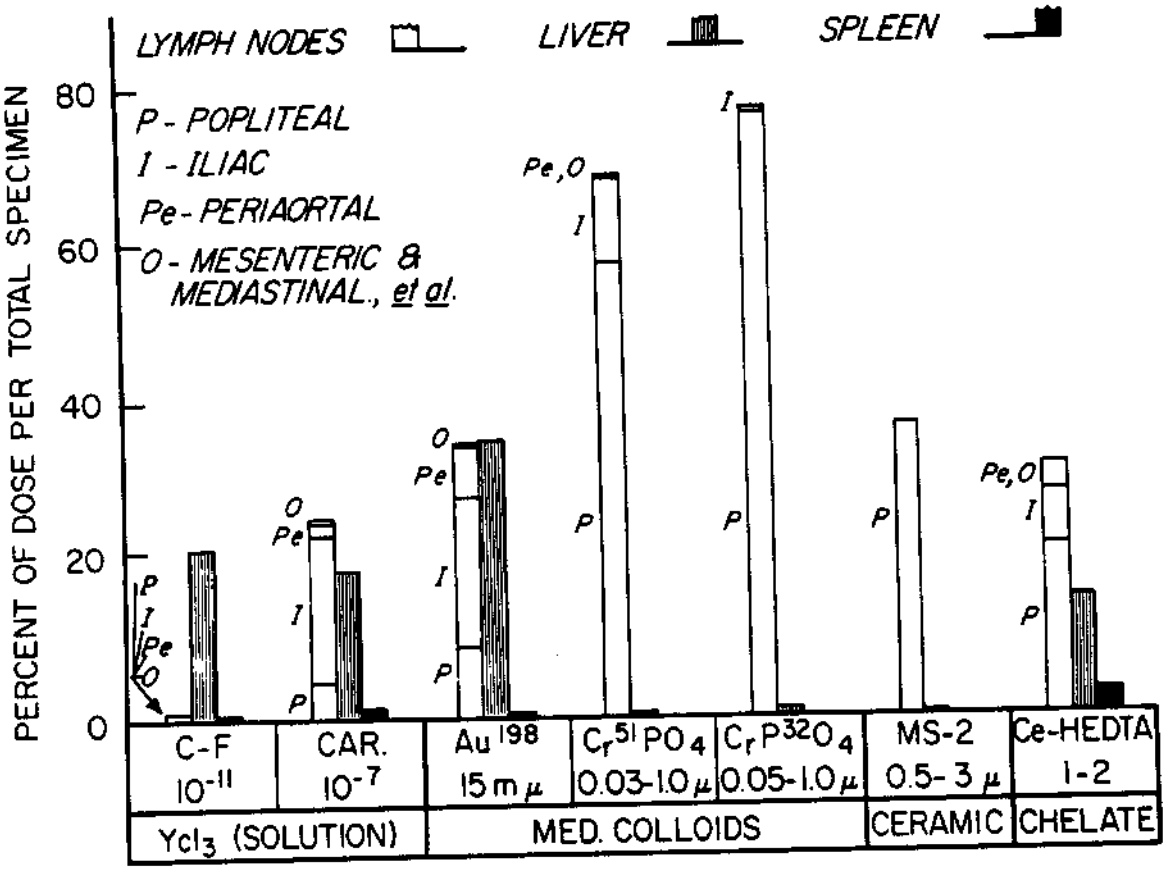

Fig. 1. Intralymphatic Localization in Dogs
(One Day)

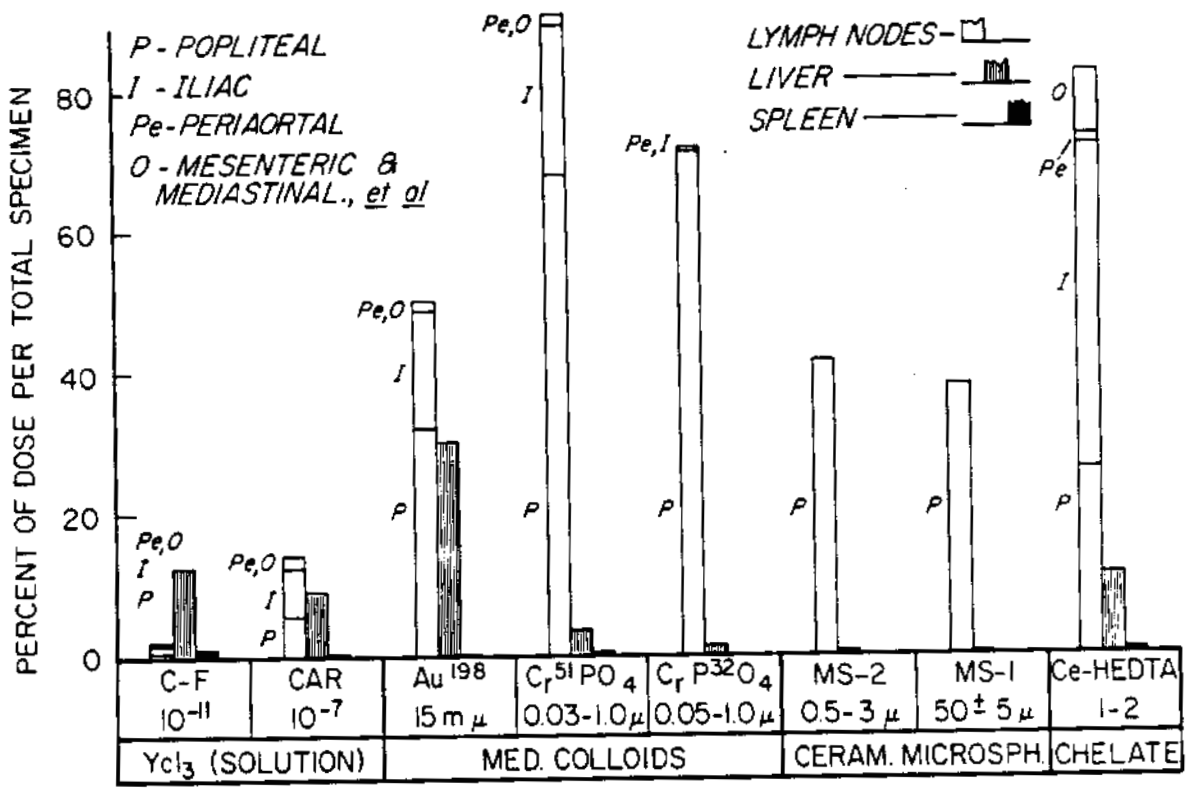

Fig. 2. Intralymphatic Localizations in Dogs
(Seven Days)

Rare-Earth Fatty Livers Induced by Lanthanon Chelates (Granvil C. Kyker and John J. Rafter)

The induction of acute fatty livers in rats by an intravenous dose of lanthanon chelate was compared for three series of compounds. These were parallel series containing cerium, neodymium, and samarium (atomic number 58, 60, and 62, respectively). Each series* included (1) chloride, (2) citrate, (3) HEIDA, (4) NTA, (5) HEDTA, and (6) EDTA; in a few studies mandelic acid and DTPA were also used. For a specific element the series of complexes progressively increase in stability from citrate to EDTA; the mandelate is rather unstable and DTPA is the most stable of all. Also, for a specific chelating agent the stability of the complex increases with atomic number within the lanthanide series. Several of the results for the cerium and samarium series were previously summarized (USAEC Report ORINS-42, 1962, pp. 22-24); the additional measurements made recently support and extend the interpretations of that report.

Early characterization of rare-earth fatty liver showed that it was not caused by elements above samarium. Below samarium, 2 mg/kg of each metal as the chloride regularly induced acute fatty infiltration. The same dose of samarium had little effect but 4 mg/kg produced the fatty change. These levels of the metal were maintained in calculating the dose of the various compounds in the series. Each dose preparation contained the lanthanon, calcium, and the chelating agent in a molar ratio of 1:1:2, respectively. A radiotracer of the lanthanon was added to each preparation (Ce^{144}, Nd^{147}, Sm^{153}).

Each of several analyzed factors correlate with the graded stability of the various chelates. Toxicity (indicated by weight loss, liver enlargement in relation to body weight, and the amount of fatty infiltration) decreases with atomic number; in this series, complex stability increases as the atomic number goes up. Cerium in citrate, NTA, and HEIDA complexes caused fatty liver similar to its chloride; HEDTA, EDTA, and DPTA gave complete protection. In the samarium series of complexes the dividing line for fatty liver induction ended with citrate. Results from the neodymium series suggest an inverted order for part of the complexes, with HEIDA and HEDTA preparations appearing lower in the series and both citrate and NTA affording protection.

*HEIDA, hydroxyethyliminodiacetic acid; NTA, nitrilotriacetic acid; HEDTA, hydroxyethylenediaminetriacetic acid; EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid.

The distribution and excretion data for the four measured compartments (liver, carcass, urine, feces) reflect quite consistently the order of complex stability; an exception appears for Nd-EDTA. A few examples show a lack of correlation between the degree of localization of the metal in liver and the degree of fatty infiltration. For samarium, the citrate and HEIDA complex localize equally and about the same as most of the compounds that cause fatty liver; yet the citrate causes fatty liver and the HEIDA-ate does not. For neodymium the same two complexes localize equally but the fatty response is reversed, with citrate protecting and Nd-HEIDA causing fatty liver. The charts (Fig. 1-4) will clearly illustrate these interpretations.

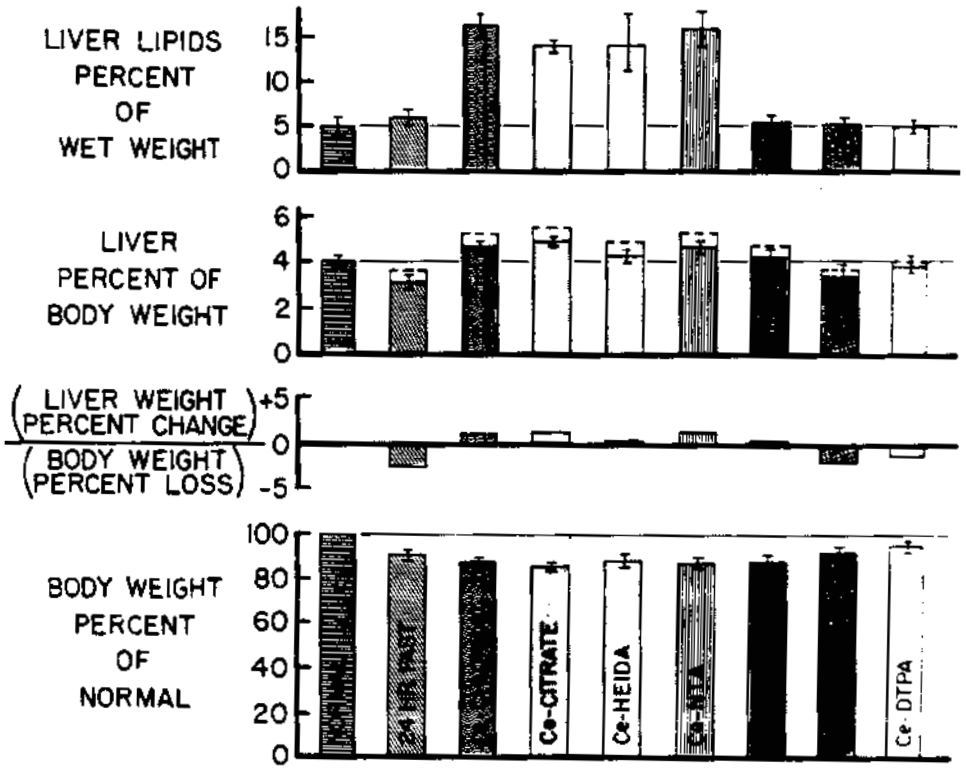

Fig. 1. Cerium Chelates - Intravenous.

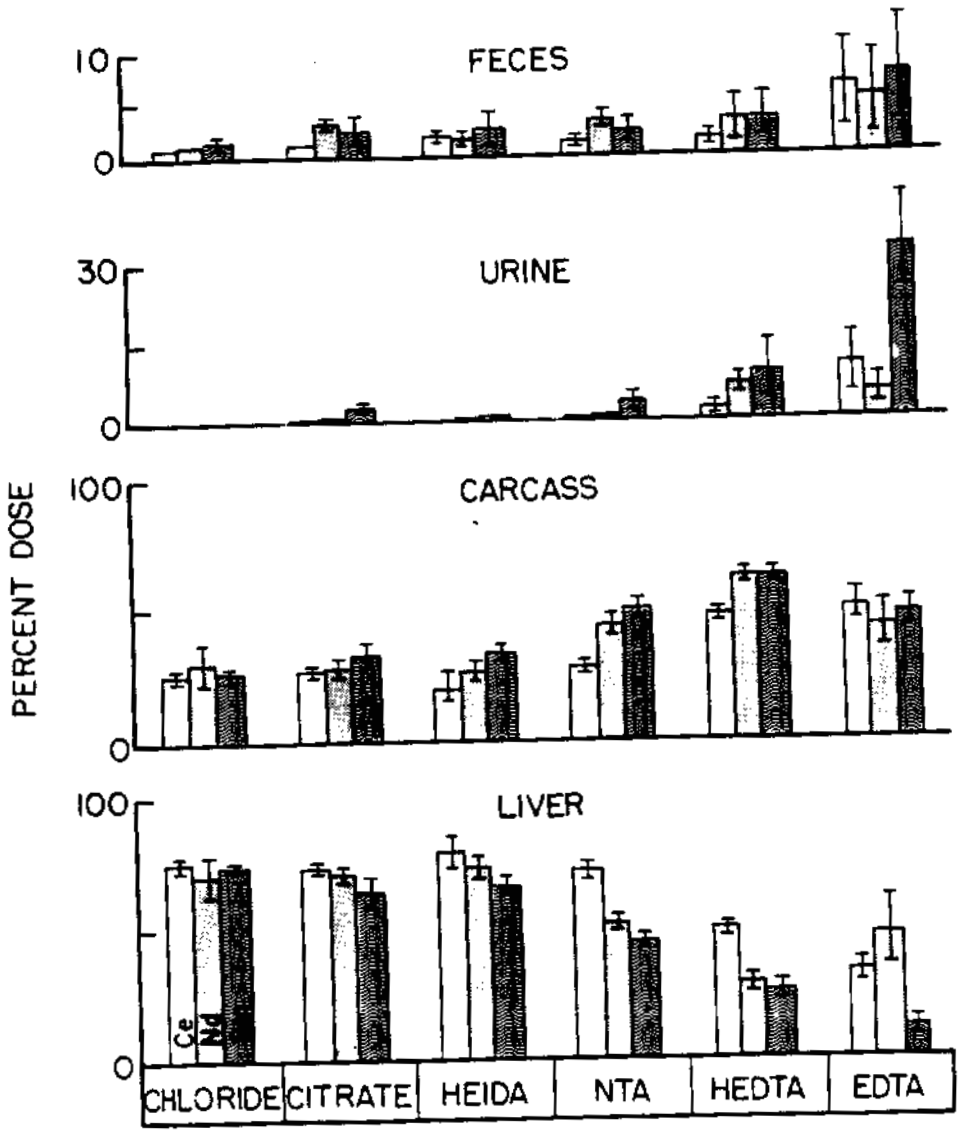

Fig. 2. Intravenous Lanthanon Chelates
Rats - 48 hrs.

