ORINS-34

OAK RIDGE INSTITUTE of NUCLEAR STUDIES

Medical Division

Report for 1959

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P. O. Box 117 Oak Ridge, Tennessee

FOREWORD

(An introduction and summary)

Marshall Brucer

During 1959 the Medical Division continued in transition. Dr. G. A. Andrews was appointed associate chairman with a primary interest in human therapy. Dr. B. W. Sitterson, now chief of clinical services, has relieved Dr. Andrews of much of the duty involved in the care of patients. Dr. D. A. White has taken over important follow-up and outpatient studies. Dr. R. M. Kniseley has returned to the Medical Division to handle other phases of clinical research.

During 1959 the medical report of the Y-12 accident was published, but the work initiated by the Y-12 accident has by no means been completed. A most promising approach to the continuation of this acute radiation work is in the problem of amino-acid analysis. If nothing else has come out of the Y-12 work, we have received an indication that this is a possible approach to be run before we can extrapolate from leukemic and other patients to accident patients.

Another important item is in the program of accident diagnosis. This relates to the whole-body counting device. Almost all the American and English whole-body counters have been studied and the entire literature has been reviewed with a compilation into a report on the ORINS design for a whole-body counter system. This was mimeographed at the end of the year.

The 1959 program report is divided into five sections: 1) Clinical; 2) Preclinical; 3) Medical Physics; 4) Training; 5) Tabulation of hospital statistics and list of publications.

The main clinical and medical physics programs have been directed toward the idea of total-body irradiation. Total-body irradiation as used in therapy (and incidentally as a method of investigating what is necessary in the treatment of accident patients) is not a new idea. The definitive studies go back to the 1930's when Heublein stated that he did get some benefit. A number of radiotherapists even today are uncertain whether this method of therapy should have been stopped. As early as 1897 Mr. Tesla, an electrical engineer, pointed out that total-body irradiation was curing sickness. The most recent work on

immunological changes helps to explain the possible meaning of these early observations. The older methods, however, of giving total-body irradiation were the weak points in the establishment of a practical basis for irradiating sick people. Three devices have been designed and are now in operation for a true total-body irradiation. One is the Navy's short-distance, cobalt-60 machine (Bethesda, Maryland). Another is at the City of Hope Hospital, a short-distance cesium machine (Duarte, California). The third is the long-distance cesium machine at ORINS. This machinery, for administering a uniform or nonuniform dose of total-body irradiation, was built and put into operation during the year. While this was being done, the clinical program emphasized a less desirable method of applying total-body irradiation, but performed some of the essential early clinical studies.

Scanning also links the medical physics and the clinical programs. During the year the linear scanner was put into operation. The calibration of the machine is not yet complete but sufficient calibration was done to begin clinical studies. The ORNL research scanner was put into operation at the end of the year. It has three main features: The sensitivity is superlative; the shielding is exceptional; and four main methods of presenting the data are attached to the machine. None of these is expected to be the final answer because none is completely satisfactory.

In the preclinical fields, one problem is outstanding: toxicity of the rare earths. The toxicity is in itself not the important item but rather the fact that the toxicity elucidates some of the metabolic functions of the rare earths. Potential clinical uses, industrial hazards, and even the basic pharmacological knowledge gained from rare-earth studies have a value far beyond immediate studies of toxicity. These substances undergo a metabolism, a transport, a distribution, and a localization that are illustrative of the entire problem of using these new agents in clinical medicine.

In any research organization, communication is of prime importance. Research data are valueless until they are available to others. Training (not textbook training, but training in the use of research results) is therefore ultimately the most important of any productive efforts of the Medical Division. This involves publication and training. The interest in nuclear medicine has grown so rapidly that the ordinary channels have been unable to cope with the need for training. A number of items in the problem of training physicians to use radioisotopes were obvious at the beginning of the year. First, we did not know (nor did anybody else) the proper subject matter for training, but we knew that we didn't know. During 1959, a program of clinical training has been set up that has defined the subject matter. Second, we knew at the beginning of the year that the method of training had to be short, had to be confined to persons already expert in one field of general medicine, and had to meet the pertinent government regulations on the use of isotopes. During the year a series of three separate one-week

courses was revised to meet these problems. Third, we knew at the beginning of the year that the didactic method of presentation alone could not achieve the objectives of the training program. No one can learn the techniques of nuclear medicine without actually performing them in the laboratory. During the year we have been able to devise a laboratory course of instruction. Six of the twenty necessary laboratory outlines have been published in draft form. Instructions for fourteen sessions are left to be completed, but until these are completed our training of physicians for the use of radioisotopes will be inadequate.

The fifth section of this report is a tabulation of hospital statistics and a list of publications. Many of our visitors ask us questions that these lists may help to answer. The list of publications is for the year 1959. Lists of all publications from the Medical Division are available from the editorial office.

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CLINICAL RESEARCH

Introduction (B. W. Sitterson)

Probably the most distinguishing feature of the clinical research program during the past year has been the increased interest and expansion in the work on the effects of radiation in human beings. Studies associated with total-body irradiation followed by attempts at bone-marrow grafts in acute leukemia patients and follow-up studies in the group of men irradiated in the Y-12 accident have continued. Much effort has gone into comparing our data with those reported by other workers who have studied similar patients. New projects pertaining to the effects of both total-body and local-port irradiation on various hematological diseases, other clinical syndromes, and normal hematopoietic tissue have been started. Bone-marrow grafting has been studied further by attempting autografts after large doses of nitrogen mustard. Considerable time has been spent trying to arrange for definitive immunological studies, which appear to be critical to the marrow-graft problem, on patients who receive total-body irradiation. A new radiation facility is nearing completion that will enhance the work on radiation effects in man.

Long-range programs involving diagnostic and therapeutic uses of internal radioisotopes continue. Some of this work is done primarily for the purpose of teaching residents and some of it constitutes definite research programs. New projects in this area include study of factors influencing uptake of iodine by malignant thyroid tissue, using the short half-life isotope iodine-132, autoradiographic studies of concentration of various radioisotopes in metastatic malignant tissue in the liver, and histologic changes induced in nonmalignant human thyroid tissue by various doses of iodine-131.

Dr. Ralph M. Kniseley rejoined the staff during the year as chief of clinical research and training and has actively participated in all aspects of the work of the clinical service.

Before leaving the staff in the first part of the year Dr. Etna Palmer managed the outpatient service, which continues to grow. Dr. Betty M. Cooper left the staff during the latter part of the year, but continues to serve in a consultant capacity as anesthesiologist for all general anesthetics. Dr. Jose B. Briones completed his appointment as

postresident assistant in radiology during the year. Since his departure the diagnostic radiology has been done by several short-term residents, trained in radiology, who have assumed this responsibility in addition to their other clinical work.

Dr. David A. White, an internist and hematologist, recently joined the staff and has taken over management of the outpatient service in addition to assisting with all other patient care.

The clinical service has continued to benefit from the generous consultant help of a number of physicians in Oak Ridge, including Dr. Robert R. Bigelow, Dr. William W. Pugh, Jr., Dr. Dana W. Nance, Dr. Henry B. Ruley, Dr. Earl Eversole, Jr., Dr. Robert P. Ball, Dr. Avery King, Dr. Raymond A. Johnson, Dr. Dexter Davis, Dr. Paul Spray, and Dr. C. J. Speas. Likewise, Dr. Eidson Smith and Dr. Robert Newman of Knoxville have been most helpful. Dr. George Minor of Charlottesville, Virginia, has continued to serve as a consultant and has made trips to Oak Ridge to perform thoracic surgery on certain patients.

Dr. Felix Pircher was responsible for a large part of the work in clinical care of patients before the end of his appointment at midyear. He also did basic clinical work with the profile scanner. Dr. Flora Pascasio and Dr. Frank Oda joined the staff July 1 with one-year appointments as research associate and resident in experimental medicine. Both have assumed major responsibilities in clinical care of patients in addition to working on specific research projects. Dr. Yoichiro Umegaki and Dr. Teruo Nagai, visiting investigators from Japan, have taken an active part in clinical rounds although their primary interests have been in special research areas.

The nursing staff, under the supervision of Mrs. Adren Sutliff, have contributed greatly to the over-all clinical program by providing care for the patients and assisting in the collection of data.

A group of interested and enthusiastic resident physicians, in various special fields, has contributed in a major way to the teaching and research activities, and to clinical care of patients.

