

RESEARCH REPORT – MEDICAL DIVISION

Oak Ridge Associated Universities



FOR THE YEAR ENDING DECEMBER 31, 1966

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INTRODUCTION

THE DIVISION of Biology and Medicine of the United States Atomic Energy Commission supports laboratories for extensive biological research at several sites, a major one being at Oak Ridge. The ORAU Medical Division is one of these laboratories and it has, as a special feature, an active clinical investigational program. Most of the financial support comes from the Atomic Energy Commission; additional funds come from the National Aeronautics and Space Administration (human radiation effects), the National Institutes of Health (immunology program in marmosets), and the Armed Forces Epidemiologic Board (infections in patients with bone-marrow depression).

The program's major objectives fall within two broad categories: to obtain information on radiation effects, especially in the human being, and to advance the clinical uses of radioisotopes. The outline on page viii gives some of the relationships of the program.

During the year, three staff members who have made fine contributions to the Division program, left for other opportunities. Dr. James Bollinger, who had worked with the lipid group, moved to the Southwest Research Institute in San Antonio, Tex. Dr. David A. White, who carried most of the responsibility for outpatient care, is now in practice at the Centerville Clinic, Fredericktown, Pa. Dr. Arthur Kretchmar moved to the University of Tennessee Memorial Research Center in Knoxville. He will continue to collaborate with the staff of the Medical Division. While here he was largely responsible for the introduction of work in theoretical and mathematical biology. Dr. Kretchmar, along with Howard Harmon, took the initial steps that led to the development of computer capabilities at Oak Ridge Associated Universities.

Joining the staff during the year were Dr. Lisbeth Kraft, Dr. Edward Balish, and Dr. Randall Wood. Dr. Kraft is a Doctor of Veterinary Medicine and she enjoys a fine reputation as an expert in animal care and in independent investigation, especially of infectious diseases in animals. Dr. Balish is a microbiologist who comes from a postdoctoral position at Argonne National Laboratory. He is developing the program on infections related to bone-marrow damage, especially that due to radiation injury. Dr. Wood had been on a postdoctoral program, working in lipid biochemistry at the Medical Division. He has now become part of the regular staff and has continued his research program with outstanding productivity.

The Medical Division's work is enhanced by productive collaborations with several scientists and institutions in Oak Ridge, Knoxville,

and at greater distances. During the year Dr. Frank Comas, radiotherapist at the Medical Division, was appointed as a staff member at the University of Tennessee Memorial Research Center, where he works one day per week, mainly in the teaching of residents. Other ties with this institution have developed or are planned. Dr. Fred Snyder and the lipid group at the Medical Division have increased their collaborative work with colleagues at the University of North Carolina.

Training activities in the Division are not emphasized in this report. We have continued to cooperate with the Special Training Division of ORAU and to provide teaching and clinical and laboratory experience for the medical courses given under the direction of Dr. Frank Goswitz. Longer periods of training in the Medical Division are provided in a variety of ways and the participants in these programs are listed on page 177.

The major symposium of the year was entitled *Compartments, Pools, and Spaces in Medical Physiology*. This was planned by Dr. C. C. Lushbaugh and Dr. Per-Erik E. Bergner, of the Medical Division staff, and Dr. W. Newlon Tauxe of the Mayo Clinic. Dr. Lushbaugh and Dr. Bergner are editing the proceedings of the meeting. The speakers included 14 from the United States and seven from other countries. Participants numbered 151, in addition to speakers, Medical Division staff members, and scientists from the Oak Ridge area.

An informal regional lipid meeting was sponsored by ORAU on March 29-30, 1966. The main purpose was to provide closer contact of faculty and graduate students engaged in lipid research within a few hundred miles of Oak Ridge, and attendance was limited to lipid groups from Vanderbilt University, University of Tennessee, University of North Carolina, University of South Carolina, and ORAU Medical Division. In addition to presentations by the participants, two invited lectures were arranged.

A low dose-rate human irradiator was built during the year with funds provided by AEC and NASA. This building consists of an outer shell 30 ft by 30 ft, an inner room 16 ft by 16 ft, plus supporting facilities. The entrance to the radiation area is through the hospital wing at the Medical Division. In the inner room there will be a homogeneous radiation field of radiation provided by 10 specially designed radiation sources. Patients can be irradiated for periods of from several hours to many days and can move about in a comfortable room during treatment. Special equipment for monitoring the patients is planned but not yet completed.

For some time the Medical Division has been seeking additional room, especially for laboratories and for experimental animals. Appropriate and needed growth in certain research activities has been prevented

A Study of Infections in Irradiated Patients

E. Balish

The most serious complication of sublethal total-body irradiation is infection. Many data exist on the increased susceptibility of, and infections that occur in, laboratory animals receiving sublethal and lethal doses of total-body irradiation. However, information is still meager on the etiology of infections in human beings after total-body irradiation at lethal and sublethal levels. Of particular interest are the infections that occur subsequent to accidental sublethal doses of total-body irradiation and that may account for possibly preventable deaths. This research project will be concerned with identifying the species of organisms involved in infections, as well as their portal of entry and response to therapy. The relative role played by commensal versus the exogenous microorganisms will be studied. Another important objective will be to ascertain whether changes in the microbial flora, which could result in the overgrowth of more virulent mutant species, might occur after treatment with total-body irradiation or local irradiation to areas of the digestive tract or the respiratory tract.

Several interesting results have shown up in the initial study of a patient treated with 100 R total-body irradiation (eight ^{137}Cs sources at 1 R/min) for chronic granulocytic leukemia. No antibiotics were given during this study and no infections were manifest. Blood samples before and after exposure were negative. A β -hemolytic *Staphylococcus albus* and *Klebsiella-Aerobacter* microorganisms were cultured from the urine two weeks after exposure. The numbers of the latter microorganisms were relatively small. However, urine samples before or immediately after (1 through 12 days) 100 R total-body irradiation were negative.