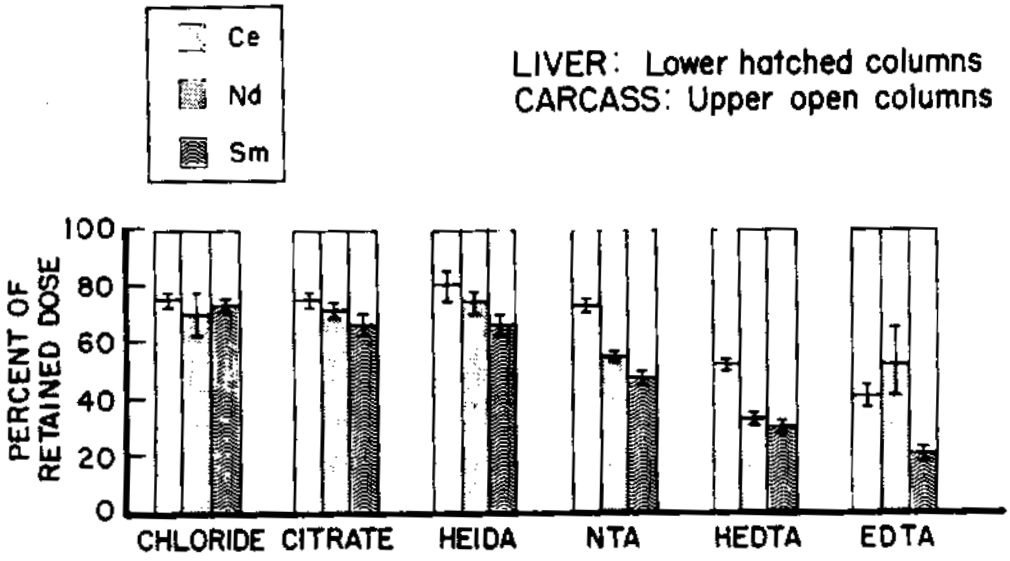

Fig. 3. Distribution of Retained Dose of Certain Lanthanon Chelates - Intravenous - Rats - 48 hrs.

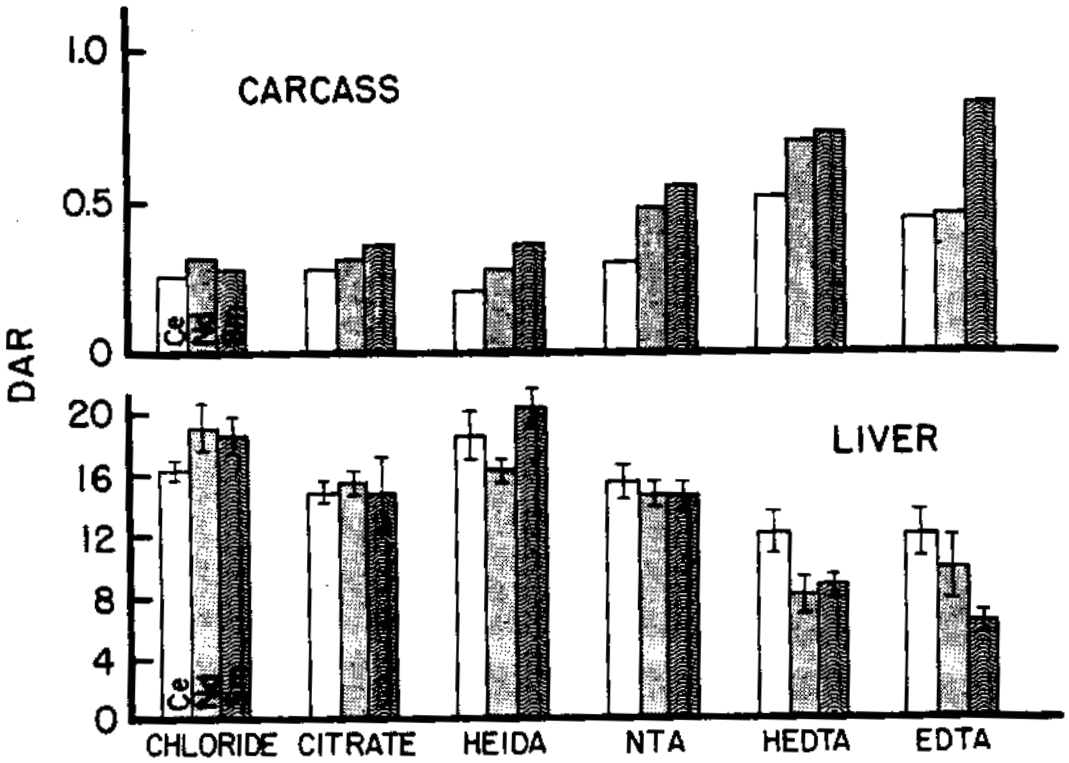

Fig. 4. Differential Absorption Ratio of Certain Lanthanon Chelates in the Liver and Carcass of Rats - Intravenous - 48 hrs.

One mechanism to explain the metabolic impact of an intravenous lanthanon cation, seen grossly as fatty liver, would assume the blocking of an essential enzyme system. Since recovery regularly occurs, the assumption would be that the cellular source of this system is not killed but that only the existing supply of enzyme is blocked or bound irreversibly. Repeated experimental evidence shows the critical damage to occur the instant the intravenous dose is delivered, whereas the prominent liver localization, which with few exceptions is an associated characteristic, proceeds gradually over several hours. These two gross characteristics may be purely coincidental, with the large eventual localization of metal in liver reflecting the metabolic inactivation or disposal of a substance that can be excreted only very slowly. To the extent that this assumed mechanism is valid, its further explanation depends on the identification of the critically bound enzyme site and its stability. These results with the series of chelates in the intact animal give a gross definition of the limits. Further study of the questions requires work on the interaction of these metals with isolated systems. This entails in vitro work with specific proteins and enzymes, which is under way.

Sephadex Fractions of Protein Bound Lanthanons (Granvil C. Kyker, Barbara Chastain, and Mary K. Ballenger)

Lanthanon cations are strongly bound by plasma in vivo and in vitro as soluble complexes of unknown structure. A few reports show that such interaction occurs with isolated protein fractions; to our knowledge this does not include work on specific enzyme systems. Since protein-metal binding can occur as the result of either chemical complexation or physical adsorption, the fractionation of metal-protein complexes based on a physical property such as molecular size should detect either type of binding. The use of recently invented cross-linked dextrans, Sephadex, offers potential advantages for application here. Various grades of Sephadex are designed to sieve or exclude molecules according to size (SG-25 to SG-200 for molecular weights from 4000 to 200,000).

Our preliminary experience with serum incubated with radioactive tracers, cerium-144 or samarium-153, on G-50 or G-75 has confirmed the expected results for these grades of Sephadex. The fractionation is done in the usual manner for column chromatography. The serum proteins elute in one main peak; usually this is followed by a small, unidentified peak (smaller molecular weight). The eluted radioactivity largely coincides with the main protein peak; recovery of the tracer is not complete during the elution with buffer, and stripping the column with EDTA brings off the remaining tracer. Elution profiles for cerium and for samarium on G-50 and G-75 are shown by Figs. 1 and 2. Both G-100 and G-200 resolve separate fractions of protein shown by the several peaks in Fig. 3 and 4, (a & b), and Fig. 4a shows that the metal is associated largely with only two of the protein fractions.

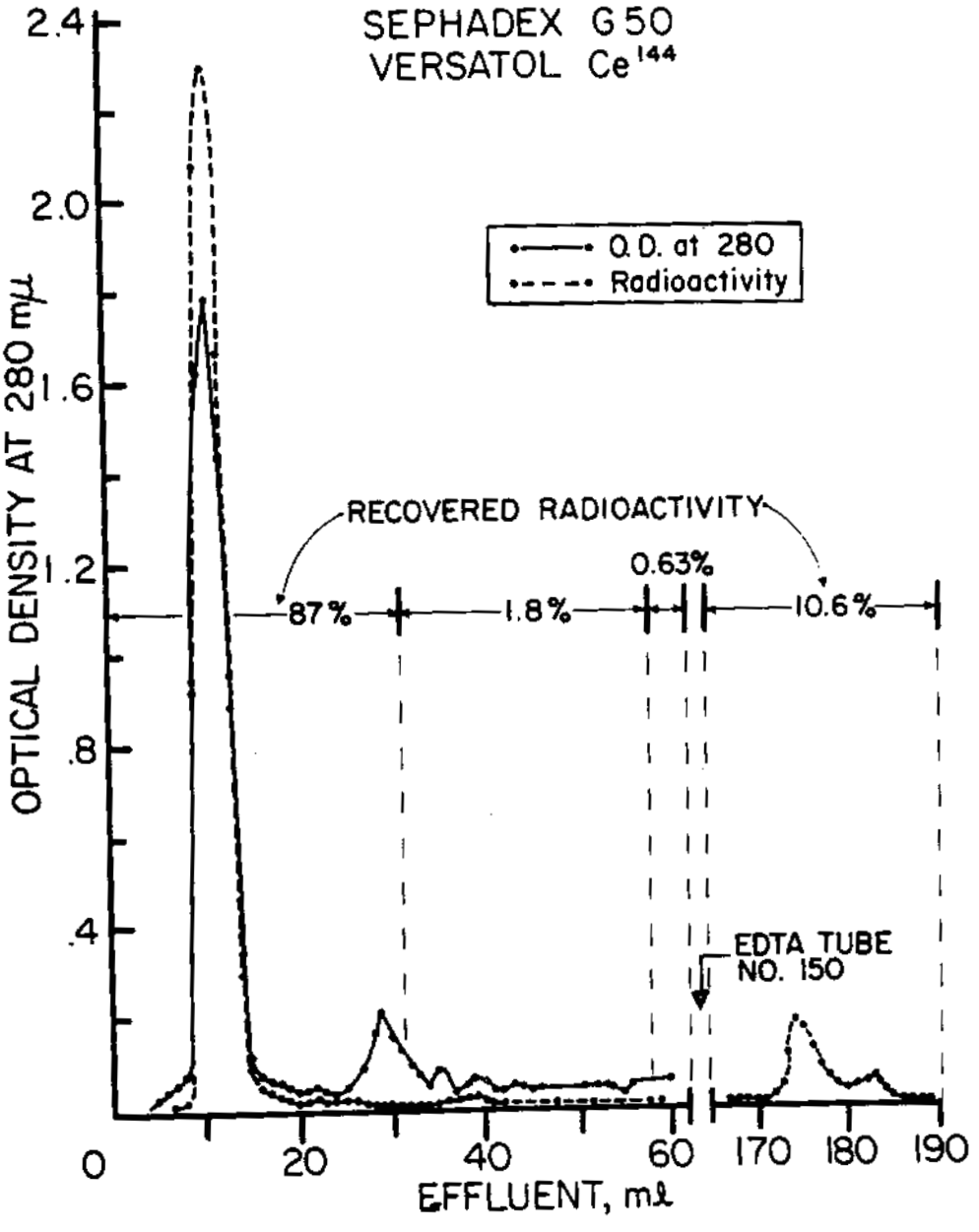

Fig. 1. Gel filtration of an incubated mixture of Versatol and cerium chloride (Ce^{144}) using Sephadex G-50 (See Experiment 2, Table 1 for column specifications).

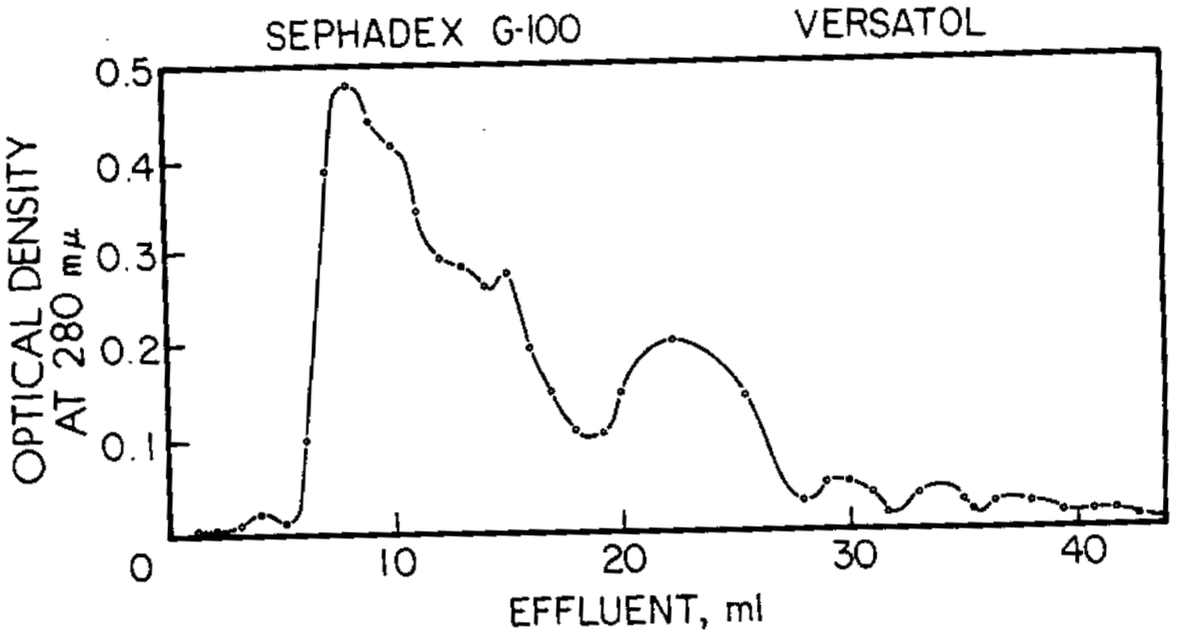

Fig. 3. Gel filtration of Versatol under conditions defined in Table 1, Experiment 5.