Short-Term Resident Physicians

Dingle, Robert W., M.D.	Boston City Hospital
Escandari, Freidoun, M.D.	Cleveland Metropolitan General Hospital
Foxman, David, M.D.	Cleveland Metropolitan General Hospital
Gazelle, Harry, M.D.	Cleveland Metropolitan General Hospital
Hieber, Robert D., M.D.	Boston City Hospital
Issarescu, Stefan, M.D.	New England Deaconess Hospital
Leon, Enrique, M.D.	Boston City Hospital
Liu, Hsing-Lin, M.D.	Cook County Hospital, Chicago, Illinois
McCoy, William T., M.D.	Massachusetts General Hospital
Meyer, James V., Jr. M.D.	University of Texas Medical Branch
Nickell, Lawrence R., M.D.	University of Texas Medical Branch
Packert, Richard C., M.D.	Massachusetts General Hospital
Papavasiliou, Paul, M.D.	Memorial Center for Cancer and Allied Diseases, New York, New York
Schatski, Stefan C., M.D.	Massachusetts General Hospital
Seeley, Clinton B., M.D.	Massachusetts General Hospital
Simon, Harold, M.D.	Massachusetts General Hospital
Thibault, Maurice, M.D.	Massachusetts General Hospital
Weylman, Walther, M.D.	Massachusetts General Hospital

Special Visiting Participants

Cunningham, Elbert C. Jr., M.D.	Oak Ridge, Tennessee
Fliedner, T. M., M.D.	Brookhaven National Laboratories
Furth, John, M.D.	New York University

RADIATION EFFECTS AND MARROW-CRAFT STUDIES

Dosimetry (R. L. Hayes)

Dose to the intestinal tract. A tracer study of the dose received by the human intestinal tract as a result of the oral ingestion of radioactivity has been started. This study was prompted by the results obtained in a previous study of intestinal-tract dose in animals. The extreme variations obtained in the animal studies raised the question of the validity of assuming an "average behavior" in intestinal-dose calculations for maximum permissible concentrations (MPC) of various radioisotopes. The study with human beings is an attempt to find the limits of normal variation among individuals in rates of passage of "nonabsorbed" radioisotopes through the gastrointestinal tract. Present studies are being carried out with tracer doses of one such isotope, lanthanum-140. Most of the subjects so far studied have been elderly, and data from young subjects will be required before any definite conclusions can be reached. Nevertheless, the data so far collected indicate that, for elderly persons, the now recommended MPC for "nonabsorbed" radioisotopes (Handbook 69) is too high by as much as a factor of 10.

When suitable detection equipment is available we plan to expand this tracer study to include measurements of the urinary excretion and body retention of partially absorbed as well as "nonabsorbed" orally ingested isotopes. Such an extension of the present study is of fundamental interest, since most maximum permissible concentrations (Handbook 69) are now based on animal distribution data rather than on data from man.

Distribution of internal radiation dose. A knowledge of distribution is a valuable aid in the planning and evaluation of total-body irradiation therapy. In this type of therapy the absorbed dose (energy absorbed per unit weight) can also be of considerable importance. Using phantom techniques, we have made depth-dose and absorbed-dose measurements for the broad-beam cobalt-60 total-body irradiator. Measurements were made in compartmentalized phantoms designed to simulate an adult, an adolescent, and a child. The study provides information on the homogeneity of radiation dose and the total absorbed energy as a function of body size. As a further aid in defining total-body irradiation dosimetry, glass dosimeter measurements

of skin dose have been made on patients given total-body irradiation therapy. Such measurements serve as an indicator of the uniformity of the dose given.

Clinical Studies. (G. A. Andrews, B. W. Sitterson, A. L. Kretchmar, F. Comas, and D. A. Ross).

Studies on total-body irradiation in acute leukemia were continued and an assessment was made of the results of the first series of cases treated. These include eleven patients with acute or subacute leukemia. Of these patients, three were adults, one was an adolescent, and the rest were children. All except one had had extensive previous drug therapy. All had active leukemia at the time of treatment and some were in critical condition. The general plan of treatment was to stop antimetabolites, to continue steroid therapy if it was already under way, and to give a large single dose of total-body irradiation. Doses varied from about 200 r to more than 900 r. Bone marrow was obtained from homologous donors cross matched for the usual major blood types. The marrow was obtained by aspiration from the posterior iliac spine, iliac crests, and sometimes from other areas. Donors were given a general anesthetic. The marrow obtained appeared grossly cellular and smears indicated a high percentage of marrow elements as compared to peripheral leukocytes. Cell counts are believed to be erroneously low, chiefly because of clumping, and were highly variable. In the most favorable aspirations, several billion cells were obtained. The volume of marrow aspirate was generally from about 100 to 300 ml. Clumps were not broken up except for passing the marrow through a needle somewhat smaller than the one used for aspiration. Marrow was administered intravenously shortly after it was obtained. In most of the patients about four separate donors were used -- one on each day during the first five days or so after exposure. In one patient, in an effort to duplicate the technique of Mathé, a single donor was selected by compatibility of major and minor red cell types. No untoward results were attributed to the marrow administration. Three patients received radiation without marrow administration. Several types of results were seen and can be summarized in the following table:

1. Incomplete suppression of leukemic process:

   Temporary suppression, then exacerbation with proliferative phase and death ----------2

   Partial sustained suppression----------3 (2 received no marrow)

2. Apparent complete or nearly complete suppression of leukemic process:

   Death in aplastic phase----------2

   Remission----------3 (1 received no marrow)

3. Early death:----------2

Twelve treatments are reported on eleven patients. One patient was treated twice with an interval of about six months between treatments.

In general, the leukemic cells proved to be strikingly sensitive to radiation and there was a massive destruction of these cells with a profound fall in peripheral white count and marrow cellularity in most cases. This destruction occurred chiefly within the first four or five days, and thus the patients with leukemia reached a phase of marrow aplasia much earlier than normal persons exposed to the same doses. Still, some of the leukemia cases showed greater resistance of the leukemic cells and never reached complete aplasia. This resistance was seen even in quite high doses and could not be correlated clearly with morphologic features of the leukemia. It appeared, however, that the older patients, with a less primitive type of leukemic cell, were more likely to show resistance. Further data would be needed to confirm this. An example of a very transient suppression of the leukemia, followed shortly by a proliferative leukemic phase, is shown in Figure 1.

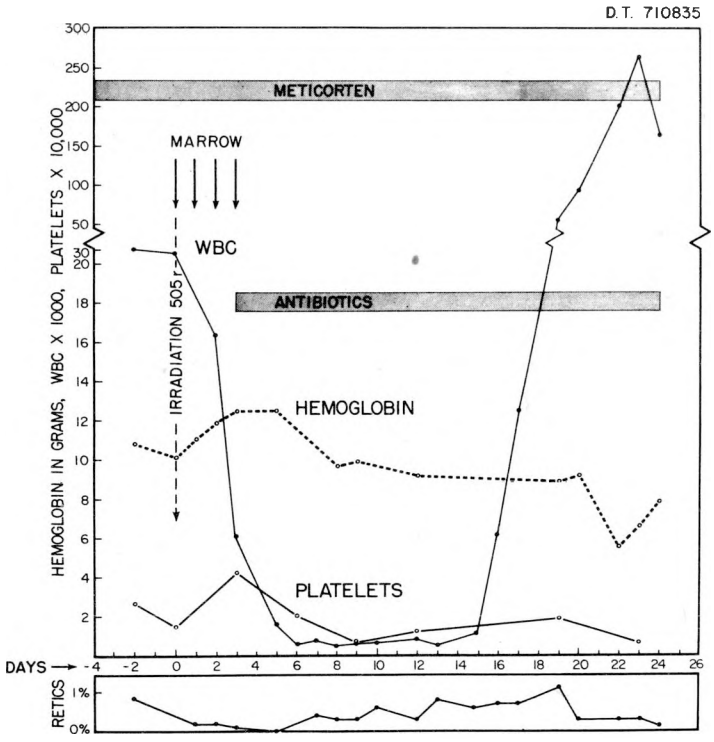


Fig. 1. Chart on patient No. 3. Only transient suppression of leukemic process by 505 r of irradiation.

Those who died with either exacerbation of leukemia or in an aplastic phase survived from periods of 17 to 25 days. It was sometimes impossible to determine the relative importance of leukemia and radiation injury as causes of death. Two very early deaths both occurred on the first posttreatment day. Deaths were due to hemorrhage and infection, with diffuse pulmonary bleeding prominent, particularly in one patient who died in an aplastic phase.

We have arrived at some conclusions about "ablation" of the bone marrow. It is not unusual for patients treated with big doses of radiation or nitrogen mustard to reach a state in which the marrow is apparently almost completely acellular. When patients die in this phase, either from hemorrhage or infection, the tendency is to say that the marrow was "completely ablated." However, the morphologic observation has great limitations and one cannot say from the appearance of the marrow that it has lost capabilities for either normal regeneration or further leukemic proliferation.