Throat cultures remained relatively stable after the exposure to 100 R. *Neisseria* and α - and β -hemolytic streptococci predominated in the throat before and for one month after exposure. Three days after exposure, β -hemolytic *Staphylococcus albus* appeared in nasal cultures. Before irradiation, a nonhemolytic *S. albus* and *Klebsiella-Aerobacter* (non-hemolytic) predominated. The β -hemolytic *S. albus* became the predominant microorganism isolated from nasal swabs within two weeks after the total-body irradiation (Table 4).

Before irradiation, the predominant microorganism isolated from gastric secretions was *Candida albicans*. The flora did not show any change immediately after exposure (Table 5). However, *Neisseria* and α -streptococci appeared as the predominant microbial flora after one day, and large populations of the latter organisms were observed up to three weeks after exposure. Moreover, during the latter period the

Hematologic Effects of Total-body Irradiation in the Human Being*

G. A. Andrews, C. C. Lushbaugh, R. M. Kniseley, and D. A. White

During the course of studies of total-body irradiation at the Medical Division, careful records have been kept of hematologic responses. Most of this information was obtained in patients with blood diseases; therefore the effects seen are not those that would be expected in the normal person. On the other hand, the responses may have considerable value in understanding the abnormal hematopoietic physiology of patients with blood diseases. To the extent that certain trends occur in all groups of patients, regardless of diagnosis, the findings are of value in pointing to general hematologic responses to total-body irradiation. These patients can be divided into three groups: (1) Those with chronic hematologic disorders, polycythemia vera, chronic leukemia, exposed to doses of 50 and 100 R; (2) those with various types of advanced neoplasm exposed to doses of 300 to 473 R; and (3) those with acute leukemia in relapse given exposures of from 206 to 384 R.

Most of the patients were irradiated in the cesium-137 total-body irradiator, which produces a uniform air dose. Dose rates were usually between 0.64 and 1.52 R/min. All the patients reported were given a single total-body exposure. Most of them were followed for a period of six weeks or longer, and none was given other forms of myelosuppressive therapy during this period.

Nonhematologic Malignancies. Figure 8 shows typical hematologic responses in the average value for four patients with cancer of various sorts (not primarily involving blood-forming tissues).

The hematologic effects are very much what one would expect in the normal. The improvement in hemoglobin was partially caused by transfusion, and therefore the red-cell values cannot be evaluated. It was our impression that these patients lacked the capacity for rapid and complete recovery that is seen in radiation accident victims, probably because as cancer patients they had been subjected to previous therapy with local irradiation and myelosuppressive drugs.

Polycythemia vera. Six patients with polycythemia vera were given 100 R exposures and the mean blood values are shown in Fig. 9. In these patients, the red-cell values had been lowered to acceptable levels by

* A condensed version of a presentation at the International Atomic Energy Agency Panel on *The Effects of Various Types of Ionizing Radiations from Different Sources on the Haematopoietic Tissues*, Vienna, Austria, May 17-20, 1966.

NASA Study on Radiation Effects

Retrospective Study on Deleterious Radiation Effects in Man

The great need for more precise knowledge about the radiosensitivity of man gave rise to a retrospective search for human radiation-exposure data that could be analyzed by rigidly imposed statistical methods. This study, supported jointly by the National Aeronautics and Space Administration and the U. S. Atomic Energy Commission, is now in its third year. The commencement of the Apollo program for human exploration of the moon's surface has created deadline pressures for completion of the part of the study focused on the effects of single and repeated exposures within an eight-day period.

Previously we reported two such studies in which first we used only data obtained from the hospital records of 100 patients treated at the Medical Division of Oak Ridge Associated Universities, and then combined that study with similar data obtained from the University of Cincinnati and the City of Hope Hospital, Duarte, Calif. We are now in process of expanding the study to include a total of about 1000 such hospital observations. We anticipate that this large amount of data will increase the accuracy of our statistical analysis so that greater reliance can be placed upon the correctness of the estimates now underlying shielding requirements of space vehicles. We also expect that correlation of medical and nursing observations of untoward changes in this large group of patients after the various levels of radiation exposure will enable predictions to be made of the kinds and severity of physiologic responses to be expected in normal man in certain radiation fields. We hope that such knowledge will supply on-ground monitors a basis for deciding whether or not an astronaut showing symptoms of distress in space is suffering from radiation exposure rather than from some other stress.

In the interest of giving our results to date the widest and earliest dissemination, the dose-response relations developed for symptoms of the human prodromal response were incorporated in Chapter 5.2.2 "Prodromal Response" and Chapter 6.0 "Lethality" in *Space Radiation Hazards*, a book to be published soon (March 1967) by the National Research Council, National Academy of Sciences under the editorship of H. Langham and Douglas Grahn (C. C. Lushbaugh and G. A. Crews).

The responsibility for data storage, retrieval and programming analysis

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has been transferred from the Mathematics Division of Oak Ridge National Laboratory to personnel of the Data Processing Center of Oak Ridge Associated Universities. The computer facilities of ORNL are still required to handle the large volume of data and perform the required analyses and statistical evaluations (A. S. Gloster and Donna Bibler).