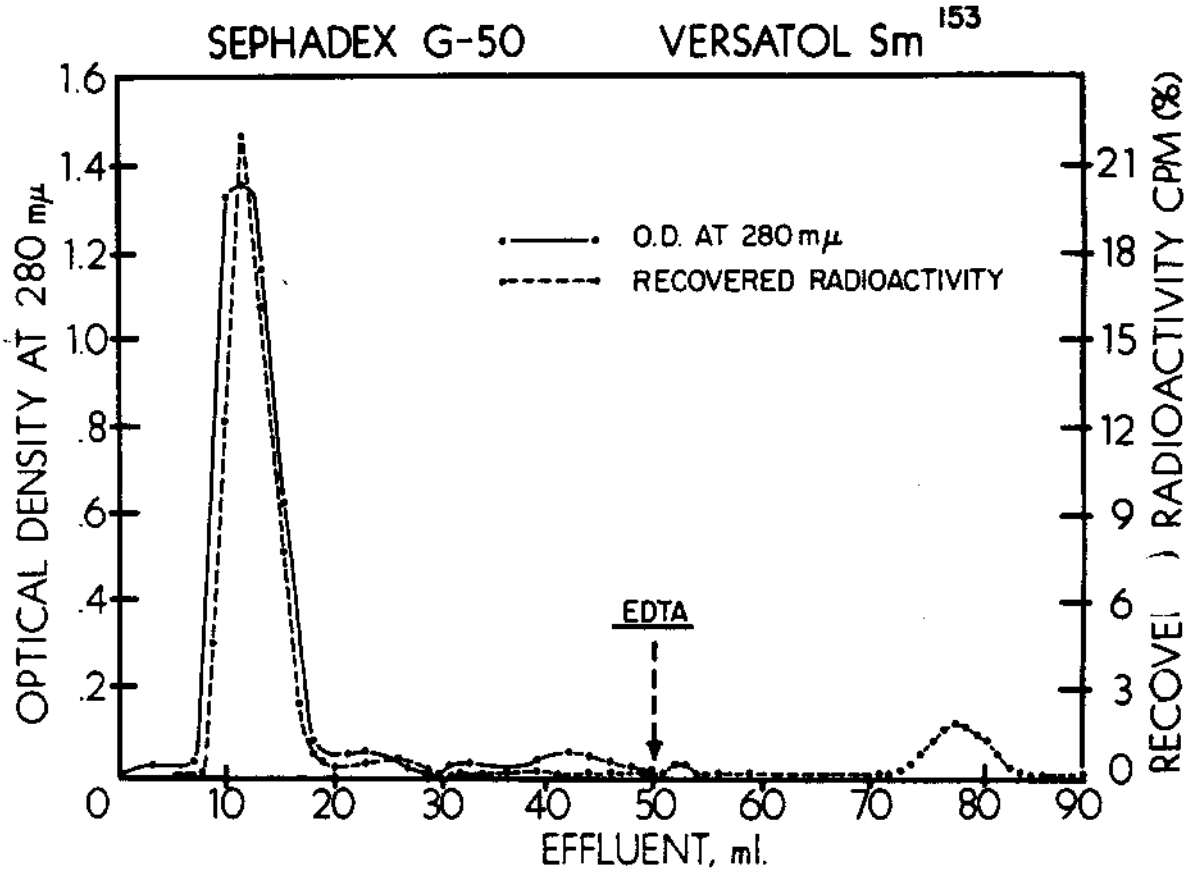

Fig. 2. Gel filtration of an incubated mixture of Versatol and samarium chloride (Sm^{153}) using Sephadex G-75 (See Expt. 4 in Table 1 for column specifications.)

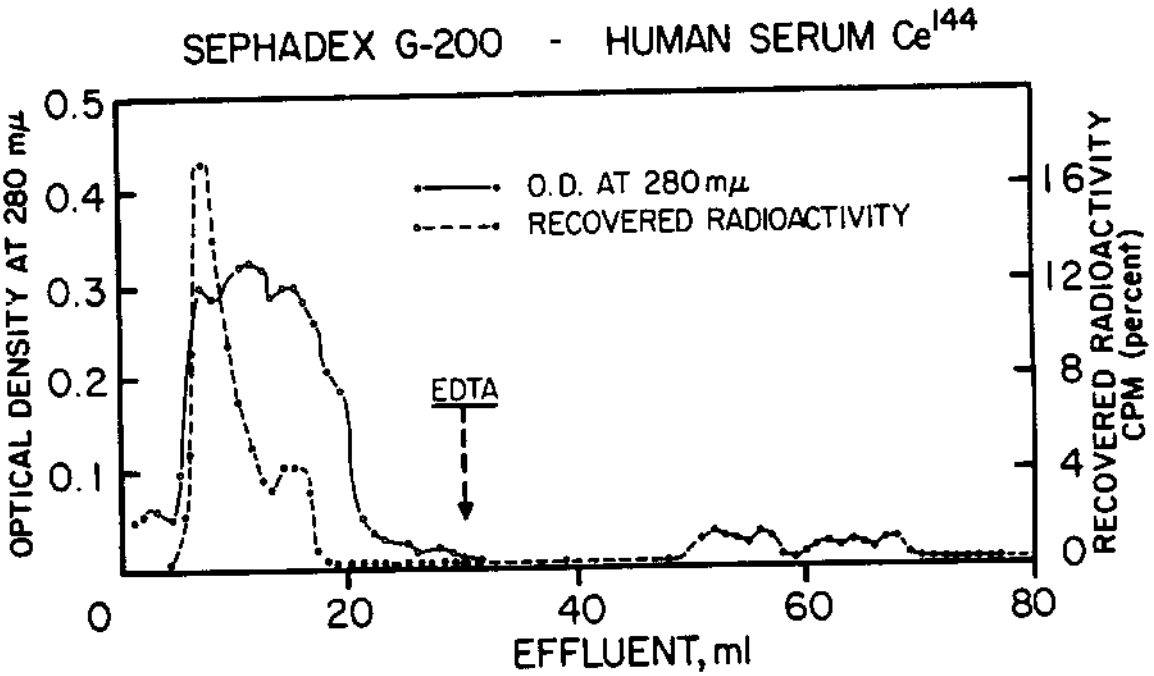

Fig. 4a. Gel filtration of an incubated mixture of human serum and cerium chloride (Ce^{144}) using Sephadex G-200. (See Experiment 6 in Table 1 for conditions.)

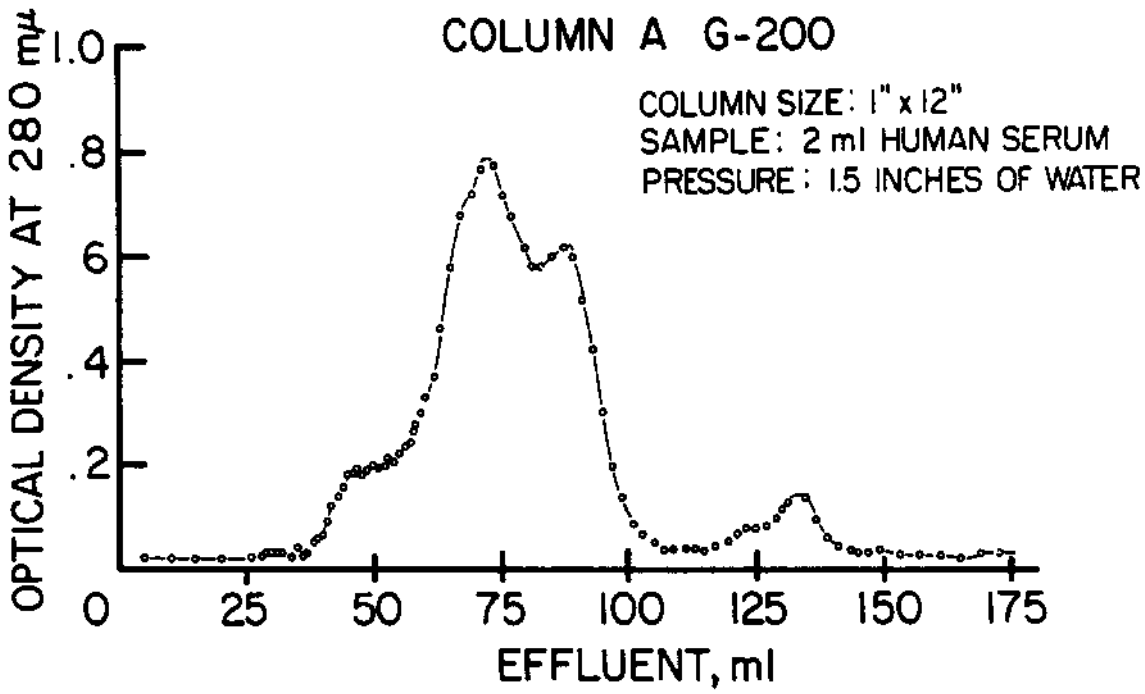

Fig. 4b. Compare with 4a: No radiotracer, different column dimensions, pressure, and flow rate. (See Table 1, Experiment 7)

Using G-100 and G-200 in the manner that was satisfactory for the lower grades has presented serious problems in maintaining workable flow rates. Published reports for these grades are limited and offer a little help. Increasing the operating pressure not only failed to increase the flow but caused several carefully poured columns to cease to flow. Columns containing graded amounts of G-200 evenly mixed with ethanolyzed cellulose (chromatographically inert for protein) or with G-25 failed to solve the flow-rate problem. For 1-in. columns, operating at low pressure (less than 2 in. of water, during both pouring and use) has proved to be the best choice of conditions. Table 1 summarizes the operating conditions and flow rates for several columns. The first four tabulated experiments compare G-50 and G-75 under similar conditions, each with Ce^{144} and with Sm^{153}. The first pair were successive runs on the same column, and similarly for the second pair. The chromatograms showed superimposed peaks of protein and tracer in these comparative runs that were broader with samarium than with cerium. The two columns of G-200 (Expt. 6 and 7) contrast conditions that differ widely; yet both gave similar profiles of protein fractions. In summary the tabulated results offer slow but workable flow rates only for columns of G-50 and G-75. These agree with the rapidly growing literature of the past two years on these lower grades which are designed, however, to fractionate molecular weights less than 40. to 50,000. We have not found procedures described for G-200, which fractionates larger molecular sizes; we have in progress studies to enable its use.

Supportive methodology (disc electrophoresis) is being standardized for identification of components in the mixtures that comprise the Sephadex fractions.

Table 1. Flow Rates for Various Sephadex Grades

Experiment	1	2	3	4	5	6	7
Sephadex	G-50	G-50	G-75	G-75	G-100	G-200	G-200
Column							
diam., cm	1.3	1.3	1.3	1.3	1.3	1.3	2.5
hr , cm	30	30	30	30	15	15	30
Bed volume, ml	28	28	28	28	14	11	50
Sample							
Versatol, ml	2	2	2	2	0.5	-	-
Human serum, ml	-	-	-	-	-	1	2
Bed volume sample	14	14	14	14	28	11	25
Tracer, μc							
Ce^{144}	9.8	-	9.8	-	-	4.8	-
Sm^{153}	-	5.2	-	5.7	-	-	-
Recovered, %	93	77	95	85	-		-
Pressure, cm-H_2O	126	126	124	125	5	200	3
Flow rate*							
ml/hr	7.8	11.7	6.0	10.8	2.1	9.0	6.0
	9.3	9.6		2.7		2.0	
						0.5	
ml/min/cm^2	.104	.156	.08	.144	.028	.120	.019
	.124	.128		.036		.027	
						.0068	

*Underscored values show changes in flow rates that occurred during runs.

RADIOISOTOPES IN DIAGNOSIS AND THERAPY

Blood Clearance of Au^{198} with an Arm Counter (William D. Gibbs and C. Lowell Edwards*)

Attempts to measure blood clearance of intravenous colloidal Au^{198} using the arm counter indicated that only 70% of the isotope disappeared with a half-time compatible with colloidal behavior; a long half-time component (approximately 30% of the extrapolated total activity) was not seen when serial blood samples were assayed.

Results of this experiment are shown in Table 1.

Table 1

Patient	Percent of extrapolated T_0 arm count remaining after 3 minutes	Disappearance T 1/2 (min) Arm counter*	Blood assay
1	65%	2.5	2.5
2	58	2.75	1.75
3	64	3.5	2.5
4	48	2.75	2.0
5	66	4.5	1.5
6	36	1.75	1.5
7	44	2.0	1.5

* Corrected for long half-time component

In all except patient 1, the half-time measured by blood assay was shorter than the value obtained using the arm counter even though corrections were made for the slow component in the latter. These data indicate that there may be a second extravascular compartment in the arm. The major extravascular compartment for which the correction was made has a dissappearance half-time that varies from 40 min to many hours. Uptake in bone marrow might explain some of this slow component.

* Visiting Internist assigned by the Public Health Service.

The results indicate that the arm counter is unsatisfactory for assessing hepatic blood clearance of colloidal radiogold.