Evidence for success of the marrow graft was very slight. In one patient who had a good remission, a series of morphologic changes in the bone marrow might have been interpreted as indicating a very transient proliferation of the graft about a week after it was given, but this interpretation cannot be strongly supported.

Studies of red cell subtypes in donors and recipients were complicated by the fact that most of the patients had received transfusions before treatment and that difficulties were experienced in attempting quantitative identification of mixed cell populations. In general the red cell subtyping studies offered little support for a true marrow graft.

One of the most striking results of this study was the demonstration that large doses of total-body irradiation alone can produce remissions in leukemia. Previous literature on this subject has been somewhat conflicting but the concensus had been that irradiation was not beneficial in acute leukemia except for localized lesions. The most striking case in the series was a patient who had a very complete, although transient, remission after 360 r of total-body irradiation. In this patient (See Fig. 2) there is no possibility that drug therapy was responsible for the benefit, and the leukemia was in a florid state at the time of treatment.

The most favorable results in this series were obtained, in general, with the lower radiation doses. No prolonged survivals were seen in patients treated with more than 600 r.

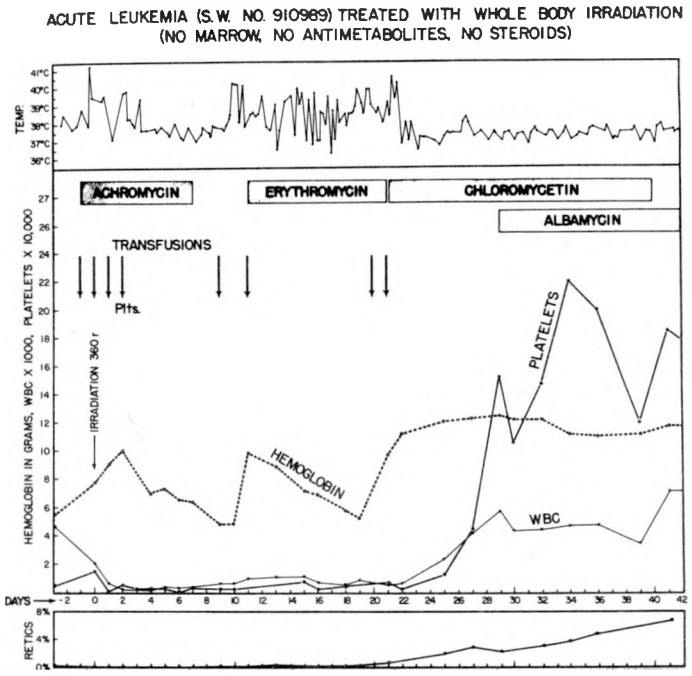
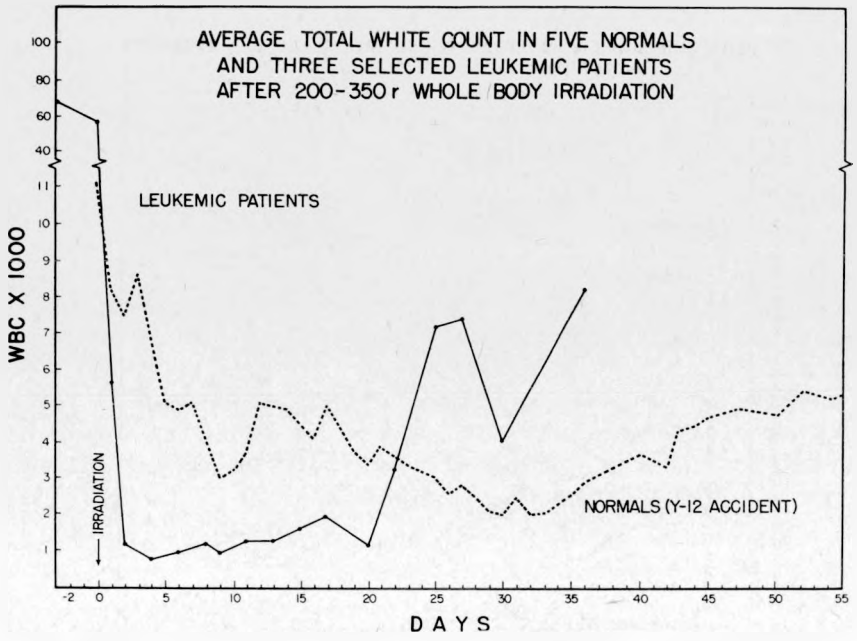


Fig. 2. Chart on patient No. 10. Remission from irradiation without marrow.

Another pertinent observation was that the regenerative phase started early in the three patients who had complete remissions. This is seen when the platelet curves, the white cell curves, and the leukocyte curves are compared with the Y-12 series of normal persons accidentally irradiated. (Figs. 3 and 4). It appears that recovery begins almost two weeks earlier in the irradiated leukemia patients than in the normal patients exposed at about the same dose. This difference in time sequence seems to involve a significant biological factor that may be of importance. Possible explanations for the earlier regeneration in the favorable leukemics can be listed as follows:

1) The type of radiation was different. The Y-12 accident included a neutron component.
2) Ages were different. The leukemics were children.
3) Marrow was cleared out faster in the leukemics, and this allowed space for regeneration.
4) Toxic products from radiation damage inhibit hematopoiesis. In the leukemics this destructive phase is completed earlier.
5) Severe pancytopenia must exist before stimulus for regeneration is activated.
6) In the leukemic, the cells that serve as precursors for eventual normal hematopoiesis are, for some reason associated with leukemia, in a state allowing more rapid proliferation once conditions are favorable.



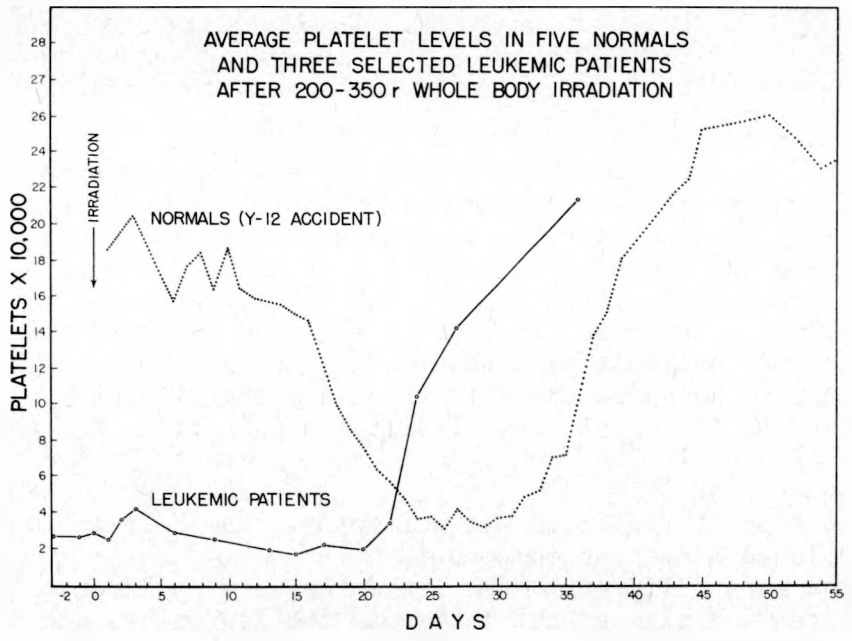


Fig. 3 and Fig. 4. Average white blood cell and platelet counts in 3 patients who developed remissions in leukemia after radiation (cases No. 1, 2, 10) compared with 5 normals accidentally irradiated in Y-12 incident with doses of 236 to 365 rads.

These two facts, (1) that irradiation alone can produce remissions and (2) that these remissions begin earlier than in normal persons treated at the same dose, are likely to lead to an erroneous belief that a marrow graft has been obtained when the results seen are due to the radiation treatment alone.

A summary of our current impressions of the treatment of leukemia by irradiation and marrow grafting is as follows:

1) Total-body irradiation alone produces remissions in some cases of acute leukemia.
2) Remissions cannot be accepted as proof of marrow transplantation, either temporary or permanent.
3) In most cases of acute leukemia, the leukemic cells appear to be radiosensitive, and there is profound cell destruction after a single large dose of radiation. This cell destruction may contribute to early deaths. Even when it appears that all the cells have been destroyed, exacerbation of the leukemia occurs later.
4) The term "marrow ablation" should be used very carefully. Many patients who survive a single dose of radiation or marrow-damaging drug have gone through a phase of apparent marrow ablation.
5) In the leukemic patient who responds favorably to total-body irradiation, the phase of marrow recovery begins about two weeks earlier than in normal patients exposed to the same radiation dose. An explanation for this fact would probably have general importance.