The data under study are from 27 participating institutions that have provided 1226 usable patient charts out of 1274 obtained (Evalyn Replinger and Dorothy Vola). At present, approximately one-half million dose-correlated observations are in the study, although not all information from all charts has been completely encoded. This volume of data required development of an efficient data management system to obtain analytic results in reasonable time periods. This development has paralleled a search for more appropriate statistical methods of data analysis for this kind of study. Probit analysis, the method used so far, was neither designed nor intended to be used in retrospect so that deviations from implied assumptions are not ascertainable with it. Even so, our use of probit analysis appears justified by its widespread use in experimental radiobiology so that relative comparisons can be made between sick and well persons and various species of small and large animals. We are also applying methods used in testing of inanimate engineering components for reliability and durability. The Weibull density function that describes wear-out failure rather than chance failure, predicting the time that any rate of failure will be evident, seems most promising, since most symptoms and signs of biological distress can be considered as evidence for "wear-out" of a homeostatic physiologic system (E. Frome).

The following abstract of one of our studies is included because it illustrates one of the many complex problems that must be solved as a basis for this program.

During the year, further refinement has been achieved in the estimates of doses required for production of specific prodromal effects. These new estimates will not be reported in detail here because they will be refined still further in the future as larger numbers of patients are analyzed.

William B. ...

Dosimetry Used in Equating Various Radiation Exposures for Determining Dose-Response Relationships

W. Beck, R. Cloutier, and F. V. Comas

The factor that can affect most the validity of our retrospective determination of human dose-response relations is the accuracy of each dose

estimate. The statistical analysis commonly used in such studies contains the assumption that the independent variable, dose, is known precisely while the biological response may not be accurately measured.

The retrospective study was divided into four phases. Phase I was a pilot study of only 100 single exposures of less than 24 hr in patients at the Medical Division.¹ Phase II was an extension of this study to test the feasibility of using data from other hospitals, and also included only single radiation exposures.² Phase III, now being completed, includes single and fractionated exposures (greater than one day and less than eight days). Phase IV is planned to study protracted exposures where exposure time was greater than eight days.

Phase I. Phase I was composed of 93 single-exposure cases of therapeutically administered total-body irradiation (TBI) and seven cases of accidental TBI. Eighty-four of these were treated with the Division's medium-exposure-rate total-body irradiator,³ and the other nine were treated with the Division's cobalt-60 temporary irradiator.⁴

The dose estimates for the 93 patients were based on a dosimetric study by Hayes, et al.⁴ The dosimetric system consisted of an aqueous ferrous sulfate solution (Fricke dosimeter) within a plexiglass phantom. The absorbed dose to the solution was taken as the patient's dose when he was exposed to the same amount of radiation.

Using this technique we determined the whole-body average dose (WBAD), and the midepigastic dose for all 93 patients. The WBAD is an estimate of the average energy absorbed per gram of tissue, where the average is taken for every gram of tissue in the body. The midepigastic dose is an estimate of the average energy absorbed per gram in the upper abdominal compartment.

The dose estimates for the seven accident cases were determined by a mock-up of the accident at its site.⁵

Phase II. Phase II extended the pilot study by the addition of 11 new ORAU cases, 29 from Cincinnati General Hospital, Cincinnati, Ohio, and 23 from the City of Hope Medical Center, Duarte, Calif., bringing the total number of patients in the study to 163.

The dose estimates for the new ORAU cases were made with the same technique described in Phase I. However, all the ORAU dose estimates were modified for patient size. The size-correction factors were determined from the experimental measurements of the absorbed doses in three phantoms representative of an adult, an adolescent and a child.

The dose estimates for the Cincinnati and the City of Hope cases were based on a comparison of phantom depth-dose measurements made at each facility.

Phase III. The addition of about 600 patients from 21 institutions with both single and fractionated exposures brought the total number under study to more than 750. Approximately 50 treatment techniques were used, and "dosage" was recorded in more than 15 different units. To make these doses comparable to those in Phase I and II, it was necessary to convert the variously reported doses to total-body average dose.

Table 17 shows the dose distribution of the 504 cases for whom total-body average doses have now been estimated.

TABLE 17
Dose Distribution of 504 Cases

Whole-body Average Dose (in rads)	Number of Cases
0-25	149
26-50	108
51-75	90
76-100	19
101-125	17
126-150	36
151-200	19
201-250	26
251-300	4
301-400	15
401-500	7
501-700	7
701-900	2
901-1100	3
1101-1300	2
	<u>504</u>

Of the 600 new cases, only 66 were treated with gamma rays from isotopic sources and 39 of these were from institutions included in Phase II. We estimated the absorbed dose for 40 of these on the basis of experimental phantom measurements made at each facility. No dose estimates for the other 26 patients could be made owing to lack of phantom data.

Doses were estimated for 310 of 535 cases of X-ray TBI with the use of a method developed by Mayneord.⁶ Mayneord's method was experimentally verified by phantom measurements before its use. With it we estimated the total-body average dose for all cases of bilateral X-ray TBI having qualities expressed as half-value layers of from 0.5 mm to

4.2 mm of copper. For most of the other cases of X-ray TBI, the free-air exposure is the only "dosage" parameter available.

There were only 34 cases of the 757 single exposures to be considered in Phase III for whom we were not able to estimate either the total-body average dose or the free-air exposure, less than a 5% loss of cases due to insufficient dosimetry data.

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Therapy with Radiation

This is a long range and continuing program which has as its objective the improved treatment of disease by means of radiation, both internal and external. Main areas of effort are (1) the best use of total-body irradiation in the treatment of lymphoma, leukemia and polycythemia; (2) local external irradiation therapy for certain types of cancer, including application of experimental methods based on animal studies; (3) assessment of total-body irradiation as compared with splenic irradiation for chronic granulocytic leukemia; and (4) continued investigations of the best use of internal radioisotopes for malignant disease, especially radioiodine for thyroid cancer.