Feasibility of Ga^{68} as a Diagnostic Agent (R. L. Hayes, J. E. Carlton, and G. C. Kyker)

Ge^{68} ($T_{1/2}$ = 280 d) decays by positron emission to Ga^{68} ($T_{1/2}$ = 68 min). A Ge^{68} cow from which Ga^{68} can be milked has been obtained from Brookhaven National Laboratory. Because of the long half-life of the parent Ge^{68} and the short life of Ga^{68}, it is believed that Ga^{68} may be a valuable diagnostic agent for bone scanning. Gallium-72 and Ga^{67} in the past were studied at the Medical Division as possible therapeutic agents for sarcoma of bone and have been shown to localize selectively in certain bone tumors, primary and metastic. Since then (1952) there has been a vast improvement in scanning equipment and it would now seem worthwhile to investigate the diagnostic value of gallium as Ga^{68} because of its short half life. Preliminary scans of Ga^{68} as the EDTA chelate (form in which Ga^{68} is milked of the Ge^{68} cow) in dogs indicate, as expected, no deposition in bone. Gallium-68 can be separated from EDTA by a solvent extraction technique. Also, there is evidence that it may be possible to milk Ga^{68} from the alumina cow with dilute HCl. Gallium-68 citrate (form used in earlier investigation) both carrier-free and also with added carrier will be investigated.

A Rapid Screening Method for Detecting Abnormal Plasma Vitamin B_{12} Binding Sites in Chronic Myelogenous Leukemia Using Arm Counting (C. C. Lushbaugh and William D. Gibbs)

The work of others has established that plasma binding sites for vitamin B_{12} are abnormally abundant in persons with active chronic myelogenous leukemia (CML). As a result of this abnormality, intravenously injected radioactive vitamin B_{12} is cleared from the blood at a much slower than normal rate in such patients. In complete remission this phenomenon disappears. In some other myeloproliferative diseases such as polycythemia rubra vera and myelofibrosis with splenomegaly, this phenomenon is occasionally encountered but, when found, raises the question of whether these diseases have not progressed into CML. In other leukemias it is not found.

Although in most patients the diagnosis of CML is easily determined, some anemic and hypersplenic states occur as preleukemic phases of CML and anticipation of this development is difficult. Also in some poorly differentiated leukemias, morphologic identification is quite difficult although possibly desirable for planning therapy. A simple method, therefore, that would measure the relative number of vitamin B_{12} binding sites and thereby

rule CML in or out would be diagnostically useful. Such a method would need to measure the amount of vitamin B_{12} remaining in aliquots of blood or plasma after the intravenous injection of the vitamin. The use of vitamin B_{12} labeled with radioactive cobalt has made this formerly very difficult determination relatively easy through well-counting scintillometry, but requires either large doses of radioactivity or large samples of blood because of the great dilution of the dose by the patient's blood volume.

In an attempt to overcome these objections, the changes in the radioactivity of a large volume of circulating blood was measured with a large liquid-scintillation well counter known as the "Arm Counter," by continuous external counting of the forearm. Counts were made during and immediately after the intravenous injection of 0.5 microcuries of Co^{60} cyanocobalamin into the antecubital vein of the other arm. The radioactivity of the arm was recorded as counts per minute on a strip-chart recorder and the net counts were graphed against time in minutes. Because the dose was not varied with patient's body weight, the initial radioactivity in the arm counter was not constant from person to person. Therefore, for purposes of comparison the curves were normalized by assigning the initial arm radioactivity the value of 1.0. The results of preliminary trial of this method in nine persons with various diseases are shown in Fig. 1. The two patients with CML and another patient with myelofibrosis did not clear the vitamin from the arm as did the other six persons. Instead, the radioactivity of their arms rose progressively or did not change after an initial increase above the 1-min value. When these curves are compared with those obtained by radioassay of peripheral blood aliquots (Fig. 2), it is evident that the assays made with the arm counter include radioactivity from a compartment in the arm in addition to that of the peripheral blood. Although this space is presumably extravascular and interstitial, these studies indicate that it also has an increased affinity for vitamin B_{12} in CML and is therefore "larger" in CML than in persons without CML.

These preliminary studies suggest the attractive possibility that "arm counting" after intravenous Co^{60} vitamin B_{12} might become a facile means of differentiating CML from other blood dyscrasias, affording measurement of abnormal and relative binding sites within 5 min or less. The possibility of the existence of increased extravascular binding sites for vitamin B12 in CML has not been suggested previously and merits further investigation.

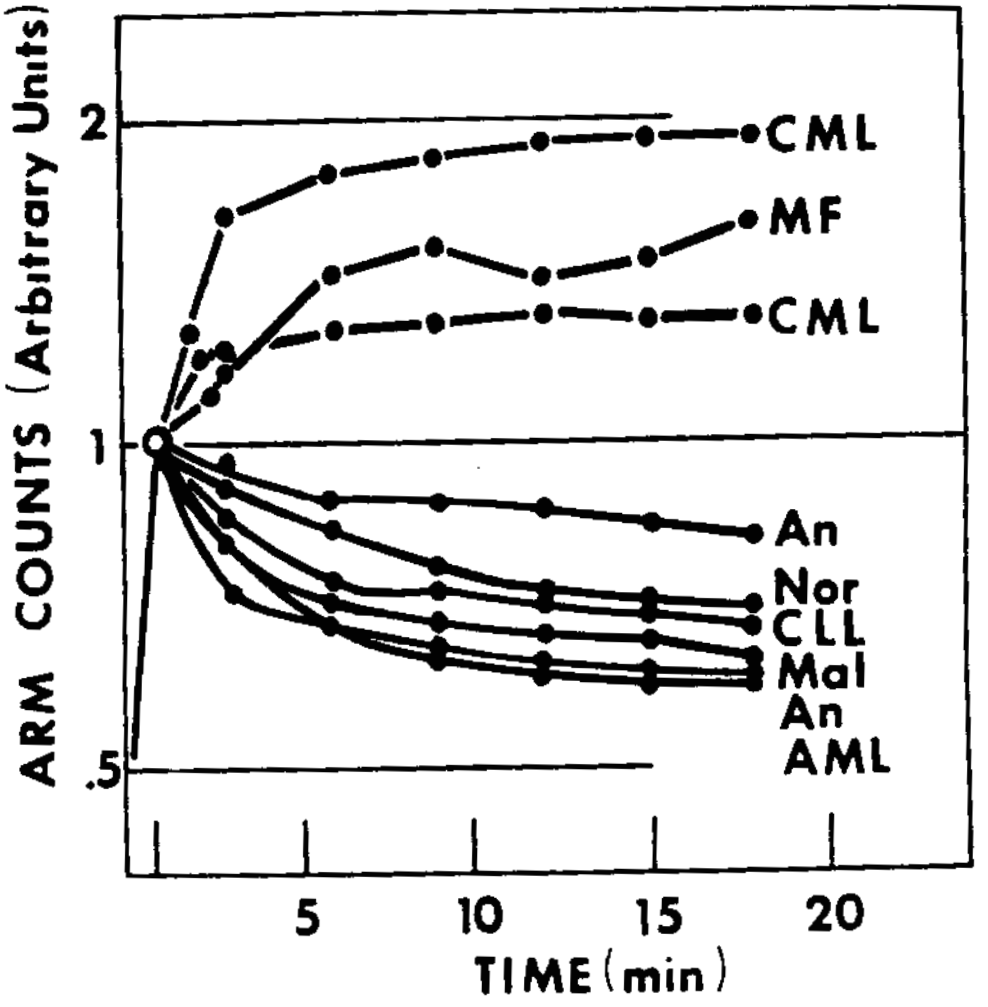

Fig. 1. Relative changes in arm radioactivity after intravenous 0.0005 millicuries cobalt-60 cyanocobalamin in chronic myelogenous leukemia (CML), myelofibrosis (MF), refractory anemia (An), chronic lymphatic leukemia (CLL), malabsorption syndrome (Mal), acute myelogenous leukemia (AML) and a normal person(Nor).

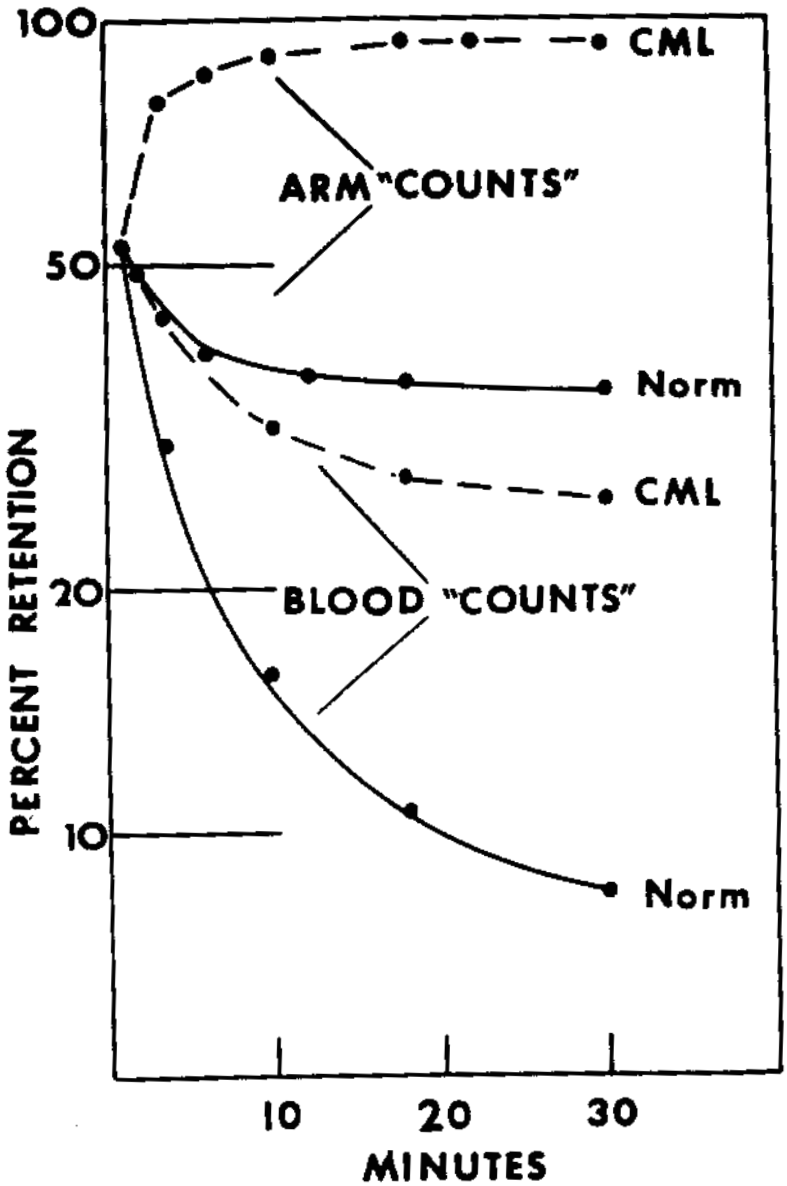

Fig. 2. Comparison of typical changes in arm and blood radioactivity in a normal person and a patient with CML after intravenous vitamin B_{12} labeled with radioactive cobalt, expressed as percentage of retention of dose to correct for differences in doses, blood volumes, and counter efficiencies.

Analysis of Patients with Carcinoma of the Ovary (F. Comas)

The patients with primary carcinoma of the ovary admitted to the ORINS Medical Division, from 1950 through 1961, were evaluated. During these 11 years, 95 women with proved diagnosis of ovarian carcinoma were admitted, of which 69 received some type of intraperitoneal radioisotope therapy. The histologic diagnoses were papillary serous cystadenocarcinoma in 43; adenocarcinoma in 19; pseudomucinous cystadenocarcinoma in 10; and the other eight had other types of malignancy. The great majority of those patients had advanced disease: 93% had pelvic and abdominal metastases, and 73% had ascites. Forty-six of the 69 were known to be dead at the time of this review.

Intraperitoneal radioisotope therapy was given in the 50 patients with ascites with the aim of alleviating or, if possible, stopping the accummulation of ascitic fluid. The isotope given was usually colloidal Au^{198}, although a few patients were treated with Lu^{177}, Y^{90}, and chromic phosphate-P^{32}. Half of this group of patients (24 or 48%) had their ascites controlled for a least one month; one-fourth (28%) were not controlled, and another fourth (24%) could not be evaluated because of the shortness of the follow-up period.

In some patients ascites appeared again after a period of remission varying from 3 months to 3 years. Since all 24 patients who obtained benefit from intraperitoneal isotope therapy are by now dead, it is possible to gauge the effectiveness of this treatment by determining whether or not ascites was controlled until the time of death. Out of the 24, 14 did not have further abdominal fluid accummulation, and 8 developed recurrence of ascites.

The therapeutic approach for treating advanced carcinoma of the ovary at ORINS Medical Division has been whenever possible to do repeated laparotomies at about 6-month intervals, removing at each time as much tumor as possible without excising important abdominal or pelvic organs. Several patients have lived for many years, relatively symptom free, especially those with slow-growing tumors. The effectiveness of this therapeutic approach can be best gauged by analyzing the dead patients with known time of death. There are 46 patients in this group; 37 of them had one or two operations, and their mean survival time was 10 months. Nine patients had three or more operations (one patient has had ten), and their mean survival time was 22 months. This difference is significant at the 1% level of probability. That these results are not overly vitiated by patient selection is shown by the observation that the incidence of ascites (a bad prognostic sign) was greater in the multiple operations group (89%) than in the group having only one or two operations (76%).

In vivo Mobilization of Ba^{137m} (R. L. Hayes and J. E. Carlton)

Cesium-137 ($T_{1/2}$ = 30 y) decays by pure beta emission to barium-137m ($T_{1/2}$ = 2.6 min), which in turn decays mainly by gamma emission to stable Ba^{137}. Wasserman, Twardock, and Comar (Science 129, 568, 1959) have reported that daughter Ba^{137m} is distributed in the rat in a pattern different from that of parent Cs^{137}, even though the half-life of Ba^{137m} is quite short. Among other findings they reported that the Ba^{137m} level in whole blood was three times that of the secular equilibrium value. We have confirmed this observation. Conceivably the overage of Ba^{137m} in blood might be used as a diagnostic test of the rate of blood flow and of general metabolic rate. Thus a determination of the ratio of excess Ba^{137m} at the time of blood sampling would be a measure of the mobilization rate of Ba^{137m} from muscle tissue, which in turn might be governed mainly by the rate of blood circulation through muscle tissue, although other factors such as cell permeability, lymph flow, etc. could be of importance as well. This ratio would be an effective measure only after equilibration of the Cs^{137} among various body tissues.