Pathologic Changes in Total-Body Irradiation (Bill M. Nelson)

Ten patients treated for leukemia with total-body irradiation (in the range of 200 to 800 r) have now come to autopsy at ORINS. Microscopic studies have been completed on all but the most recent one. With the possible exceptions of the bone marrow, the histologic observations so far fail to demonstrate responses in various tissues that would differ from the expected effects of the same doses of radiation given locally rather than to the entire body. The marrow in two patients dying 17 and 18 days (after doses of 790 and 500 r respectively) was quite aplastic, without evidence of residual leukemia. No morphologic (or clinical) evidence of successful marrow transplantation was found in any of the six patients given intravenous infusions of fresh marrow from homologous donors. The six patients surviving more than three weeks (24 days to 219 days) all died with leukemia and with little, if any, apparent residual effect of the radiation in the sections of marrow. All ten deaths could be attributed to hemorrhage or infection. At least five deaths can be attributed to leukemia, since death occurred after recovery from the irradiation. The two early deaths (within two days after irradiation)

may also be credited to leukemia, although radiation effects were detected at autopsy. A patient with granulocytic leukemia, dying in the fourth week after radiation, was found to have fibrosis of the marrow. This fibrosis seems to have been present before treatment and no fibrosis was seen in the other patients.

Further analysis of the autopsy observations is needed to investigate possible distinguishable features of the deaths attributable to radiation. In particular, the frequent occurrence of severe pulmonary hemorrhage is of interest. The lack of characteristic gastrointestinal lesions is noteworthy and suggests the possible use of higher doses.

Follow-up on Y-12 Accident Patients (B. W. Sitterson, A. L. Kretchmar, and G. A. Andrews)

In March 1959, about nine months after exposure, the five high-dose patients from the Y-12 radiation accident were admitted to the hospital for three days to obtain studies under conditions of diet and physical activity comparable to those under which earlier studies had been done. The three low-dose patients were seen as outpatients at this time. All eight patients were seen again as outpatients in August 1959.

Subjectively, they still reported varying degrees of undue fatigue, weakness and stiffness in muscles, popping noises in joints, insomnia, irritability, and nervousness. Some of these difficulties were most prominent in low-dose patients, with no apparent correlation between the dose of radiation and the severity of these symptoms. In general they seemed to have improved slowly and progressively. Physical examinations showed no significant abnormalities and no objective findings to account for these symptoms.

One patient (Patient D) had a recurrence and increase in severity of the vague abdominal pain that was noted during the period of hospital care immediately after the accident. Upper and lower gastrointestinal X-ray studies showed no abnormality. Treatment with antispasmotic and antacid medications resulted in noticeable improvement. This patient also developed symptoms of memory lapses and disturbance in balance when walking downhill, but not on level ground or going uphill. A consultant neurosurgeon examined him and found no significant abnormal neurological observations. The consultant's opinion was that the symptoms were functional.

Hematological studies have shown that the hemoglobin, hematocrit, white blood cell count, differential count, and platelet count of all the patients are within normal limits.

The results of special biochemical studies are reported in a separate section of this report.

Ophthalmological and slit-lamp examinations have been done on all the patients by a consultant ophthalmologist at approximately six-month intervals. Sperm counts have also been obtained at periodic intervals, with participation on a voluntary basis.

Comparison of Y-12 Patients with Yugoslavian Accident Victims (G. A. Andrews)

In connection with the total-body irradiation program and the interest in marrow transplants, we at the Medical Division are comparing the Y-12 accident victims, the Yugoslavian accident victims, and the leukemic patients given total-body irradiation. It is generally agreed that the physical data on the Yugoslav victims are not adequate to allow a clear estimate of the dose of radiation. Whether or not the apparently successful bone-marrow transplant was life saving in these patients is a question that is difficult to answer in the absence of clearly established dosage. Efforts are being made here to compare the hematologic response of the Y-12 and Yugoslav groups of patients on the assumption that the blood changes and clinical observations may help to indicate relative dosages.

In a dosage comparison based on hematologic effects, it is clear that there are some qualitative differences between the two groups of patients. The Yugoslav group showed much greater granulocyte and lymphocyte depression, but the platelet depression, except in the patient who died, was generally no more severe than in the Y-12 group. Biological and clinical information indicates with considerable certainty that the one Yugoslav victim who died received a much higher dose than any in the Y-12 group. At the other extreme the one Yugoslav who recovered without bone marrow probably received a dose lower than the average of the five in the Y-12 group. The most critical comparison is between the four Yugoslavs who received bone marrow and recovered and the five in the Y-12 group. For the purposes of such comparison, the blood data were averaged for each group and a comparison was made for the averages. An analysis of these charts indicates the following:

Reticulocytes: Neither group had complete suppression of reticulocytes. The Yugoslavs were somewhat lower on the fifth day than the Y-12 group. Subsequently an increase occurred and the two groups remained almost identical, with a secondary depression between the twenty-fifth and thirty-first days. After that a striking reticulocytosis was seen, considerably more profound in the Yugoslav than in the Y-12 group, with both groups reaching their peak level at about 47 days after exposure.

Lymphocytes: The early lymphopenia was much more profound in the Yugoslav group and lymphocyte levels remained distinctly lower throughout the first two months. Both groups showed some increase in lymphocytes at the fortieth day, and then another slight slump during the subsequent three weeks.

Platelets: The initial platelet levels were somewhat lower in the Yugoslav group, but at about the twenty-second day the curves become identical. After the twenty-seventh day, however, recovery in the Yugoslavs took place a little more rapidly than in the Y-12 group. On the other hand, later in the recovery phase between the fortieth and fiftieth days, the Y-12 levels were higher than those in the Yugoslav group. Platelets were counted by the Brecher and Cronkite method in the Y-12 group and by the Feissly method in the Yugoslav group.

Granulocytes: Granulocytes were much more profoundly depressed in the Yugoslav group and reached much lower levels between the twenty-sixth and thirty-fifth days. Recovery seemed to occur at about the same time in the two groups.

In attempting to assess this information in terms of evidence relating to the success or failure of the marrow graft in the Yugoslav patients, several serious problems are presented: 1) The dose of radiation is poorly established for the Yugoslavs. 2) Comparison of clinical and laboratory data is handicapped by the differences in methods used and by the fact that the Yugoslavs were given many types of medication and treatment. 3) The bone marrow was given to the Yugoslavs rather late, shortly before spontaneous recovery might have been expected, if it is assumed that the Yugoslavs followed the same pattern as the Y-12 group.

In spite of all these difficulties we would like to present the following tentative conclusions:

1. The four intermediate-dose Yugoslav victims probably received a higher radiation dose than the five Y-12 victims. This is based upon clinical data (early and profound epilation, eventual cataracts) as well as upon hematologic comparisons.

2. The recovery in the Yugoslav group occurred in about the same time as in the Y-12 group. If the radiation dose was higher for the Yugoslavs and recovery is normally later with a higher dose, then it might be argued that the marrow treatment had hastened recovery somewhat. In view of the uncertainties involved, it appears that the only strong evidence for function of the graft is in the red-cell identification studies reported by the French.

Attempted Autografts with Large Doses of Nitrogen Mustard. (A. L. Kretchmar, B. W. Sitterson and G. A. Andrews)

A clinical study, with a carefully controlled series of patients, has been completed in which autologous bone marrow grafts were attempted after a large single dose of nitrogen mustard had been given.

Patients in this series were selected without any knowledge of whether they would be given marrow or serve as controls. The selection was made on clinical grounds and, as far as possible, patients with metastases to bone marrow were excluded. After the decision to include a patient in the series, assignment to the "control" or "graft" group was made on the basis of a table of random numbers. The patients in the series were numbered in consecutive sequence and those numbered 2, 6, 7, and 10 were given an infusion of autologous bone marrow. One other patient who was given autologous marrow (and who had been treated before this method of selection of control subjects was instituted) was also included in the analysis of results.

One milligram per kilogram of nitrogen mustard was given to all patients. This dose was administered intravenously in 20 to 30 ml of saline over an interval of 10 to 15 minutes, including the time required for dissolving the nitrogen mustard and manipulating the infusion apparatus. This was done while the patient was still under general anesthesia and after bone marrow had been aspirated. The aspiration was for diagnostic purposes in the control group, but in the autograft group enough additional material was aspirated to obtain a total marrow volume of 140 to 150 ml. An interval of 4 to 6 hours separated the time of administration of mustard from the infusion of the marrow. During this interval the marrow suspension (diluted with 1/3 volume of citrate-salt solution) was kept in a plastic bag at room temperature. (In the first two trials the marrow was kept at refrigerator temperature until it was warmed to room temperature and immediately infused.)