Total-body Irradiation

F. V. Comas, C. L. Edwards, G. A. Andrews, Helen Vodopick, and R. M. Kniseley

The study of total-body irradiation has continued and Table 20 indicates the numbers of patients treated this year in the various diagnostic categories, and a summary of the experience of previous years.

No large doses were given this year. Several children with acute leukemia are being carried on combined drug therapy. We plan to offer

TABLE 20

Total-body Irradiation Treatments Given at the Medical Division

	50 R	100 R	200 R	300-900 R	Total
Through 1965:	29	56	3	25	113
Calendar Yr 1966:					
Acute Leukemia					
Chronic lymphocytic leukemia		3			
Chronic granulocytic leukemia		2			
Lymphosarcoma		2			
Multiple myeloma					
Polycythemia vera		1			
Thrombocytosis		1			
Lymphoma	1				

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high dose (300 R) total-body irradiation to these patients when it becomes apparent that no further remissions can be expected from drug therapy.

It has been possible to treat two patients with chronic granulocytic leukemia with 100 R to compare the results with those in a small series treated earlier with 50 R.

Specific research results of the total-body irradiation program are covered to a considerable extent in other portions of this publication.

Spleen and Liver Irradiation in Chronic Granulocytic Leukemia

F. V. Comas, C. L. Edwards, Helen Vodopick, and G. A. Andrews

Irradiation of the spleen in patients with chronic granulocytic leukemia results in a lowering of the peripheral white cell count. The purpose of this study is to quantitate this effect and to clarify its mechanism.

The study protocol consists of irradiating the spleen and liver alternatively to compare the effects of spleen irradiation with that of irradiating an organ other than the spleen. The radiation dose is adjusted so that the product of the midplane dose (in rads) by the volume irradiated (in cm³) equals 4×10^6 . This dose is divided in 10 equal fractions to be given in a period of three weeks. The blood count is followed at close intervals for at least 75 days and bone marrow is aspirated before and several times after irradiation.

- (1) Constancy of response after spleen irradiation is striking.
 - (a) The white cell count begins to fall immediately after the first radiation dose, and continues dropping in an exponential manner, with a half-time of nine days, for the next six weeks, after which it begins to rise again.
 - (b) Regardless of the pretreatment count, the lowest white cell count attained is between 3 and 7% of the initial value, *Table 21*.
 - (c) The platelet count decreases much less, to a mean value of 36% of the initial count, and its fall is neither immediate nor exponential.
 - (d) The myeloberythroid ratio in the bone marrow always decreases.
 - (e) The spleen volume shrinks to about 17% of its pretreatment volume.

TABLE 21
White Cell Count Relative to Preirradiation Values
3 Weeks After Spleen and Liver Irradiation
with $4 \cdot 10^6$ ergs (nominal)

	No. of Patients	Mean	95% Conf. Lim.
Spleen	8	50%	2.6 - 7.4
Liver	6	38.1%	8.1 - 68.1

$P < 0.01$

Range of spleen control counts 72 000 - 243 000
 Range of liver control counts 67 000 - 224 000

(2) The response to liver irradiation is variable, but in general the white cell count and bone-marrow changes are much less pronounced than after spleen irradiation.

These results indicate that the response to spleen irradiation in patients with chronic granulocytic leukemia is highly predictable if the radiation factors are kept constant (dose, time, and volume). The patient's general condition and severity of the disease do not seem to influence the response, provided the changes observed in the white cell count are normalized to the pretreatment value. The results do not provide enough information yet to explain the mechanism of effect.

Radioisotopes in Diagnosis

The objective is to apply radioisotopic test compounds in (1) the detection of tumors, (2) the measurement of functional disorders, and (3) the diagnosis of metabolic defects. The agents are radioactive compounds, either already available or newly synthesized and tested in animals; the subjects are patients in the Medical Division's research hospital; and the procedures involved emphasize whole-body counting and scanning. Special techniques for radioassay and autoradiography on biopsy and autopsy material are applied to these problems.

Nonhomogeneity of Marrow in Acute Leukemia

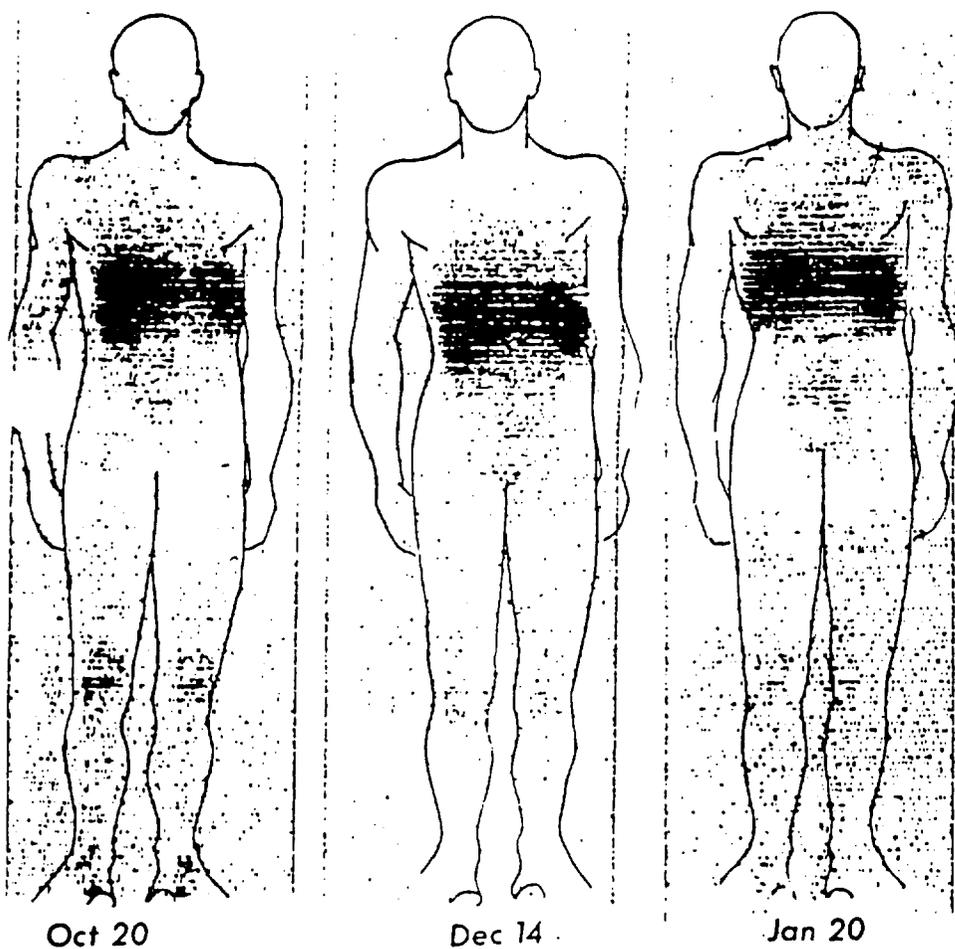
C. L. Edwards, Helen Vodopick, and R. M. Kniseley