Studies with rats have shown that the Ba^{137m}:Cs^{137} ratio of blood taken from the aorta rises with time after I.V. Cs^{137} administration. The rise continues for as long as 24 days postadministration. This is apparently caused by a more rapid drop-off in Cs^{137} activity in the vascular compartment as compared to that in the extravascular compartments. When, on the other hand, the percentage of extravascular Ba^{137m} mobilized into the vascular compartment was determined by taking whole-body counts and blood samples, this was found to be constant from day 1 to day 24 post-Cs^{137} administration.

Various attempts to alter the circulation and general metabolic rate of rats have produced significant differences in the percentage of Ba^{137m} mobilized. The following table indicates these results.

Animal State	% Ba^{137m} mobilized	Standard deviation
Stunned	7.4	±1.2
Anesthetized	3.2	±0.5
Anesthetized + hypothermia	1.8	±0.2
Anesthetized + hyperthermia	4.7	±0.3

Each value is the average of five determinations.

Attempts to alter the circulatory state with a vasoconstrictor and a vasodilator have failed to show any significant effects on the percentage of Ba^{137m} mobilized. Two dogs that were studied showed highly variable day-to-day Ba^{137m}-Cs^{137} blood ratios when blood was drawn while the animals were in a conscious state. This variation may have been due to day-to-day variations in excitability since good agreement was obtained when the dogs were anesthetized.

Lanthanum-140 as a Measure of the Completeness of Stool Collections
(R. L. Hayes, J. E. Carlton, and Bill M. Nelson)

Measurement of a patient's ability to absorb an orally administered substance may be invalidated by incomplete collections of feces. Because of forgetfulness or embarrassment the patient may fail to report that a stool was lost. The result may be a high absorption value that often would not be recognized as erroneous. At the Oak Ridge Institute of Nuclear Studies the frequency of unreported, incomplete fecal collections has been as high as 30% in studies of gastrointestinal motility. The orally administered La^{140} used in these studies is not absorbed from the intestinal tract; thus incomplete fecal collection is immediately apparent from the sum of the radioactivities of the stool samples. Hence La^{140} might be used to verify the completeness of fecal collection for a variety of clinical tests, especially the absorption of iron-59. It was also hoped that, when a stool was missed, the proportion of La^{140} recovered could be used to estimate the unabsorbed Fe^{59} that had been lost, thus providing an acceptable result from data otherwise invalid.

Lanthanum-140, as an unabsorbable tracer given with an oral dose of other substances, generally will verify the completeness of stool collections for gastrointestinal absorption tests. However, when collections are incomplete, the proportion of La^{140} lost cannot be used to calculate the loss of unabsorbed Fe^{59} because the rates of passage through the intestinal tract are different. A distinct retardation of Fe^{59} excretion was observed. In one subject as much as 23% of Fe^{59} administered was excreted after the La^{140} had been completely recovered. Lanthanum-140 can be of use in studying this phenomenon, which probably can be attributed to exfoliation of epithelial cells containing iron taken up from the intestinal tract.

Collimation for External Counting of Cr^{51} in the Spleen: A Three-Dimensional Integral Analysis Using Isoresponse Data (Bill M. Nelson, Vichai Pochyachinda*, and Makumkrong Wasanasomsithi*)

The sequestration of erythrocytes by the spleen can be demonstrated by the increase of radioactivity in the spleen after intravenous administration of erythrocytes labeled with chromium-51. This accumulation of activity in the spleen can be measured by detectors outside the body and such measurements have been reported useful in predicting which patients with hemolytic anemia would be helped by splenectomy. The purpose of the present study was to investigate the features of importance in the design of a collimator for external counts of Cr^{51} in the spleen.

Two collimator systems were used for a 2-in. crystal: one a cylindrical "flat-field" collimator, extending 85 mm from the face of the crystal; and the other with the same crystal, housing, etc., but with the collimator removed. Isoresponse curves were obtained for each system by applying the front face of each detector to a tank of water in which a small source of Cr^{51} was systematically measured at all points in a 3-dimensional grid. From the isoresponse data we obtained a simplified, 3-dimensional integration of the counting efficiency for each locus of activity in the water. Thus it is possible to compute by simple arithmetic the contribution of activity in a specific volume relative to the contribution of activity elsewhere in the tank. Various clinical conditions including splenic or hepatic enlargement can be simulated and studied by arithmetic substitutions for different dimensions and activities in the tabulated 3-D analyses for each collimator.

These analyses were used for the evaluation of two practical considerations of collimator design: (1) "Specificity" and (2) "Reproducibility." Specificity is optimal when the sensitivity to radioactivity in the spleen is greatest relative to the sensitivity to activity elsewhere in the body. If the counting rates are statistically adequate, reproducibility is optimal when collimator design minimizes the errors due to clinically inevitable variations in positioning of the detector on the body surface. When these considerations are applied to the 3-D analyses of the two collimating systems, taking into account the distribution of Cr^{51} in the spleen, liver, and body wall, the "no-collimator" system is shown to be superior. The same method of 3-D analysis can be used for other purposes.

* IAEA Fellows, 1962, 1963 from Bankok, Thailand

Intermittent Corticosteroid Therapy in Malignant Lymphocytic Diseases (D. A. White)

During the past year we have given intermittent corticosteroid therapy to six patients with refractory lymphosarcoma or chronic lymphocytic leukemia. The rational for this therapy was a report by Khuri, et al., of the Washington University School of Medicine, presented at the IX Congress of the International Society of Hematology in Mexico City in 1962. (Proceedings have not been published.)

Three patients have chronic lymphocytic leukemia and three have lymphosarcoma. All had received 50 r or 100 r total-body irradiation on at least one occasion. All had been treated also with other more conventional forms of therapy. All the patients had one or more cytopenias in the peripheral blood. Two patients received 125 or 150 mg of prednisone daily for more than two weeks before the drug was stopped, and treatment was resumed within a few days in the dosage of 150 mg weekly or on two successive days each week. The other four patients received initially 25 to 100 mg of prednisone two days a week.

In three patients the benefit has been impressive. The treatment probably was life saving for one of these patients. Two other patients have had less marked improvement. The last patient was recently placed on prednisone two days a week and there has not been sufficient time to evaluate his response. Favorable results have been observed in both lymphosarcoma and chronic lymphocytic leukemia.

We have found intermittent steroid therapy a valuable form of treatment for selected cases of advanced malignant lymphocytic diseases.

A Unilateral Renal Function Test, Using Radio-Hippuran and Localized Abdominal Compression (Richard Steckel*)

Present unilateral renal function studies are imperfect in reliability and in convenience for the operator and the patient. Intravenous pyelography, aortography, and renal angiography, catheterization of the individual ureters (Howard test), and radioisotope renograms have been used in the diagnosis of unilateral renal disease. Intravenous pyelography leaves much to be desired in sensitivity. Aortography, renal angiography, and ureteral catheterization are complicated and specialized techniques. The radioisotope renogram is at present a nonprecise method of assessing renal function: matching two scintillation detectors and obtaining comparable

* Visiting Radiology Resident, ORINS, from Massachusetts General Hospital.

counting geometry for the two kidneys make quantitative comparisons of renal function difficult. Radio-Diodrast is excreted not only by the kidneys, but also by the liver, further complicating interpretation of the renogram. Radio-Hippuran is said to obviate this difficulty, but recent studies have shown that it, too, may be excreted in significant amounts by the liver, or at least concentrated there.[1,2]

A recent report, describing temporary unilateral ureteral obstruction by external compression, suggests the possibility of a simple approach to the study of comparative renal functions.[3] It has been shown, both by intravenous pyelograms and by selective creatinine clearance studies, that obstruction of a single ureter at the pelvic brim can be achieved by localized abdominal compression with a simple appliance. The appliance is roughly pyramidal with a 10-cm square base and a height of 10 cm, and it is constructed of unfinished wood. The rounded apex of the pyramid is placed on the abdomen between the anterior superior iliac spine and the umbilicus. It is then used to compress the abdomen, with an abdominal compression band and a blood pressure cuff. Obstruction of the homolateral ureter is obtained during the period of compression (approximately 140 to 160 mm of mercury), at the level of the ala of the sacrum. If the appliance is correctly used, the contralateral ureter continues to transport urine to the bladder.

The present test uses two intravenous injections of 25 microcuries of radio-Hippuran. An indwelling venous needle is used to collect serial blood samples after each injection, at 2-min intervals. A single scintillation counter is placed over the bladder. After injection of 25 microcuries, over a suitable observation period (20 min), the increment in bladder activity is determined. When the increment in bladder activity is divided by an average blood activity for radio-Hippuran during that period, a total "renal clearance" for radio-Hippuran is obtained. Then the second dose of 25 microcuries is given intravenously, and serial blood samples and bladder counts are obtained as before. However, unilateral ureteral compression is applied externally just before this second dose, and it is maintained while the determinations are being made (20 min). Similar calculations for this second collection period will yield a radio-Hippuran clearance value for the unobstructed kidney alone. When compared with the renal clearance for both kidneys (first injection), a quantitative comparison of individual renal functions is obtained.

Preliminary studies with three patients have shown that the individual renal function study described above may be of value. Matched scintillation counters, as used in the radioisotope renogram, are not required. A single counter is placed over the bladder, and matching counting geometry for two separate counters is avoided. The recent report of Bernstein and Hamby on the use of unilateral abdominal compression to achieve ureteral obstruction indicates that temporary, acute obstruction

of one ureter does not significantly alter the function of the opposite kidney.[3] No significant sequelae have followed obstruction of this short duration. The obvious complexity of the Howard test and of angiography and the specialized equipment required contrast sharply with the relative simplicity of this individual renal function.

References

1. Abbott Laboratories Brochure, 1960. I^{131} radio-Hippuran for intravenous use in studies of kidney function and in diagnosis of kidney disease.

2. C. T. Dollery and C. M. E. Matthews, Distribution of Hippuran labelled with I^{131} in the kidneys and liver. Brit. J. Exp. Path. 43: 329-332 (June 1962).

3. L. M. Bernstein and W. M. Hamby, Unilateral urine sampling utilizing external ureteral compression, New Engl. J. Med. 268: 1093-1099 (1963).

MEDICAL INSTRUMENTS DEVELOPMENT

The opportunity to make clinical trials on patients, combined with the unusual concentration of electronic skills in Oak Ridge, makes the Medical Division a logical focus for the development and testing of new instruments for clinical use.

The division has continually enjoyed the expert collaboration of members of the thermonuclear group at Oak Ridge National Laboratory - C. C. Harris, P. R. Bell, Jack Francis, and D. A. Ross. Dr. Ross (who was formerly on the Medical Division staff) has made a special contribution in the design of the low-level whole-body counter. Important recent projects have emphasized refinements in area scanners; C. C. Harris has played a major role in the design and construction of these devices.

Whole-Body Counting Instrumentation (A. C. Morris and D. A. Ross*)

At the ORINS Medical Division we have a clinical need for whole-body counting instrumentation covering a continuous patient-activity range from therapeutic doses down to the natural body background. Since the required instrument-sensitivity range is more than 100,000,000 to 1, ORINS will require three separate whole-body counting systems to achieve this coverage. The sensitivity range for each of these systems is given in Fig. 1, as is also the sensitivity response for the ORINS linear scanner.

The high-level counter has been constructed and is in clinical operation; the diagnostic-level instrument is nearing completion; and the low-level counter is now under construction. Once the three systems are in operation, the retention of many radioactive isotopes and compounds of research interest can be followed for long intervals of time. A description for each of these counter systems follows.

High-Level, Whole-Body Counter (Cyclops)

This instrument is located on the second floor of the hospital D-wing and is now being used in clinical studies. The detector is a 2- x 2-in. crystal

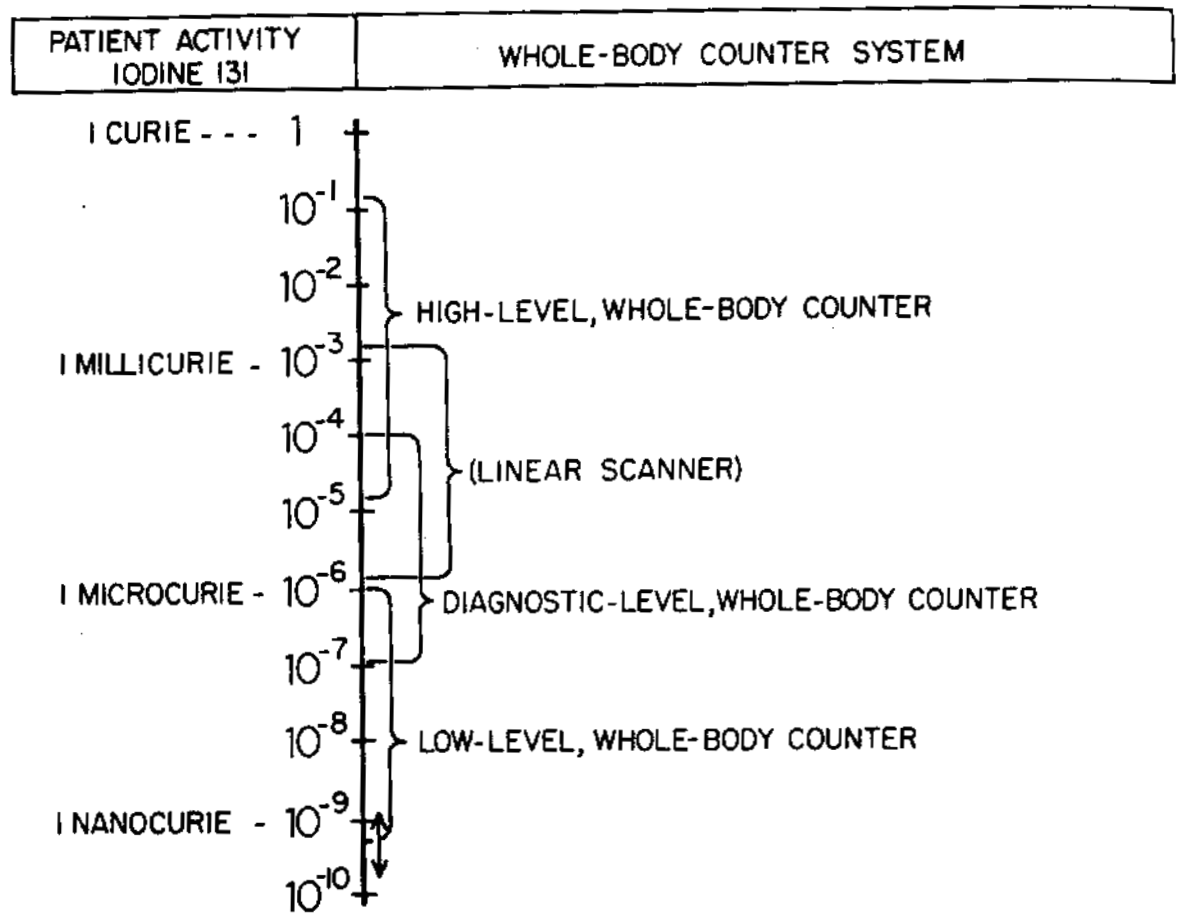

Fig. 1. Sensitivity ranges for the ORINS Whole-Body Counters.

mounted in a collimator near the ceiling and is aimed at the reclining subject on a low bed on the floor. The collimator restricts the view of the crystal roughly to the solid angle subtended by the bed. A curved lead attenuator is used when the counting rate becomes so high that the instrumentation starts suffering from electronic indigestion. The attenuator is designed so that it passes only 10% of the incident radiation from the 364-kev peak of I^{131}.