There was no significant difference in the degree of depression of white blood cells and platelets, of the time required for beginning of marrow regeneration, and of the rate of regeneration between the control subjects and the group that were given autologous marrow transfusions. Regeneration of platelets was a little more rapid in one of the autologous-marrow group. There is no evidence that the infusion of marrow had any effect on the clinical course or on the degree of marrow depression that was induced by this large dose of mustard. We conclude that spontaneous regeneration of bone-marrow function after 1 mg/kilo of nitrogen mustard begins in the third week after the administration of the drug and is dramatic in its rapidity; normal levels of circulating white blood cells and platelets may be reached in the twenty-fourth to twenty-eighth day after mustard. The autologous

marrow graft does not effectively contribute to this regeneration. It may be that at larger mustard doses or with many times the number of autologous cells used in this series (4×10^6 to 1.3×10^9), a significant contribution from the autograft would be apparent.

Recent and contemplated studies relating to the marrow problem.

a) Low-dose total-body irradiation. We are giving single doses of 50 r to various hematologic disorders and are studying the effects on blood and bone marrow. We have treated only a few patients, but we believe that this type of study will help to show the radiosensitivity of various cell types and may yield other information on radiation effects. From the literature one can learn that many patients have been treated with total-body irradiation in this dose range, but variations in technique, time-dose relationships, and methods of study make comparison of the data difficult.

b) Effects of local irradiation to the spleen on "hypersplenic states." Dr. Comas, Dr. Nelson, and other members of our staff have been studying patients with various diseases believed to have some degree of hypersplenism. In some of these patients they have obtained red cell survival curves with chromium-51 before and after radiation to the spleen. The treatment to the spleen has been with a dose somewhat higher than is usually used for lymphomas or hematologic disorders.

c) Effects of local irradiation on marrow. By means of multiple aspirations as compared with similar marrow studies on areas not in the field of radiation, we have made preliminary study of the effects of local irradiation to bone marrow. This study should yield worth-while information on the mechanism of production of hematologic depression by radiation that does not include all the body's marrow. We expect comparison with total-body irradiation effects to be valuable.

BIOCHEMICAL STUDIES OF RADIATION EFFECTS

A. L. Kretchmar

Two systems, manual[1] and automatic,[2] involving the separation of amino acids by cation-exchange resin have been used.

Preliminary work with known solutions of amino acids indicated that recovery of material from these solutions was 100 per cent. The variability of routine analyses was 4 to 9 per cent. The greater variability was associated with experiments where the amounts of amino acids put on the column were below optimal levels. These experiments were done with the manual method.[1] Certain mechanical problems with the fraction collector and the automatic pipetting machine contributed to the variability. Because of this, a simple modification that reduced splattering was made in the funnel of the device for collecting fractions, and a commercially available all-glass pipetting machine was substituted for the original model, which had stainless steel valves.

The position, in the effluent diagram, of all the amino acids was pH and temperature sensitive so that these conditions had to be especially carefully controlled.

The automatic system of analysis, as performed by the Spinco Amino-Acid Analyzer, was found to have greater resolution than the fraction collector method and to greatly reduce the time required for an analysis. The resolving power of this apparatus was especially good when columns were poured with resin that was a mixture of particle sizes; this mixture was obtained by combining 20 per cent by volume of fraction B with 80 per cent of fraction C (resin particles separated hydraulically by the procedure of Hamilton).[1] These columns run at a higher pressure than the columns poured from resin-fraction C. For example, column 1B operated at 50 pounds pressure initially (compared to 43 pounds). This column, after 6 months of use, operated at 70 pounds pressure. The life of the columns seems, therefore, to be limited by this gradual build-up in operating pressure. The analytic capabilities of the column have not altered, but the buffer lines and associated couplers can not handle pressures of more than 75 to 80 pounds for the extended period of operation required.

Table 1 represents data collected on a standard amino acid mixture, over a period of 9 months. These standardizing runs are made

periodically to check the reagents and buffers.

Table 1

Recovery of Amino Acids from a Standard Solution (Effluent pH 3.25)

The figures represent calibration constants

Amino Acid	Average "C" constant	Standard Deviation
Aspartic Acid	19.2	0.6
Threonine	19.0	0.4
Serine	19.8	0.4
Proline	3.4	0.1
Glutamic Acid	19.1	0.6
Glycine	19.2	0.5
Alanine	19.4	0.6
Valine	18.5	0.6
1/2 Cystine	9.4	0.4

These check runs indicate that the analytic precision has been between 3 and 4 per cent, an improvement over the manual method.

In addition to these two analytical facilities, an automatic recording apparatus has been constructed that can perform, simultaneously, an analysis and a collection of the effluent in suitable fractional volumes. This equipment has been used as a second analytical instrument because of the heavy load of analytical work, but its capabilities are primarily designed for large sample-volume experiments where the effluent fractions can be used for isolation and for chemical identification of peaks in the analytical diagrams. When the apparatus is used in this type of study, 1/9 of the column effluent is split off and run through an automatic analyzer while the major portion is directed to a fraction collector and preserved in variable-sized fractions for further studies.

Daily Variation in Levels of Urinary Amino Acids and Variation due to Change in Protein and Caloric Intake.

Two subjects were maintained on selected diets for periods that varied between 1 and 3 weeks. The diets were designed to give information about amino acid excretion on low, intermediate, and high protein intake. The effect of a caloric excess and of a caloric deficit was also studied.

Table 2 summarizes the results for a metabolic period of 7 days. These data are derived from 7 consecutive 24-hour collections

beginning on the 8th day of dietary control. The body weight was essentially constant during the experiment. The protein intake was approximately 100 g per day and the caloric level was adequate.

TABLE 2

Daily Variation in Levels of Amino Acids Excreted in Urine
(Both subjects were on the same controlled diet)

Amino Acid	Subject A		Subject B	
	mg/day*	C.V.**	mg/day*	C.V.**
Histidine	94 ± 9	17	193 ± 13	15
Glycine	77 ± 3	11	65 ± 5	20
Glutamine	70 ± 3	11	130 ± 14	26
Taurine	38 ± 4	22	50 ± 6	28
1-Methyl Histidine	63 ± 2	6	64 ± 4	16
3-Methyl Histidine	52 ± 1	2	65 ± 3	11
Serine	30 ± 2	11	42 ± 3	19
Tyrosine	16 ± 1	12	21 ± 1	14
Alanine	12 ± 1	8	32 ± 3	19
Phenylalanine	12 ± 1	12	11 ± 1	24
Threonine	13 ± 1	12	22 ± 2	17
1/2 Cystine	4 ***		12 ± 1	21
Leucine	6		7 ***	
Isoleucine	4		5	
Cystathionine	3		4	
Valine	3		5	
Glutamic Acid	3		4	
Methionine	2		3	
Beta-aminoisobutyric acid	6		36 ± 2	11
Beta-alanine	0		3	

* mean of 7 consecutive 24-hour collections with standard error of the mean.

** $\frac{\sigma}{M}$ 100 = C.V.; this is percentage variation from the mean of a single determination.

*** Standard error and coefficient of variation was not calculated for these minor constituents since integration of the low peaks is obviously subject to error.

Biochemical individuality[3] is apparent in the significant differences in levels of excretion of a number of amino acids, even though the dietary sources of these acids were the same for both subjects. (The same amount and kind of protein were ingested by both subjects but subject A required fewer carbohydrate and fat calories.) This individuality is also expressed by a greater variability in the levels of excretion of subject B.

The variability in the amount of amino acids that are excreted (10 to 15 per cent by subject A, and 15 to 20 per cent by B) is several times the analytical error, and is assumed to represent fluctuation in endogenous metabolism, since the same daily menu was ingested during each of the days of the experiment.

The variation in excretion of taurine by both subjects is greatest. This is not so distinctive for subject B who also excretes glycine, glutamine, phenylalanine, and cystine with a variability of 20 per cent or more.

The closeness of regulation of the amount of each of the amino acids excreted is not, in general, a function of the amount per se. The coefficient of variation in the excretion of glycine and of alanine by subject A is similar, even though the amount of glycine is 6 times that of alanine. A similar example may be noted in the data for subject B, who excreted 9 times as much histidine as tyrosine with the same fluctuation of 15 per cent in daily levels.

The dietary intake definitely influences the levels of excretion of seven amino acids. These substances are listed in Table 3, together with excretion levels during several different dietary periods. The first three were consecutive periods of 7 days each. The 4th was 7 days, 3 months later, and the 5th and 6th were consecutive and of 7 days each but 3 months later than the 4th. The whole study, therefore, involved the same two subjects maintained on 4 different diets at intervals over approximately 6 months.

The excretion of 1-methyl histidine is dependent entirely on dietary intake (Table 3). This amino acid practically disappears from urine when the dietary intake of protein is very low, indicating that endogenous excretion is very slight or nonexistent.