Acute leukemia, we are accustomed to think, is a diffuse and homogeneous involvement of the entire cellular marrow. Ordinarily, in any one patient, samples aspirated from different sites show little variation in the type of the cells present. Aided by bone-marrow scanning we have encountered a situation that varies from this pattern and is of extreme interest to students of acute leukemia.

The patient, a 49-year-old man, after an acute recurring febrile illness in March and April 1965, treated with antibiotics, was found to have acute aleukemic leukemia. Blood values showed a moderate pancytopenia, but abnormal leukocytes in the blood were not sufficient to make the diagnosis obvious. He was treated with 6-mercaptopurine and several transfusions. Gradually his blood values and general condition improved. In October he had a hemoglobin of 14.3 grams with platelets $185,000/\text{mm}^3$ and leukocytes of $3650/\text{mm}^3$. In spite of the clinical and hematological improvement, the bone-marrow aspirates from the posterior iliac spines and sternum showed acute leukemic cells almost exclusively. On October 20, 1965 (*Fig. 42*), a bone-marrow scan with the use of technetium-99m sulfur sol colloid showed decreased uptake of the tracer compound in the usual marrow sites and an unusual concentration in the region of the knees. Aspiration from the proximal right tibia (November 5, 1965, see *Fig. 43A*) disclosed a marrow of increased cellularity with a pronounced erythroid hyperplasia. Megakaryocytes and granulopoietic cells were present though moderately decreased.

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Scattered leukemic cells were seen among the normal elements, but the picture was in striking contrast to aspirates taken from the sternum and iliac spines (Fig. 43B). Repeat aspiration one month later (December 4,



Bone Marrow Scans ^{99m}Tc colloid 4mc
Pt E.B. 511445 male age 49 Acute Leukemia

42. Sequential whole-body bone-marrow scans in a 49-year-old man with acute leukemia.

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1965) from the proximal end of the right tibia showed the diffuse leukemic pattern that had been encountered in aspirates from other sites and this correlated with deterioration of the patient's hematologic and clinical status. In spite of continued supportive and chemotherapeutic efforts, his condition continued to decline. Death occurred on February 22, 1966.

Until recent years, scanning the marrow organ had not been customary or feasible, and observers have been unable to adequately understand those patients who have evidence in the blood of a good deal of normal hematopoiesis when the marrow appears completely filled with blast cells. (This is not limited to patients responding to therapy.) In this patient, the marrow scan provided a direct explanation for the clinical hematologic response and pointed to the site of compensatory normal hemopoietic activity in the region of the knees. Furthermore, correlation was good between the subsequent marrow scan and the later

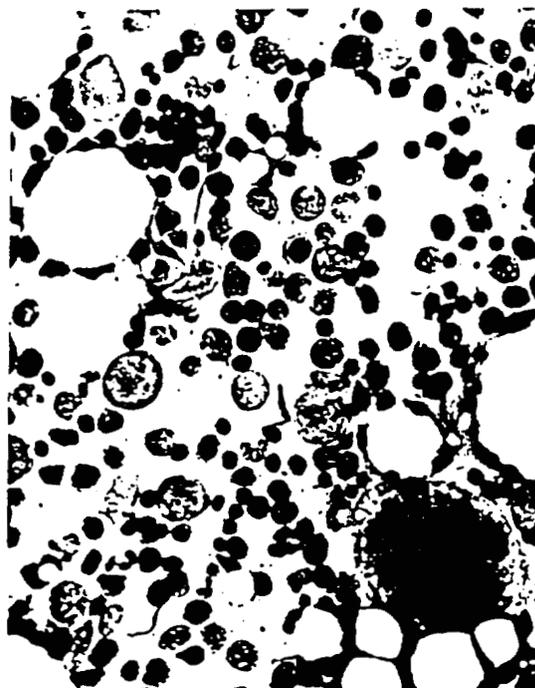


Fig. 43A. Marrow aspirate in November, 1965, from proximal tibia showing erythroid hyperplasia. Note the presence of megakaryocyte and granulocytic precursors.