The routine practice is to administer a known dose to the patient and from the same stock bottle to measure an appropriate amount into an 8-liter water-filled bottle used as a standard. Throughout the period of study, which often extends over a week or more in the range of this counter, the patient, standard and background counts are made consecutively, and values for percentage of retention are computed. For I^{131}, with an activity range from 50 microcuries to 150 millicuries, a 1-min counting time is routine. Ten-minute counts will give reasonable statistics down to 10 microcuries. The lead attenuator is used whenever a subject contains more than about 10 millicuries.

This high-level whole-body counter is advantageous in that it is relatively inexpensive to construct, the counting times are short, and the patient is in a comfortable position. Our experience with a series of patients has demonstrated that this instrument provides an accurate means of following whole-body retention of gamma-emitting isotopes and compounds in the high-dose range.

Diagnostic-Level, Whole-Body Counter

To bridge the sensitivity gap between the high-level and low-level counters, a medium-range instrument has been designed and will soon be in operation. The basic arrangement of this counter is shown in Fig. 2. Radiation detection is accomplished by means of four 3- x 3-in. scintillation crystals mounted in a collimating lead trough 1-1/4 in. thick and weighing 3000 lb. This trough-like collimator allows all the crystals to view the entire length of the patient, and all detected counts will be summed into one input for the spectrometer system. The subject will recline on a special X-ray stretcher, which is radiolucent. This stretcher will when be wheeled over the collimator-detector assembly, and the count will be made.

Calculations predict that this system will be more than 200 times as sensitive as the high-level counter and will operate most usefully in the 0.1 to 100 microcurie range. Since a large amount of tracer work at the Medical Division is done in this activity range, this counter will fill an important need in the research program.

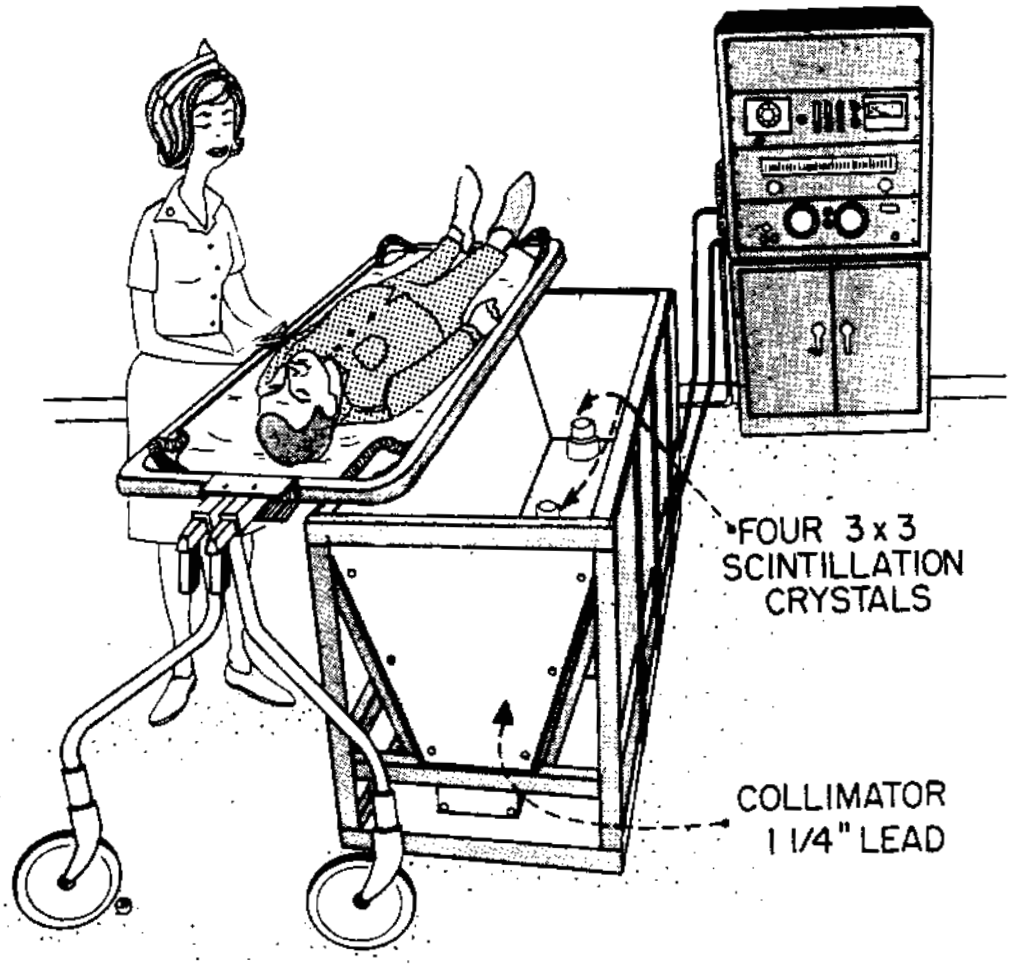

Fig. 2. The ORINS diagnostic-level whole-body counter.

Low-Level, Whole-Body Counting Facility

Over the past several years the Medical Division has been engaged in the design of a whole-body counter having a minimum-background characteristic, and this facility is now under construction at the north end of the hospital D-wing. When completed, this counter will be used in retention studies at low tracer-dose levels and also for other investigations at or near the natural body background.

To achieve the required minimal background within the 8-foot-cube counting volume, three major background-reducing measures are being used. First, the counter is being constructed underground so that radioactive discharge material in the air from some of the Oak Ridge area installations will have a minimum effect. Although the actual amount of radioactivity released is relatively small, other highly sensitive whole-body counters operating in this area have been plagued with varying background counts caused by this airborne contamination. Second, ORINS is testing all construction materials lying within 6 ft of the counter for radioactive content. Materials giving a high background count are eliminated. And third, this counter will use a "graded shield" having five distinct attenuating layers, each successive layer reducing the background to an additional extent. The layers of this shield proceeding from the outside earth to inside the steel box, or "Cave," are 12 in. of low-potassium concrete, 24 in. of crushed olivine ore, 5 in. of low-activity steel plate, 1/4 in. of low-activity lead sheet, and 1/16 in. of low-activity stainless steel. This configuration is shown on p. 62 of the Medical Division Research Report for 1962 (USAEC Report ORINS-42). The attenuation of this shield is very high; for example, when counting the 1460-kev peak of potassium-40, the reduction for just the steel and olivine components of this shield is 14 half-value layers, or an attenuation factor of more than 20,000 to 1.

The search for low-activity materials was first directed at finding a concrete that would be suitable for use in the building near the counter. An acceptable mix has been found and its spectrum is compared with the spectrum of a standard concrete purchased locally (Fig. 3). As can be seen in the figure, for the K^{40} range the "low-activity" concrete response is only 10% of its "standard" counterpart, and this special concrete is being used in the current construction.

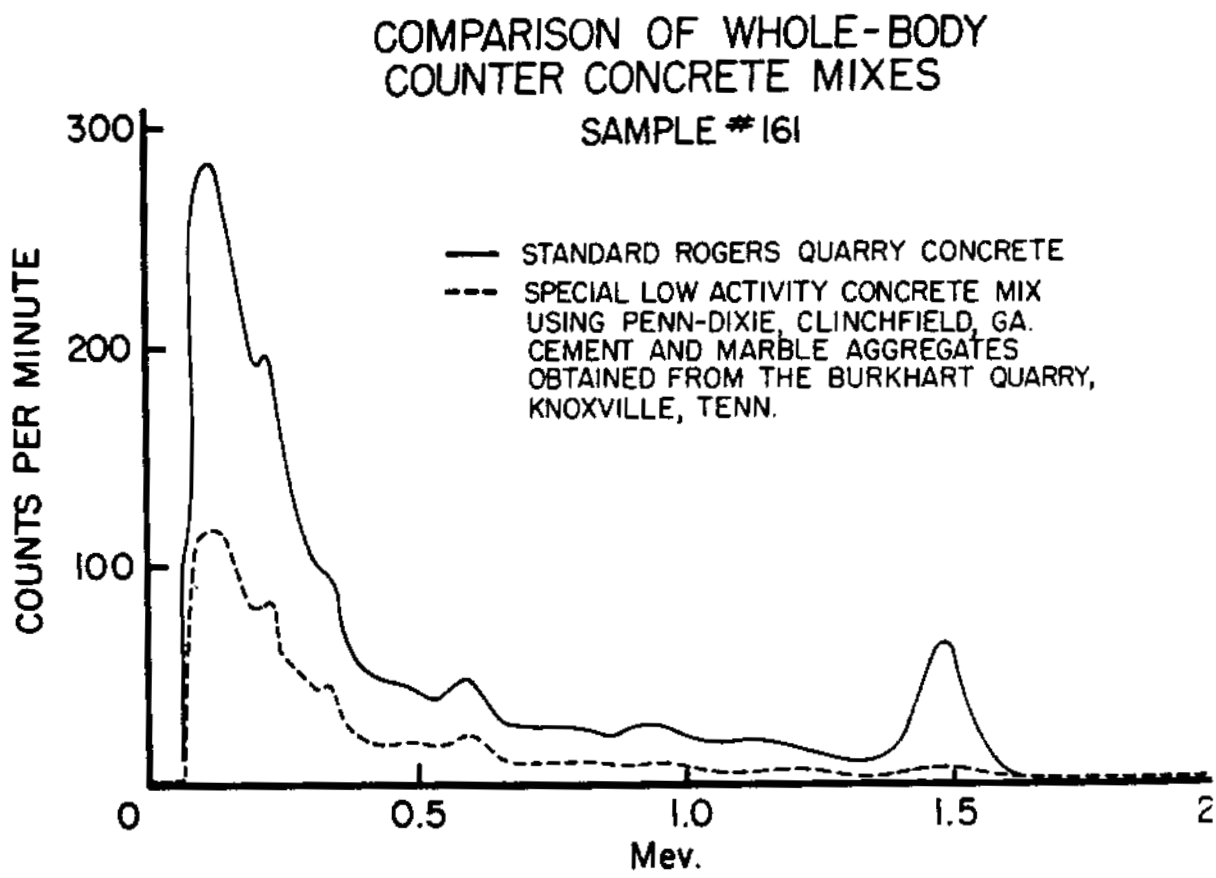

Fig. 3. Comparison of spectra of the locally available concrete mix and the special low-activity mix used in building the ORINS low-level whole-body counting facility.

Other counting experiments were performed in an effort to locate suitable steel, lead, and stainless steel. The rather extensive tests made on steel indicate that probably no steel of current manufacture is suitable for use in low-level counting enclosures. The new-steel samples counted by ORINS have come from all parts of the country and give background counts, in the fallout-energy range, 200 to 500% more than similar old-steel samples.

The detecting system inside this counter will consist of eight, ultra-low-background 5- x 4-in. scintillation-crystal detectors arranged above and below a patient who is supported by a canvas sling. The height and spacing of the detectors will be adjusted to give a uniform counting response along the patient's length.

Whole-Body Scanner (A. C. Morris, Jr.)

A whole-body scanner has been designed to scan an area 25 x 72 in. The focusing-type collimator used weighs about 250 lb, has a 4-in. focal distance and is mounted under the patient. A 5- x 4-in. scintillation crystal serves as the detector and feeds pulses to the single-channel spectrometer and binary scaler system. Patient support is provided by a high-strength 1/8-in. fiberglas-filled sheet of epoxy resin, which is attached to the upper portion of the scanner framework. Scanning speeds are adjustable up to a maximum of 40-in./min and the line spacing may be varied from 1/16 in. to 1-3/16 in. in 1/16-in. increments. Line spacing, scan width, and scan length parameters may be controlled from a remote panel. Synchronous motors are used to drive the collimator and by this means artifacts from speed variations are eliminated.

Two remote recording systems are at present being incorporated into the scanner. Both these recorders are driven by means of selsyn motors, which are geared down by a ratio of 5 to 1, so that a 60-in. patient produces a 12-in. record, a convenient size. One recorder will use a mechanical "dot-tapper" marking on a carbon-backed white paper. The other recorder will use a crater-lamp light-flasher, exposing a sheet of 14- x 17-in. photographic film. Pulses of light from the crater-lamp may be set in length from 20 to 120 microseconds in six steps, and the pulse frequency varies in accord with the detected count rate. The photoscan data-recording speed is not limited by any mechanical mechanism and therefore can be made to operate at higher rates than the mechanical "dot-tapper." Both the mechanical and photographic recorders for this scanner will have individual adjustments for "dot factor" so that each may be used in its optimum range.