Taurine excretion is definitely influenced by dietary protein intake but, in contrast to 1-methyl histidine, there is excretion from endogenous sources. The excretion during the first 100-gram-dietary-protein period is higher than during the second; this is also noted for the two 20-gram periods. This result suggests that there is some type of "buffering" in the system for taurine excretion and the levels of taurine in the urine do not rapidly follow fluctuations in dietary intake. The urinary excretion during periods of low protein intake is not greatly different from that when a moderate amount of protein is ingested. The catabolism of sulfur-containing amino acids would seem, therefore, to involve a certain minimal level of taurine formation and excretion.

Histidine excretion is influenced by dietary intake of protein and is affected in a way that is qualitatively similar to the effect of diet on taurine excretion. The remarkable feature in the data for

TABLE 3

EFFECT OF DIET ON EXCRETION OF AMINO ACIDS IN URINE. AVERAGE EXCRETION LEVELS ARE GIVEN IN mg/day.

Amino Acid	Subject A						Subject B					
	Dietary Protein Level						Dietary Protein Level					
	100 g	100 g	ad lib	180 g	* 20 g "x"	** 20 g "D"	100 g	100 g	ad lib	180 g	20 g "x"	20 g "D"
1-Methyl Histidine	49	63	49	46	trace	trace	68	64	71	518	trace	trace
Taurine	66	38	31	123	51	49	78	50	73	136	74	59
Histidine	82	94	107	131	69	57	224	193	211	270	193	155
3-Methyl Histidine	46	52	48	92	38	36	64	65	69	102	51	47
Glutamine	65	70	82	80	56	51	130	130	138	168	104	94
Beta-aminoisobutyric acid	6	-	4	13	trace	5	33	-	36	49	10	21
Beta-alanine	trace	trace	trace	trace	trace	trace	4	4	3	17	trace	trace

* This diet contained 20 g of protein and excess calories.
** This diet contained the same 20 g of protein but was deficient in calories.

this amino acid is the great but consistent difference in levels of excretion between the subjects. Despite similar changes in excretion that can be related to diet, subject B consistently excreted about 2 to 3 times as much histidine as A. This consistent difference in the level of excretion, despite variation in diet, is also apparent in the excretion of glutamine.

Excretion of 3-methyl histidine and beta-alanine varies with the level of intake of protein in the diet. The levels of excretion are slightly but consistently different between the two subjects.

The level of beta-aminoisobutyric acid is quite different in urine from these subjects. It seems probable that changes in dietary protein intake or in the type of foods ingested may influence to some extent the levels of excretion of this acid. The individuality of excretion is not changed, however.

A group of four amino acids is shown in Table 4. The levels of excretion of these acids varies in different periods and also is different for the two subjects. These differences are not so consistent nor so great as in the acids listed in Table 2.

Summary:

1. The general range of variation in the amino acids in urine collected during consecutive 24-hour intervals was 10 to 15 per cent for subject A and 15 to 20 per cent for subject B.

2. This variation is probably attributable to endogenous variations in metabolism since both subjects ingested the same amount and type of protein and this degree of variability is several times larger than the analytical error.

3. Variation in taurine excretion is greatest in both subjects and averages 25 per cent.

4. The variation in levels of excretion is not a function of the amount excreted.

5. The excretion of 1-methyl histidine is dependent upon an exogenous source and this substance practically disappears from the urine when a meat-free diet, low in protein, is ingested.

6. Taurine excretion can be correlated with the amount of protein in the diet but there is an endogenous source of excretion so that some is excreted even on a low-protein diet.

7. Histidine, 3-methyl histidine, glutamine, and beta-alanine levels are also affected by changes in dietary protein intake.

TABLE 4

EFFECT OF DIET ON EXCRETION OF AMINO ACIDS IN URINE. AVERAGE EXCRETION LEVELS ARE GIVEN IN mg/day.

Amino Acid	Subject A						Subject B					
	Dietary Protein Level						Dietary Protein Level					
	100 g	100 g	ad lib	180 g	20 g "X"	20 g "D"	100 g	100 g	ad lib	180 g	20 g "X"	20 g "D"
Glycine	77	77	110	94	84	70	68	65	74	88	74	56
Serine	29	30	36	35	23	23	42	42	47	56	30	27
Tyrosine	16	16	19	21	10	10	25	21	19	26	19	17
Phenylalanine	11	12	15	13	7	7	14	11	11	10	4	7

8. Beta-aminoisobutyric acid is excreted at different levels by these subjects on all diets. The influence of diet on the levels, in either one individual, however, is not great.

9. Excretion of glycine, serine, tyrosine, and phenylalanine shows rather small differences that are perhaps due to differences in dietary intake. These changes are not so definite as in the other amino acids.

10. The minor amino acid constituents in urine are excreted at levels that are not altered by the changes in diet included in this study.

Effect of Total-Body Irradiation on Amino Acid Excretion by Normal Men.

The accidental exposures to significant doses of total-body irradiation that occurred in Oak Ridge and the fatal exposure that occurred in Los Alamos have provided an opportunity to study the effect of irradiation on the amino acid excretion of normal men.

Table 5 summarizes the results of taurine analysis; all of these analyses were made by the column chromatographic technique.

TABLE 5

TAURINE EXCRETION IN URINE OF ADULT MALE HUMANS AFTER ACCIDENTAL EXPOSURE TO TOTAL-BODY IRRADIATION

Subject	TAURINE (mg/day)		
	Days after Exposure		
	1	4	dif. (4-1)
A (365 rads)	228	273	45
C (339 rads)	204	262	58
D (327 rads)	105	194	89
B (270 rads)	190	210	20
E (236 rads)	213	231	18
F (69 rads)	-	109	-

Total-body exposures to doses of 236 rads and above have induced an increased taurine excretion. In all five of these men the excretion on the 4th day is higher than on the 1st day after the accident. The increment of excretion between the 4th and 1st days is not very closely correlated to the dosage of irradiation, although those exposed to more than 300 rads have greater increments in taurine excretion than those below 300 rads. The exposure to only 69 rads has apparently not induced an elevation in taurine excretion.

Table 6 shows the effects of exposure on the excretion of beta-aminoisobutyric acid (BAIB). It is well known that levels of excretion of this substance vary among individuals, so that a change in excretion with time after exposure, and not any single determination, is the observation that might be correlated with the dose of total-body irradiation.

TABLE 6

BETA-AMINOISOBUTYRIC ACID EXCRETION IN URINE OF ADULT MALE HUMANS AFTER ACCIDENTAL EXPOSURE TO TOTAL-BODY IRRADIATION

Subject	BAIB (mg/day)		
	Days after Exposure		
	1	4	dif. (1-4)
A (365 rads)	74	55	19
C (339 rads)	38	18	20
D (327 rads)	42	30	12
B (270 rads)	9	4	5
E (236 rads)	48	-	-
F (69 rads)	-	14	-

The expected variation of levels among individuals is noted in column 3. In contrast to taurine, the excretion of beta-aminoisobutyric acid is highest on the 1st day after exposure and by the 4th day has returned to levels that are probably not much elevated. The time-course of excretion of BAIB has not, however, been completely studied by the column method as yet. The increment in BAIB excretion between the 1st and 4th days may be correlated more closely than taurine excretion to dosage of irradiation, but the range of exposures is so narrow that the usefulness of BAIB excretion as a biochemical dosimeter has not been tested in this accident.

The results of analyses of urine from the Los Alamos patient are summarized in Table 7.

This subject excreted an abnormal quantity and distribution of amino acids. Some regions of the chromatograms were altered by the presence of unknown compounds. In the region of methionine-isoleucine-leucine, beta-alanine, and beta-aminoisobutyric acid the presence of these unknowns made integration of the amino acid peaks impossible. An effort will be made to isolate and identify these substances.

TABLE 7

AMINO ACID EXCRETION BY LOS ALAMOS ACCIDENT VICTIM
C.K. Fatally Irradiated 12-31-58

Amino Acid	1st Spec. 14 hr after accident	2nd Spec. 17 hr 45 min after accident	Last Spec. 22 hr 25 min after accident	Total	Oak Ridge Subject** A (365 rads) First day
	mg	mg	mg	mg	mg/24 hr
Taurine	44	80	91	215	222(166)
Threonine	*	*	*	-	43(33)
Serine	1.3	1.6	2.5	5	62(63)
Glutamine	3.3	3.2	4.4	11	188(151)
Glutamic Acid	1.2	0.8	0.5	3	6 (4)
Glycine	2.6	2.6	3.4	9	186(175)
Alanine	2.6	3.0	3.0	9	55(42)
Valine	0.5	trace	0.2	-	9(11)
Cystine	6.8	5.5	7.8	20	47(19)
Cystathionine	0.2	0.2	*	-	8(14)
Methionine	*	*	*	-	5(4)
Isoleucine	*	*	*	-	*(*)
Leucine	*	*	*	-	10(10)
Tyrosine	1.1	0.7	1.2	3	35(36)
Phenylalanine	2.0	1.1	1.4	5	20(30)
Beta-aminoisobutyric acid	*	*	*	-	74(35)
1-Methyl histidine	0.1	1.3	1.8	3	13(2nd day)
Histidine	0.1	1.8	3.4	5	225(2nd day)
3-Methyl histidine	2.9	6.3	10.1	19	54(2nd day)

* Integration uncertain.