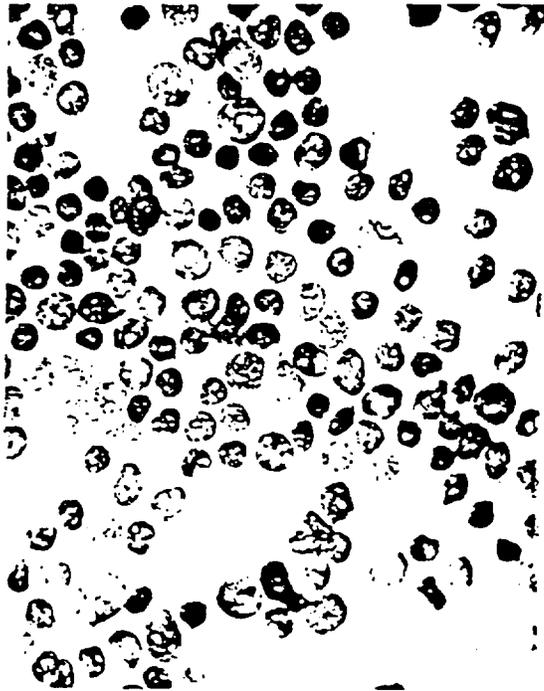


Fig. 43B. Aspirate on October 29, 1965, from sternum showing the diffuse proliferation of blast cells, and absence of normal hemopoietic activity.

tibial marrow aspirate. As the patient relapsed the scan showed less activity around the knees, and the marrow contained a much larger proportion of blast cells.

This study demonstrates the usefulness of the bone-marrow scanning technique in broadening our understanding of a hematologic process. It also suggests that in the pathogenesis of acute leukemia, fatty marrow is capable of responding with the production of normal sustaining hemopoietic elements in the face of persisting diffuse leukemic infiltrations of the marrow in the trunk regions. These observations raise an interesting speculation that perhaps some patients with acute leukemia under optimal management, even though incurable, may be amenable to more prolonged palliation if a greater and more persisting colonization of fatty marrow with normal elements can be achieved or if therapy can be directed selectively to the more abnormal areas of marrow.

Bone-marrow Scanning with Radioactive Colloids

R. M. Kniseley, C. L. Edwards, G. A. Andrews, R. Tanida, and R. L. Hayes

Bone-marrow scanning, as a nondestructive technique of delineating the hemopoietic marrow organ in patients, yields information helpful to the clinician and investigator.¹ Two fundamentally different radio-pharmaceutical approaches to bone-marrow scanning are possible: (1) radioactive isotopes of iron and (2) colloids labeled with radioisotopes. The former is based on the fact that with active hematopoiesis, most of a tracer dose of iron injected intravenously is transported to the bone marrow and incorporated in erythrocytes unless there is an iron excess. On the other hand, colloids are removed from the blood by the reticuloendothelial cells of the liver, spleen, and bone marrow. There is ample evidence that in normal animals and in many clinical conditions the distribution of radiocolloids in the bone marrow closely correlates with the distribution of erythropoiesis as detected by the distribution of radioiron or "red marrow."

Of the two approaches to bone-marrow scanning, that of using radio-iron has the more appeal because of greater interest in the distribution of the erythropoietic marrow. Iron-52, however, is the only isotope of iron that can be given in quantities sufficient for area scanning, but owing to its short half-life and difficulties in its production, it is limited to only a few institutions. For general use, radiocolloids are more promising.

There are, therefore, two basic problems toward which our attention has been focused in 1966: (1) correlation of bone-marrow distribution of radiocolloids and radioiron in various abnormal situations and (2) improved radiocolloids for scanning of bone marrow.

Iron-59 is a readily available isotope, but owing to its long effective half-life and radiation, it may be given in doses of only 0.05 millicuries or less, activity too small for practical rectilinear scanning with present equipment. Profile scans, however, can be made with the Division's linear scanner. Comparing a ⁵⁹Fe scan with the radiocolloid scan permits us to correlate the distribution of "erythropoietic marrow" and the "phagocytic marrow."

An example of a patient in whom there was a discrepancy is a woman, age 19, with an aplastic anemia following chloramphenicol therapy. In *Fig. 44*, the whole-body scan after the intravenous injection of technetium-99m sulfur-sol colloid shows (as did the profile scan) the usual distribution of the colloid in the liver, spleen and the bone marrow of the trunk. The profile scan with ⁵⁹Fe exhibits localization in the liver but little in the normal sites of hemopoiesis. The group at Donner Laboratory using ⁵²Fe and technetium colloid have even more graphi-

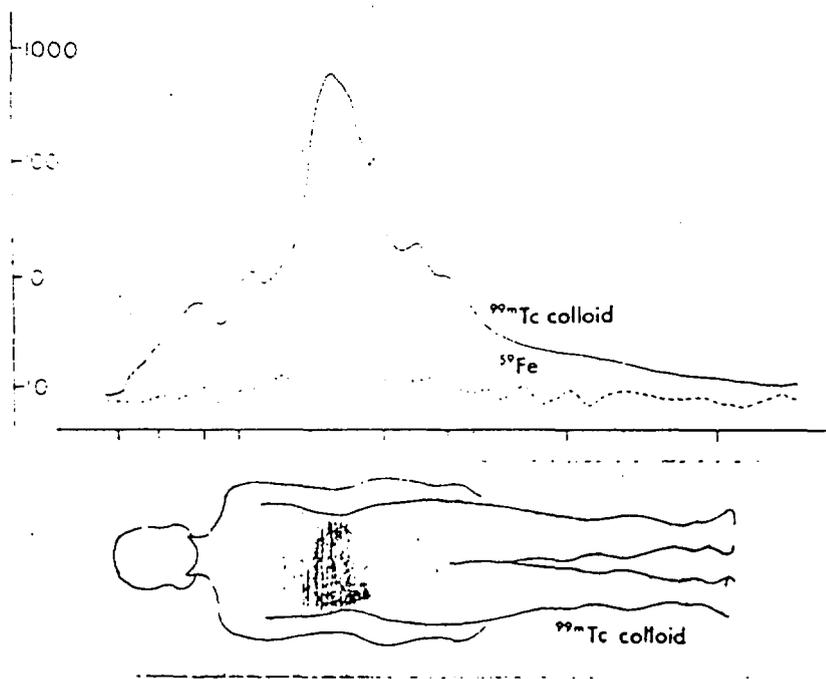


Fig. 44. Whole-body scan and linear scans of a 19-year-old woman with aplastic anemia subsequent to therapy with chloramphenicol. The distribution of colloid follows the usual pattern in the trunk region. The ^{59}Fe fails to localize in the usual regions of hemopoiesis.