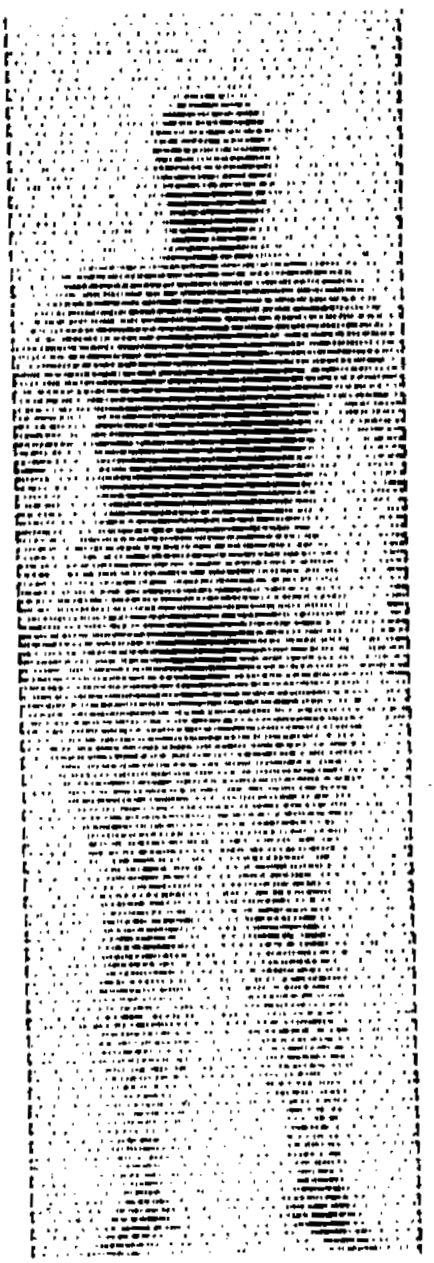

Fig. 1. Scan of a surgically athyroid patient made 5 hr after a 5-millicurie dose of I^{131} as NaI.

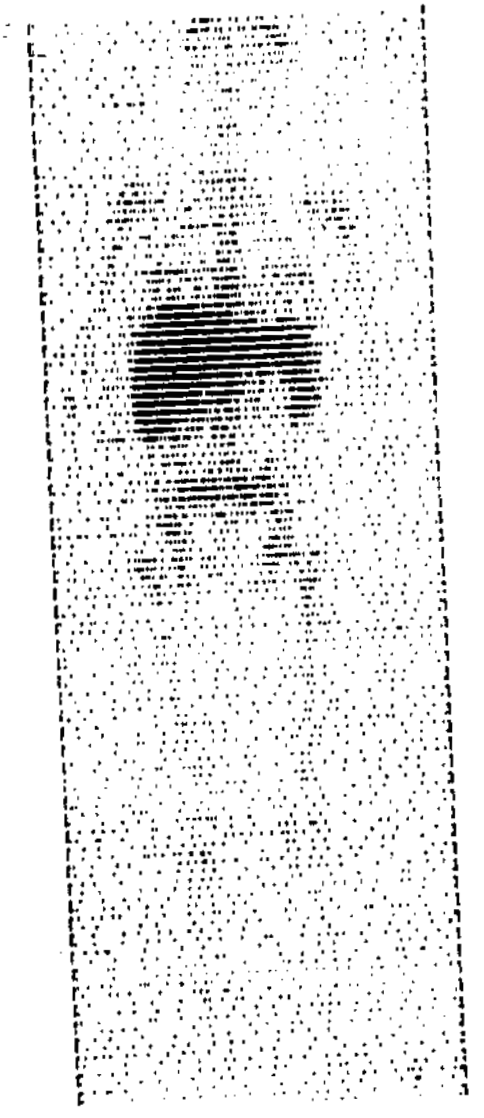

Fig. 2. Scan of a patient with acute granulocytic leukemia who had been given 2.5 millicuries of Au^{198}.

Some preliminary test scans made with this whole-body scanner are shown after doses larger than normally to be used. Figure 1 is a scan of a surgically athyroid patient made 5 hr after a 5-millicurie dose of I^{131} as NaI. In Fig. 2 the patient, who had acute granulocytic leukemia, had been given 2.5 millicuries of Au^{198}.

Arm Phantom (Orhan Ternar* and A. C. Morris, Jr.)

During the past year the Medical Division has been using a commercially available "ARMAC" arm counter for some *in vivo* retention, absorption, and other dynamic isotope studies. One recurring problem, which arose in connection with these studies, was the lack of an adequate phantom with which to count backgrounds and to establish the various contributions and recording-response rates for the instrument system.

A phantom has been designed and constructed, and consists of a plexiglass shell of arm size containing many compartments commensurate with the volume of bone, muscle, and blood vessels in the upper arm, lower arm, and hand (Fig. 1). Each compartment may be filled and sealed separately so that contribution studies may be performed independently for each section. The phantom has a length of tubing inside, representing the blood vessels, which opens to the outside through small ports in the end of the phantom. By passing radioisotope solutions through this tubing at various rates, the instrument response may be investigated.

This arm phantom is now in use at the Medical Division and has helped improve the accuracy of studies made with the ARMAC counter (Fig. 2).

A New Large-Volume Radioactive Sample Counter (Derodymus)
(William D. Gibbs)

An instrument was designed to measure accurately the radioactivity in bulky samples of variable shape and size without necessity for corrections for spatial distribution of radioisotope in the sample. Such an assay system was needed particularly for quantitating I^{131} in total 24-hr fecal specimens.

* IAEA Fellow from Istanbul, Turkey.

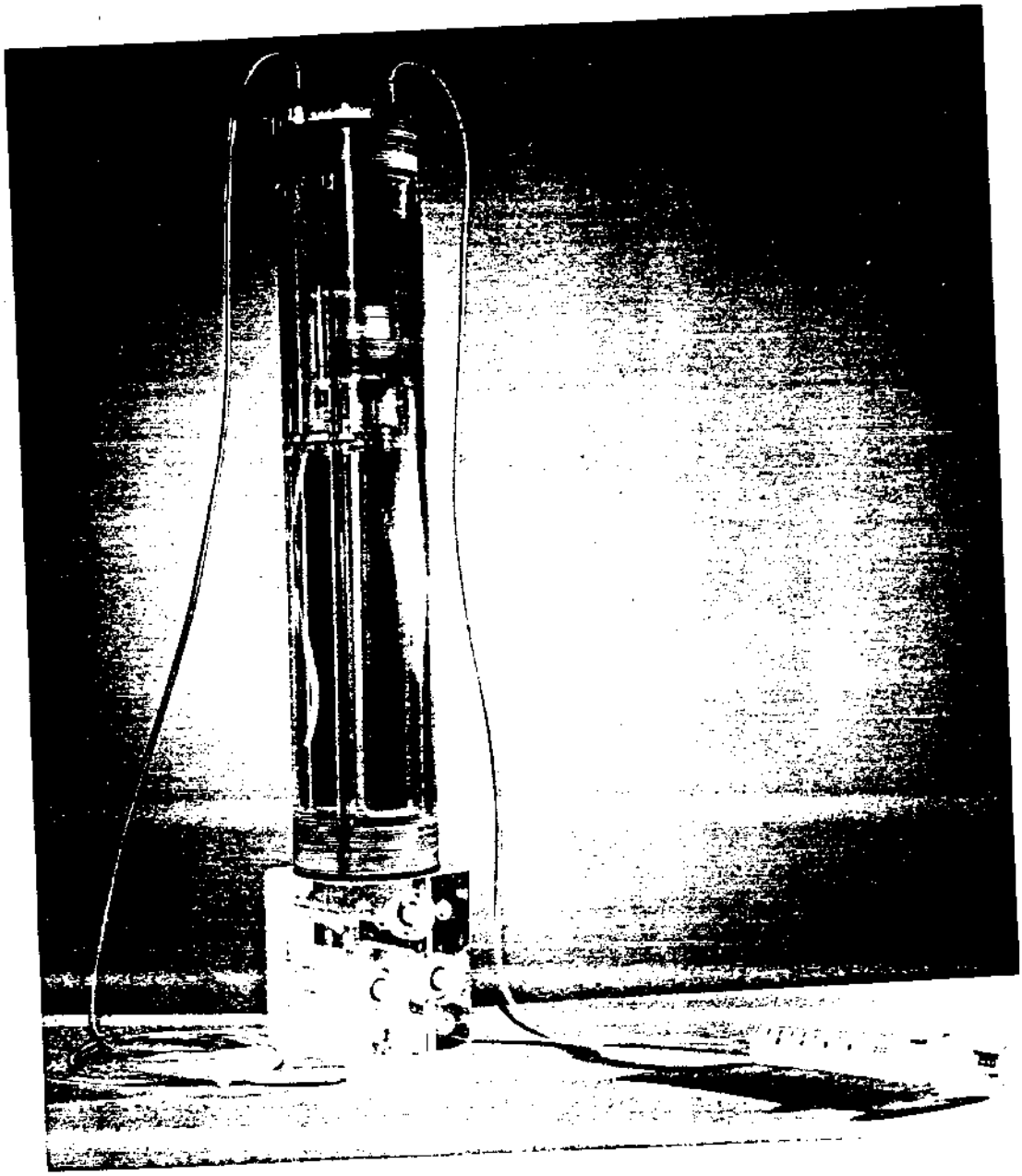

Fig. 1. ORINS arm counter.

Fig. 2. ORINS arm phantom being placed in the arm counter.

The best design for this purpose was found to consist essentially of two, vertically opposed, 2- x 2-in. NaI crystal detectors, 24.75 in. apart; viewing a chamber, shielded with 4 in. of lead, whose floor, made of 1/8-in. plexiglass, was 12.25 in. above the face of the lower crystal. Although each detector can be calibrated separately, single spectrometer and scaling units are used to obtain an integrated count from the two crystals.

The operation of the instrument, named for Derodymus, a two-headed monster of the Greeks, is based upon the premise that a threshold for counting exists above which the counting rate is almost independent of sample size or isotope distribution. This involves the concept of counting intentionally a certain amount of scatter radiation. For I^{131} this threshold, determined empirically at first, was found to be 200 kev. When a 200 kev window width is used with it, results (Table 1) are obtained showing that even when sample volume is varied from 10 to 500 ml, or the position of activity in this volume is also varied widely, the total counts obtained vary less than 2% from those of the standard 150 ml solution. This degree of accuracy is obtainable with 0.02 to 300 microcuries of I^{131}.

Table 1

Percentage of I^{131} Gamma Activity Counted Relative to Standard Activity in 150 ml Volume (arbitrarily chosen to be 100%)

I^{131} Distribution	10	50	100	150	200	300	400	500
Solution	99.5	100.1	100.1	100.0	99.5	98.9	98.2	98.0
Point source floating on water	100.6	101.9	102.1	102.3	100.1	101.5	100.0	99.7
Point source at bottom	100.4	101.4	102.2	101.5	101.1	100.8	99.5	99.1

Counting conditions determined for other radioisotopes for this device are shown in Table 2.

Table 2

Isotope	Threshold (kev)	Window (kev)
Fe^{59}	440	top off
Cr^{51}	190	200
Hg^{203}	180	200

Because of the relative unimportance of size and spatial configuration of the sample in the counting chamber, whole-body counting of live as well as dead small animals or organ aliquots can be done with similar accuracy.

SCANNING AND WHOLE-BODY COUNTING

The Medical Division program has explored external scintillometry in clinical studies ever since the availability of the first scintillation crystals. Rectilinear (area) and profile scanning have been applied to long-range studies of patients with functioning thyroid cancers. Organ scans have been made of the brain, thyroid, liver, spleen, kidneys, serosal cavities, and sites of local radioisotope injections using a variety of radioisotopes. Currently we have concentrated mainly on scanning of the bone-marrow organ, but are continuing to test new diagnostic compounds and instrumental refinements. Whole-body counting more recently has shown promise as a clinical tool and instrument development is described in an earlier section. The study on I^{131} in the box turtle (made during the Summer Research Participation Program) has been a useful model for showing the application of whole-body retention measurements in compartmental analysis.

Clinical Scanning of Bone Marrow (R. M. Kniseley, G. A. Andrews, Ryosaku Tanida and C. Lowell Edwards)

The 1962 ORINS Medical Division Report (USAEC Report ORINS-42) described the initial results obtained on scanning the bone marrow with radioisotopes. Using the ORNL research scanner we have shown focal marrow lesions caused by radiation or neoplasm, and delineated quantitative and distributional changes in the marrow organ of patients with a variety of hematopoietic disorders. Colloidal Au^{198} has been the best compound for use up till now.

Bone-Marrow Scans (till November 15, 1963)

Diagnosis	No. Patients
Acute leukemia	13
Chronic granulocytic leukemia	1
Chronic lymphocytic leukemia	2
Lymphosarcoma	4
Hodgkin's disease	3
Carcinoma of the breast	3

Bone-Marrow Scans (till November 15, 1963) (cont'd.)

Diagnosis	No. Patients
Myelofibrosis and polycythemia vera	7
Miscellaneous neoplasms	5
Acquired hemolytic anemia	1
Monocytosis associated with ileitis	1

The area scans of the pelvic bones appear to be of the greatest clinical usefulness; here there is relative freedom from radioactivity in other structures. The distribution of Au^{198} in the chest is more difficult to interpret because the variable amount of activity in the lungs is difficult to distinguish from that in the marrow. In some patients there is very little in the lungs, but in others the lung fields are more prominent than the thoracic bones and a light area corresponding to the heart can be seen clearly. Evidence for radioactivity in the lungs was found in a patient who had had a right pneumonectomy; activity was distinctly greater over the left lung field.

Marrow scanning may prove useful clinically in determining the sites at which other bone-marrow studies can best be done; for example, in radioactive iron uptake and turnover studies, it has been customary to place the detector over the sacrum. Scans in this series suggest that the greatest amount of cellular marrow is over the sacro-iliac region lateral to the midline. Marrow scans have also helped in selecting sites for aspiration study.

Scans are of great value in showing the size of the marrow organ, but results are often not what would be expected in relation to cellularity at aspiration sites, or the general hematologic picture.