** Parentheses enclose average excretion 9 months after accident.

Despite the general reduction in amount of most of the amino acids, C.K. excreted more than 200 mg of taurine. Subject A from the Y-12 group also excreted taurine in this range. If it can be suggested that the general reduction in quantity of other amino acids affected the excretion of taurine also, then C.K. would have had very high excretion of taurine except for the impaired renal function. It is known that his blood urea-nitrogen was rising during the survival period so that there is good evidence of retention of nitrogenous substances.

Normal renal function produces a characteristic qualitative pattern of urinary amino acids. This is evidenced by the high excretion of glycine relative to alanine acid of tyrosine relative to phenylalanine. In circulating-blood plasma there is more alanine than glycine and the quantities of phenylalanine and tyrosine are about equal. In addition, the normal kidney retains valine, isoleucine, leucine, lysine, and arginine; these are prominent components of the plasma amino acids, but are minor urinary constituents. This characteristic qualitative pattern is absent from C.K.'s urine. Glycine and alanine were excreted at equal levels, and phenylalanine predominated over tyrosine. Cystine and 3-methyl histidine were relatively prominent and together with taurine were the major constituents. There was retention of glutamine, glycine, and histidine, usually major constituents in urine.

The Y-12 subject shows definite elevation of urinary taurine, cystine, and beta-aminoisobutyric acid. Glutamine and glycine levels are variable so that the significance of the elevation of these constituents and of threonine and alanine is doubtful. The low level of excretion of 1-methyl histidine suggests that the intake of meat was low and, therefore, the elevated excretion of taurine cannot be ascribed to a dietary change.

In our experience, total-body irradiation does not induce a generalized amino-aciduria. Rather, certain specific alterations may lead, when they are understood, to a more complete picture of the mechanisms by which irradiation induces metabolic damage in man.

A Comparison of the Effects on Taurine and Beta-aminoisobutyric Acid Excretion of Total-Body Irradiation with the Effect of a Large Dose of Nitrogen Mustard.

The increased excretion of taurine and beta-aminoisobutyric acid by normal human beings after sublethal and lethal irradiation has been discussed in the previous section. It is of interest, also, to consider the changes that occur in patients with leukemia after total-body irradiation and in patients with a variety of malignancies after a large, single, intravenous dose of nitrogen mustard. The data from these groups are particularly interesting since samples can be collected before irradiation or administration of mustard and base-line data can be obtained.

A beginning has been made in this work and data from two patients are summarized in Table 8.

TABLE 8

EFFECT OF TOTAL-BODY IRRADIATION OR NITROGEN MUSTARD ON TAURINE AND BAIB EXCRETION

Patient, disease, and treatment	Days relative to treatment	Amino acid and Excretion level, mg/day Taurine	BAIB
810936, acute leukemia	Before	36	9
800 r TBI	After - 1	47	110
	2	31	119
	4	105	25
910958, bronchogenic	Before	103	8
carcinoma, 1 mg/kg	After - 1	420	62
nitrogen mustard	4	305	6

Eight hundred r total-body irradiation with gamma rays from cobalt-60 induced a 13-fold increase in beta-aminoisobutyric acid excretion. This effect was prompt and short-lived. The effect on taurine excretion was also prominent, approximately a 2.5-fold increase. The peak elevation in taurine excretion appears later than that for beta-aminoisobutyric acid. This delayed taurine peak also occurred in subject A of the Y-12 group.

One milligram per kilogram of nitrogen mustard produced a prompt, 8-fold increase in beta-aminoisobutyric acid. This effect had completely subsided by the 4th day after treatment. The effect on taurine excretion was also prompt. An increase of 4 times occurred on the 1st day after treatment. The taurine excretion was still 3 times the pretreatment level on the 4th day after mustard therapy.

This work is not sufficiently far along to permit generalizations, except that the radiation-induced increase in taurine and beta-aminoisobutyric acid, occurring in normal, previously healthy men, has also been induced in a patient with acute leukemia and by nitrogen mustard therapy. In all the conditions so far studied, there has been evidence of severe bone-marrow depression. The drop in peripheral blood cells occurred sooner in the patient with leukemia and in the patient given nitrogen mustard than in the Y-12 accident victims. The promptness of the increase in beta-aminoisobutyric acid was similar in all three groups, however. There may be a difference in the effect of mustard and irradiation on taurine metabolism, although the data suggesting this are very limited.

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Separation of thiocyanate from body fluids. (D. S. Allen and B. L. Byrd)*

Thiocyanates are normal constituents of saliva, blood plasma, and urine. By far the commonest quantitative method of determination has been the colorimetric measurement of the ferric thiocyanate complex. Despite the fact that many constituents of these body fluids are known to interfere in the color development of this complex (including such substances as iodides, hydroxy acids, and trichloroacetic acid), many investigators have merely removed proteins by means of trichloroacetic acid, added ferric ion, and measured the ferric "thiocyanate" complex colorimetrically. The wide variation in the reported levels of thiocyanates in body fluids suggested that a method of removing interfering substances before developing the color of the complex might be a significant step forward in quantitative determinations of the thiocyanates.

This investigation has explored the possibility of using ion exchange resins for separating thiocyanates from substances that interfere with color development in the ferric thiocyanate complex. Thiocyanates are quantitatively removed from a solution by an ion exchange resin, then complexed off the column by means of a ferric nitrate solution. The color must be developed in artificial light and measured colorimetrically within 15 minutes. Several methods of inhibiting color fading have been tested. Optimum conditions for removal of thiocyanates from the ion-exchange column have been worked out.

Liquid scintillation counting of labeled thiocyanate. (D. S. Allen and B. L. Byrd).

Beta counting of sulfur-35-labeled potassium thiocyanate (KCNS) would be much simplified if the liquid-scintillation method could be used. To determine the feasibility of this method it was of interest to know whether aqueous solutions of KCNS would appreciably quench a xylene-dioxane-naphthalene scintillator. Sulfur-35-labeled SO_4 was used as the activity and varying amounts of aqueous KCNS were added. The average counting efficiency of five sulfur-35 samples was 7.4 ± 0.1 per cent. There was a progressive decrease in efficiency of counting with increase in amount of KCNS. Two hundred thirty-five micrograms of KCNS in 50 ml of liquid scintillator were counted with a 6.1 per cent efficiency.

Dr. Kretchmar, and Dr. Kurt Kraus, Oak Ridge National Laboratory, have assisted in this work. Dr. Allen did this work during his stay as research scientist with the Special Training Division. He has now returned to the Department of Chemistry, New York State College for Teachers in Albany.

* Although these studies do not involve radiation effects, they are included here as a part of the long-range program in biochemical research.

RESEARCH WITH INTERNAL ISOTOPES

The Clinical Staff

Scanning

The clinical staff of the Medical Division has continued to take an active interest in problems of scanning. Our chief emphasis has been on a close correlation between scans made in the same patient with varying types of equipment. Every effort is made to obtain direct information on the isotope distribution by assay and autoradiograms of tissues obtained at operation and autopsy, and to correlate these findings with the information on the scans. During the past year distinct advances have been made in the equipment available and in the knowledge of interpretation.

The linear scanner in clinical studies. The ORINS linear scanner (described elsewhere) has been given clinical trial by Felix J. Pircher, and others, with the following isotopes: arsenic-74, gold-198, chromium-51, copper-64, iron-59, iodine-131, iodinated human serum albumin I^{131}, rose bengal I^{131}, diodrast I^{131}, phosphorus-32, and yttrium-90. Doses varied from 0.04 microcurie to 157.3 microcuries. The scanner is an excellent instrument for clinical investigations, and its usefulness is beyond doubt for the following reasons:

1. The procedure for obtaining the record is relatively simple and of no inconvenience to the patient. It takes only ten minutes for one scan.

2. The scanner aids in understanding the kinetics of isotope distribution. The site of the isotope in various phases of distribution can be studied efficiently.

3. It is useful for accurate localization and definition of an isotope within the body, a frequent clinical demand. This is well demonstrated in scans with gold-198 intrapleurally, and iodine-131 in patients with thyroid cancer.