cally demonstrated differences in marrow patterns in certain disorders.²

During the past year, 48 scan studies were performed on patients with various hematologic disorders. Although we have encountered some unexpected abnormalities and variations in the distribution of the marrow, we have been generally disappointed in the quality of the technetium-99m scans with the use of present equipment. Most of these scans were made with a sulfur colloid of $^{99\text{m}}\text{Tc}$ to take advantage of its radiation characteristics that allow a larger millicurie dose with less irradiation compared to ^{198}Au . Our scans with $^{99\text{m}}\text{Tc}$ sulfur colloid have been inferior to those made with the ^{198}Au colloid. The suspected reasons are the physical characteristics of the colloid giving a higher blood level (less completely removed), nonuniformity of the colloidal preparations and the attenuation of the 140 keV gamma at varying depths of bone, with considerable scatter being detected without discrimination from the photopeak.

Using ^{68}Ga ferric oxide colloid to scan the bone marrow of animals. Hayes *et al.*³ have obtained encouraging results. Pilot studies have been attempted on two patients with leukemia. The scans obtained were suboptimal but its localization in the reticuloendothelial system compared favorably with that of $^{99\text{m}}\text{Tc}$ sulfur colloid (Fig. 45). Diminished concentration in the marrow is demonstrated on both scans and correlates well with a myelofibrotic picture histologically. The liver and massive spleen are seen on both scans but the resolution appears to be better in the ^{68}Ga ferric oxide scan. The blood clearance studies with ^{68}Ga

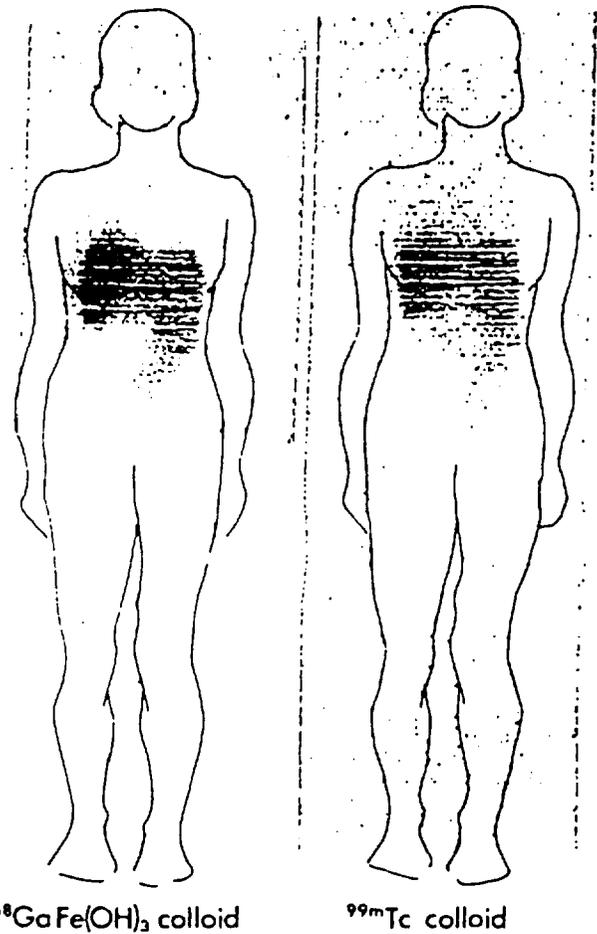


Fig. 45. Scans of patient with chronic granulocytic leukemia after ^{68}Ga -labeled ferric oxide (left) and technetium-99m sulfur colloid (right).

ferric oxide colloid and technetium sulfur colloid reveal a rapid early blood disappearance with a half time of 2 and 7 min (Fig. 46). The gallium colloid, however, cleared more completely with a significant difference within 30 min of the infusion.

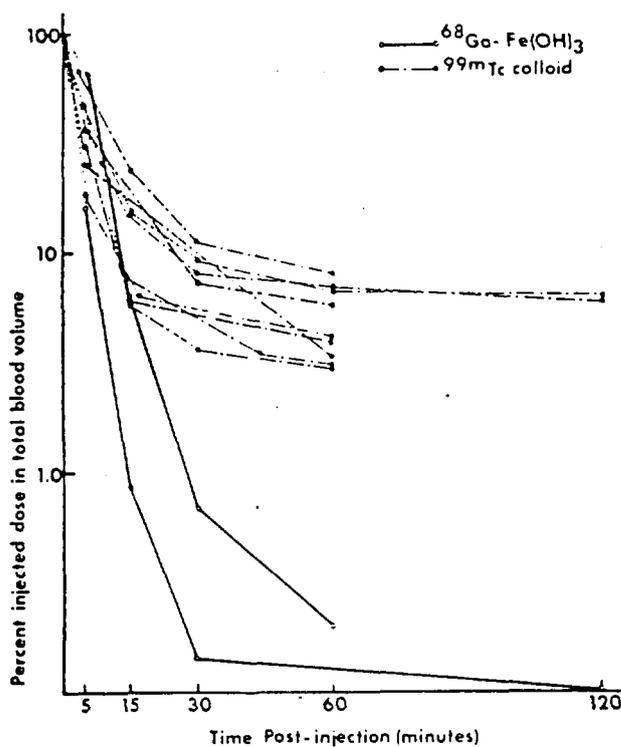


Fig. 46. Disappearance curves from blood (solid lines) from two patients after intravenous doses of ^{68}Ga -labeled ferric oxide colloid. Compare with disappearance curves obtained from patients given technetium-99m colloid.