Experience up to the present indicates that in granulocytic leukemia, subacute or chronic, there is normal or increased uptake. In chronic lymphocytic leukemia we have seen a normal uptake in one patient and a greatly decreased uptake in a patient with an advanced state of the disease with a so-called "packed marrow." In acute leukemia variable results have been seen - increased, normal, and decreased colloid deposition. There is a lack of obvious correlation with the state of the disease or treatment.

Scanning of the Subarachnoid Spaces after Intrathecal Injection of I^{131} Albumin
(K. F. Hübner and D. W. Brown*)

The visualization of the subarachnoid space of the spine with radioactive tracer techniques was first described by Bauer and Yuhl, and there have been several subsequent reports. This method has proved to be useful for the localization of lesions obstructing the subarachnoid space. Failure of this procedure to gain wide acceptance can be attributed to the relatively poor quality of scanning machines available heretofore. With the availability of the ORNL research scanner, reassessment of the procedure has been carried out on eight patients.

Procedure: The patient's thyroid gland is blocked with stabile iodide. After the routine lumbar puncture, 100 microcuries of I^{131} serum albumin are injected intrathecally. The total volume of undiluted test material is about 1 ml. The needle is removed and the patient is allowed to return to his room with no restrictions on activity other than those usually imposed after lumbar puncture.

Two hours later scanning is begun with the ORNL scanner, which has a tungsten-shielded, 37-hole, focusing, gold collimator and a 3-in. crystal. The scanning is begun at the tip of the coccyx and carried upward to the head. The present procedure takes about 45 min. Three of eight patients developed headaches of a 24-hr duration, a frequent complaint after lumbar puncture; otherwise no untoward reactions or symptoms were noted.

Complete compression of the spinal cord was demonstrated in three patients, one with lymphosarcoma, one with multiple myeloma, and one with a metastatic lesion from a liposarcoma. In one patient the precise location shown by the scan was confirmed by a pantopaque myelogram.

The clarity of scans that can be obtained with this instrument makes myeloscintigraphy a much more valuable procedure. The dose of 100 microcuries is innocuous. Radioiodinated serum albumin leaves the subarachnoid space very rapidly, as proved by follow-up scans at 24 hr. Once the I^{131} albumin enters the blood stream, it behaves as if it had been injected intravenously. Intravenous doses larger than 100 microcuries are used routinely in many clinical procedures without adverse effects. The advantages of this procedure over routine myelography with pantopaque or other iodized oils are (1) the apparent lack of danger of myelitis, pantopaque pulmonary embolism,

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Fig. 1. Normal scintigram of the subarachnoid space of the spinal canal 3 hr after injection of I^{131}-labeled albumin. Dispersion throughout the spinal canal is accompanied by some spreading into the nerve-root sheaths.

Fig. 2a. Pantopaque myelogram showing compression of the spinal cord at T 10 (metastatic tumor. liposarcoma).

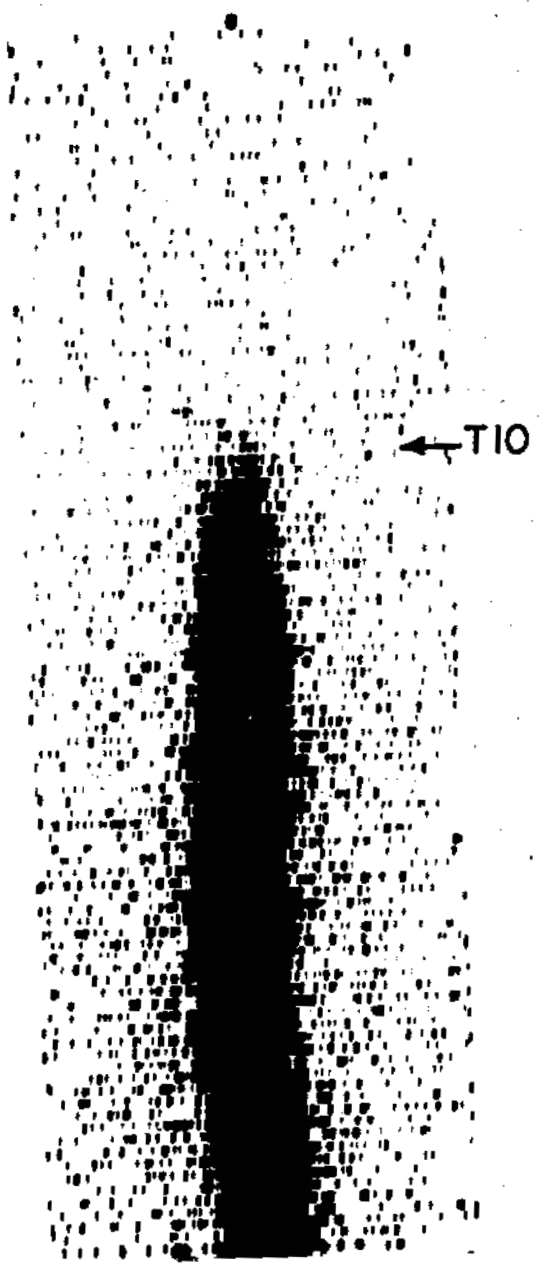

Fig. 2b. Scintigram showing block at T 10.

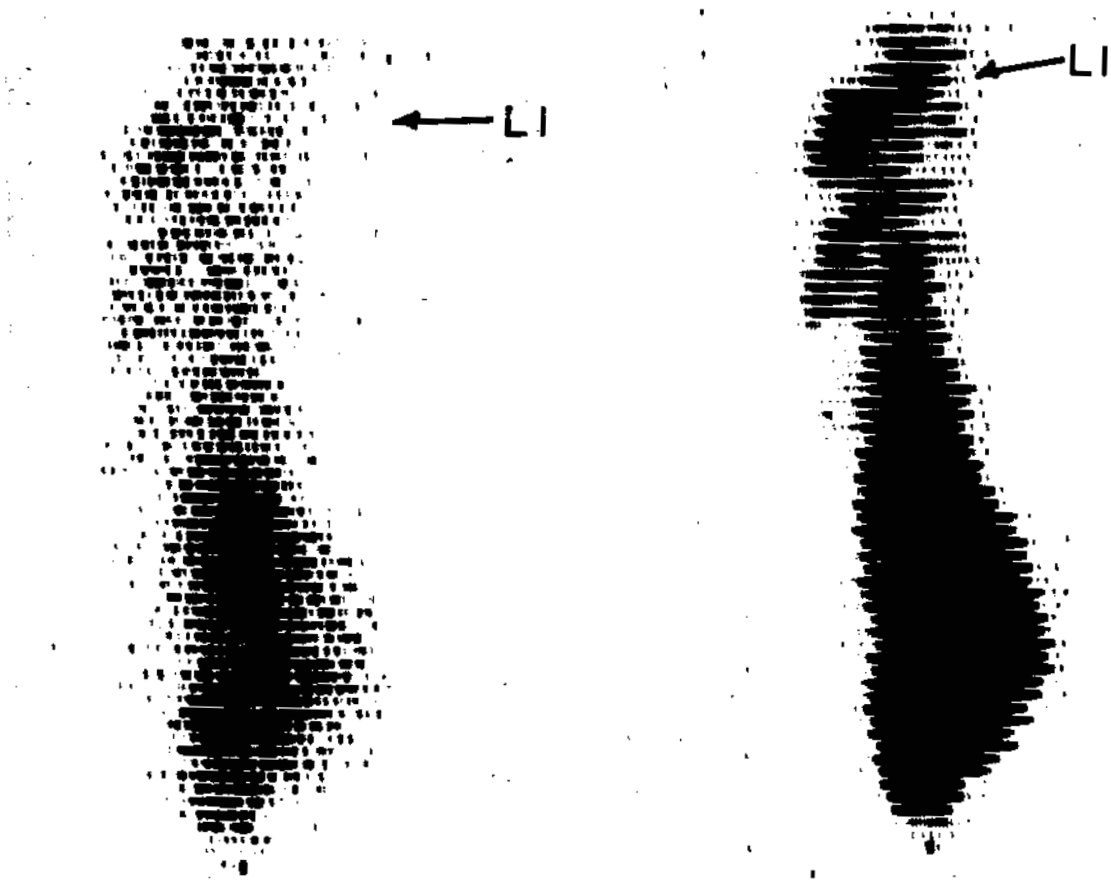

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Fig. 3a. Scintigram of the lower spinal cord 1 hr after injection if I^{131}-labeled albumin. Distention of the caudal sac is regarded as a normal variation. Note that impaired spread along the right nerve-root sheaths correlates with clinical symptoms of leukemic epidural infiltrations.

Fig. 3b. Rescan of Fig. 3a, an electronically obtained image made by using the ORNL rescan device to enhance the contrast of the primary scan.

and other complications of pantopaque myelography make it safe; (2) the radioiodinated serum albumin does not have to be removed; (3) tilting of the patient is not necessary, for the radioiodinated serum albumin particles disperse very rapidly throughout the spinal canal. See Figs. 1 - 3.

* * *

The cooperation and assistance of C. C. Harris and his colleagues at the Oak Ridge National Laboratory is gratefully acknowledged.

* * *

Whole-Body Retention of NaI^{131} in Man Measured with the ORINS High-Level Whole-Body Counter (W. D. Gibbs, Joe Gray, and G. A. Andrews).

The whole-body retention curve of I^{131} activity after administration of NaI^{131} can be used as an index of the retention of iodine in the body; if large amounts are retained it can usually be assumed that most of it is in thyroid tissue or functioning thyroid cancer. Other factors in retention are the amount localized in other iodine-concentrating tissues (stomach, salivary glands) and the efficiency of circulation and renal excretion. Geometric considerations sometimes make it difficult to measure the uptake in metastatic thyroid tumors. Here the whole-body retention is of value, and serial studies help to indicate the changes in function of the tumor. The ORINS high-level whole-body counter was developed to investigate this problem using therapeutic doses (about 100 millicuries) of NaI^{131} as well as large diagnostic (scanning) doses (0.5 millicuries). In the past two and a half years, on 57 patients, 155 studies have been made: 11 hyperthyroid; 5 euthyroid; and 139 athyroid. Of the athyroid group 84 were in patients known to have residual functioning thyroid carcinoma by area scanning of metastatic sites.

The results to date are summarized in the following tables:

Table 1

Number Patients	Number Studies		Range of 3-day retention (percent dose)
5	5	Euthyroid	28 - 36
11	11	Hyperthyroid	31 - 92
41	139	Athyroid	0.3 - 23
	55	No functioning tumor	0.3 - 12.5
	84	Functioning tumor	1.0 - 23

Table 2

	Less than 1% retention	More than 10% retention
Athyroid		
No tumor	9 of 55 studies (16.4%)	2 of 55 studies (3.6%)
Tumor	2 of 84 studies (2.4%)	6 of 84 studies (7.1%)

These results appear to show that in most cases hyperthyroidism can be differentiated from the euthyroid state by this method. In athyroid patients with thyroid cancer, if the tumor takes up 10% or more of the dose, the retention is also easily detected. However, very small amounts of tumor, or very poorly functioning tumors, are not always distinguished by this method, because their function may be less than that attributable to variations in the uptake in salivary glands and stomach, and variations in rate of clearance of extracellular fluid.

Comparative Study of Thyroid and Whole-Body Retention of Iodine in Box Turtles (W. D. Gibbs, E. D. Wilson*, H. Hodges, and C. C. Lushbaugh)

Recently a whole-body counting method[1] was described for measuring thyroid function radioisotopically in species of animals where the size or location of the gland makes such measurement difficult or impossible with a collimated NaI crystal. To test this new method and to evaluate the new large-volume sample counter[2] as a whole-body counter for small animals, a comparison of thyroid and whole-body retention of I^{131} in the box turtle (Terrapene carolina carolina) was made.

The turtles were injected intraperitoneally with 5 microcuries of carrier-free NaI^{131}. Whole-body counts were done immediately after injection, at 3 days, and then for 5 weekly intervals. After 35 days the turtles were killed, thyroids were removed and assayed, and the residual (whole-body-less thyroid) radioactivity was determined. Comparison was then made of the percentage of retention of the initial dose in the whole-body and thyroid gland. Immature turtles weighing less than 100 grams comprised one group. Two groups of mature turtles were studied: one shortly after collection in midsummer and the other in autumn just before hibernation.

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The results in Table 1 reveal an amazingly high thyroid uptake for such a slow-moving animal, and a range of uptake in the sexually mature turtles well within the hyperthyroid range for man and other animals. Although the definite hyperthyroid response of the autumnal group might be due to the prolonged period of captivity, the high iodine content of the lettuce, tomato, and meat diet that was avidly consumed during this time would seem to exclude this interpretation. The high intrathyroid versus extrathyroid iodine ratio found in this group in comparison with the others would imply instead that thyroidal entrapment or binding of iodine was increased before hibernation.

A histologic sampling of these thyroid glands showed that these high retentions were accompanied by change from low cuboidal to high columnar epithelium and vacuolization of the colloid, thus confirming this unexpected observation of heightened thyroid activity.

These studies showed, also, that the new large-volume specimen counter was an efficient, accurate means for radioassay of I^{131} even in a geometrically bizarre small animal. They revealed, in addition, that the accuracy of the whole-body counting method of determining thyroid function depends upon the intrathyroid versus extrathyroid bound iodide ratio. Where this ratio is large, as in man, whole-body retention and thyroid retention will be synonymous; where it is small, owing to loss of bound iodide by the thyroid into other pools, the whole-body assay will summate these activities and provide a means for determining the relative size of the thyroid and extrathyroid bound iodide compartments.

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Table 1

Comparison of 35-day Thyroidal and Whole-Body Retention of I^{131} in Box Turtles

Groups	Whole-body retention		Thyroid retention		Thyroid Content		Intra-/Extra-thyroidal ratio
	Mean (% Total Dose)	Range	Mean (% Total Dose)	Range	Mean (% whole-body retention)	Range	
Midsummer immature (10)*	33.3	18-49	27.8	11-41	82.4	59-96	4.7
mature (30)	51.2	10-85	38.7	7-66	75.5	45-99	3.1
Autumn mature (30)	62.0	32-84	55.9	20-82	89.4	53-100	8.4

* Number of animals

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