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Skeletal Scanning with Gallium-68 Citrate

C. L. Edwards, J. Ahumada,* and R. L. Hayes

Studies carried out on animals by Hayes¹ and preliminary experience in patients² indicate that gallium-68 can be a clinically useful agent for the detection of metastatic bone lesions when administered with 2 to 4 mg of stable gallium citrate per kilogram of body weight. Bone scans of the entire skeleton, or of areas of specific interest, have been made on 75 patients to whom 103 doses of gallium-68 citrate were administered. For the most part the patients were inpatients at the ORAU Medical Division hospital and were being treated for malignant neoplastic or hematologic diseases. Some outpatients with known neoplastic disease were referred from the local medical community.

The scans were interpreted as normal in 52 patients and abnormal in 23. Of the 52 normal scans, two were of patients with known neoplastic disease of the skeleton and were, therefore, "false negative." Of the patients with abnormal scans, the skeletal lesion was a malignant neoplasm in 14. The remaining nine were due to a variety of lesions, i.e., eosinophilic granuloma, healing fractures, osteosclerosis, possibly osteomyelitis, and acute arthritis. Many of the patients had multiple lesions, some undetected before scanning. In two patients suspected of having bone metastases, the gallium-68 citrate scans succeeded in locating the lesions where X-ray examinations failed. The earlier demonstration of the lesions allowed earlier palliative teletherapy.

Scans made with gallium-68 citrate are being compared with those using other bone-scanning agents. In two patients, scans were made with gallium-68 citrate and then strontium-85. In the first patient, both scans demonstrated lesions not visible on roentgenograms (*Fig. 47*). In the second patient, both failed to demonstrate the lesion, which proved to be a giant cell tumor with malignant features. In a third patient, the scan made with gallium-68 citrate demonstrated a known lesion, which had been proven by biopsy, as well as another lesion not accessible to biopsy. He was not scanned with strontium-85 until five months later, but neither lesion was detected at that time. It remains to be established that the second lesion was actually a neoplasm.

Six patients experienced a reaction characterized by tremor, chills, fever, nausea and apprehension. In at least one patient the reaction was aggravated by a very low serum ionized calcium. The reactions have caused us to modify our techniques for the production of the radio-pharmaceutical in an effort to eliminate all possible sources of pyrogens.

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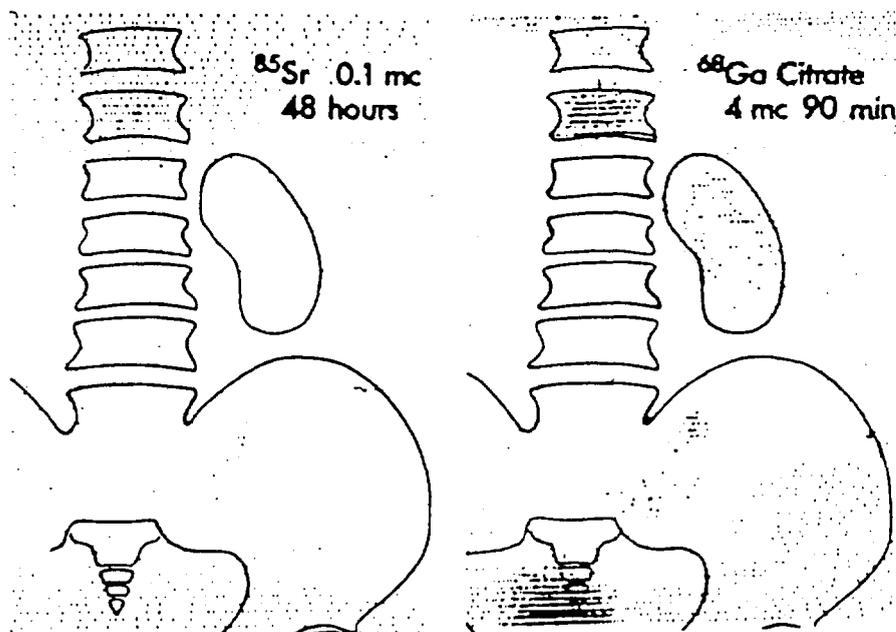


Fig. 47. Comparative scans made with ^{68}Ga citrate and ^{85}Sr of a patient with metastatic prostatic carcinoma indicating lesions in the first lumbar vertebra and the left sacroiliac region.

The uncertainty of chemical toxicity of the carrier gallium mitigates recommendation of broad usage of this preparation as yet. An LD_{10} of 20 mg/kg body weight was suggested by Brucer, et al., in 1953.³ Chemical toxicity was thought to be responsible for the bone-marrow depression and dermatitis seen in patients who had received gallium-72 as treatment of osteogenic sarcoma. They also observed lethal nephritis in dogs given much smaller doses of gallium than those given to any other species.

We have seen no evidence of nephritis or dermatitis in patients at the 2 to 4 mg/kg level. Eight patients have been found to have an unexplained, temporary depression of white blood cell values (to below 10 mm^3 or to 50% of preinfusion value) within the first 60 days after infusion. All had significant malignant disorders and six had ma-

lignant diseases of the lymphatic or hematopoietic systems. Observations on more patients without bone-marrow involvement will have to be completed before a definite conclusion can be drawn regarding the effect, if any, of the stable gallium carrier upon the bone marrow.

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