4. It provides semiquantitative information.

5. This technique for determining isotope distribution could be used to supplement the information obtained from a whole-body counter, if one were available here.

6. Localization of metastases (as in thyroid carcinoma) offers information in one dimension only, and this in connection with area scanners can lead to precise localization. Here the linear scan shortens the time needed for area scanning.

7. The instrument has value in measuring the uptake of high-level doses as in the treatment of thyroid carcinoma. In these situations other instruments often are too sensitive and cannot be used.

8. Pure beta emitters such as yttrium-90 and phosphorus-32 can be detected and observed by measuring the Bremsstrahlung.

Autoradiographic studies of distribution of isotopes used for liver scanning. Studies of local metastases in the liver by external scanning techniques, done here and elsewhere, have been hampered by lack of direct information on the location and distribution of radioisotopes in the tumor and in the normal liver tissue. Without direct knowledge of the actual distribution of the radioisotope it is very difficult to evaluate problems of sensitivity and efficiency of scanning techniques. Sometimes operative procedures and autopsies have yielded precise information about the location of the metastases, and this can be correlated with the scans. Such information is not available in any large number of cases and does not give much help in comparing the distribution of various radioisotopes. During the past few months Dr. Frank Oda and others in the Medical Division have been carrying on a study in rats in which liver tumors have been produced. The various radioisotope preparations that might be useful for liver scanning are injected and the distribution is determined by autoradiogram and tissue assay. Radioactive colloidal gold and iodine-131-labeled rose bengal show an almost complete absence of concentration in the liver tumors, so that the latter can be seen as negative areas in autoradiograms. Iodine-131-labeled albumin produces a much more variable distribution, and the results in this phase of the study are incomplete. The iodine-131 rose bengal passes through the liver rapidly. This has the advantage of producing a low radiation dose but leaves hardly enough time for scanning. Correlated with this investigation are further studies on distribution of these materials in patients with liver lesions.

Radioiodine

Iodine-132 studies in thyroid carcinoma. Flora Pascasio, B. W. Sitterson, and G. A. Andrews have been using radioactive iodine to treat metastatic carcinoma of the thyroid in patients who have undergone total thyroidectomy. Several measures, thought to influence favorably the uptake of iodine by the malignant tissue, are in general use. Little information is available, however, from definite studies concerning the effectiveness of these measures in humans.

Iodine-132, with a half life of 2.3 hours, should permit repeated uptake determinations within a relatively short time. Thus the effect of various measures on iodine uptake could be determined on the same tumor mass in one patient with elimination of variables arising from long-time intervals between uptake studies. Preliminary work using iodine-132 to determine the effect of withholding thyroid medication, giving exogenous thyroid stimulating hormone, and prior administration and omission of antithyroid drugs has been done in two patients with suitably large and isolated tumor masses. Standard thyroid uptake technique, with a B filter to shield the tumor mass and obtain body background, was used. The dose of iodine-132 for each uptake determination was one millicurie.

Results indicate that using the standard thyroid uptake technique may not be feasible. Because of variation in size and shape of tumor masses, use of the B filter does not appear to give reliable body-background counts and some other way of determining this will be necessary. Also some reasonably accurate method of determining the depth of tumor masses within the body is needed to arrive at the proper distance between skin and the detector. In the two patients studied, the counts obtained at various time intervals after the dose seem to indicate that by the time the tumor has reached its peak uptake the isotope has already decayed to such low levels of activity that counting is difficult. Consequently it may be necessary to use larger doses of iodine-132 or another isotope of iodine with a somewhat longer half life.

Long-range study of carcinoma of the thyroid. We have studied a considerable number of patients with carcinoma of the thyroid. Further follow-up and additional cases have been obtained in the group with small areas of functioning thyroid tissue (either tumor or normal thyroid) left in the neck after attempted total thyroidectomy for carcinoma. Results continue to indicate the value of complete thyroidectomy and use of rather large test doses of iodine-131 to locate metastases.

Treatment of hyperthyroidism and effects of iodine-131 on thyroid tissue. The clinical staff has treated a small number of cases of hyperthyroidism and obtained some further information on histologic effects in the thyroid gland. We are also beginning to study surgical

biopsy of thyroid glands treated with iodine-131.

Carcinoma of the ovary, effusions, and colloidal radioisotopes.

We have treated a small number of patients with effusions during the year and have obtained some further information on the comparison of colloidal gold-198 and yttrium-90. A small amount of carrier (in the range of 25 mg), along with the therapeutic dose of yttrium-90, appeared adequate to ensure localization of the radioisotope, and apparently cut down on the adverse reactions to the stable yttrium. We have seen several cases of carcinoma of the ovary and have continued the long-range study on combined therapy with repeated surgery and intraperitoneal gold-198.

Hematologic Disorders.

An increasing emphasis on hematology characterized the year. This was largely related to the program in radiation effects and an increased number of patients with acute leukemia and other blood disorders. In addition to the data already mentioned, we have obtained valuable information on long-range follow-up of patients with polycythemia. Examples of myeloproliferative transitions are becoming more common in our series of cases. In these patients studies with iron-59 and chromium-51 are quite puzzling. Splenectomy seems occasionally to be indicated, but the criteria for recommending it are far from clear.

Calcium-47 studies in patients.

With the receipt of the first available calcium-47, Dr. Flora Pascasio and members of the staff studied the metabolism of calcium-47 in four patients. Two received oral doses of 0.190 millicurie; two received intravenous doses of 0.095 millicurie. Fecal and urinary excretions were determined and blood levels were obtained. Daily linear scans were taken for 12 days and it was possible to obtain satisfactory external counts, in great contrast to the situation with calcium-45, a pure beta emitter. The patients were on a low calcium diet two days before and during the 12 days of the procedure. No stable calcium was added to the activity. At the time of administration, the specific activity was about 0.24 millicurie per milligram. The calcium was given as calcium chloride. The ratio of calcium-47 to calcium-45 was approximately 2:1.

The following summarizes the observations:

Plasma levels:

Oral doses: Peak of activity appeared 1 to 3 hours after administration. Significant activity was detected at 30 minutes.

Intravenous doses: Peak activity appeared at 2 minutes. At the 7th to 8th day the plasma levels were similar in both groups.

Urinary Excretion:

Peak excretion was in the first 24 hours for both groups, 5 per cent in patients with intravenous doses, 3 per cent in those with oral doses. During the 12 day period, peak excretion was 13 to 18 per cent of total activity given for intravenous doses, and 5 and 12 per cent for oral doses.

Fecal Excretion:

Total: Oral: 40 per cent of dose in one patient.
Intravenous: 10 to 12 per cent of dose.

Linear Scans:

With oral doses, the intestinal activity was apparent up to three and four days. The later oral-dose scans and the intravenous-dose scans showed a profile consistent with general skeletal deposition. The largest activity was over the thorax and abdomen with small peaks at knee and ankle.

CLINICAL TELETHERAPY

F. Comas

The volume of patients in the clinical teletherapy program increased slightly over 1958. Nine hundred treatments were given to 64 new patients. (This includes localized and total-body irradiation). About half the treated patients suffered from lymphomas of several types. Special interest was centered on patients with carcinoma of the urinary bladder, forming part of a long-range study on management of this disease with radiation therapy. This is preceded by an exploratory laparotomy to accurately map out the extent of the primary tumor and regional lymph-node metastases, in an effort to minimize "geographical misses." Among the patients so far treated, two have shown tumor persistence at the primary site, in spite of vigorous irradiation. The third has completed therapy too soon to be evaluated.

PRECLINICAL RESEARCH

Granvil C. Kyker

Programmatic activities during 1959 dealt largely with experimental studies and partly with training. The facilities for experimental work experienced both some enlargement and improvement; a new building occupied a year ago increases laboratory space by a third or more. Important instrumentation was added during early 1959. The group has remained the same; the improved laboratory facilities have, however, greatly enhanced accomplishments.

Summaries of experimental work are presented under three main areas: 1) New Isotopes and Methods, 2) Rare-Metals Metabolism, and 3) Biochemical Studies. The second and third areas developed progressively out of the first, which began about seven years ago to evaluate certain rare-earth radioisotopes selected for their potential medical usefulness. The second area evolved a few years later when the need arose to define the toxic and metabolic effects of carrier, which had become a major influencing factor on internal radioisotopic patterns. The acute hepatic lesion, first observed about three years ago during the course of study in the second area, opened the third. During 1959, as well as the year before, the area of biochemical studies has developed to comprise half or more of the total effort. Efforts in this third area have gone almost totally to the characterization of numerous influencing factors and the mechanism of the acute fatty infiltration that occurs in liver after intravenous treatment with any one of various rare earths in the cerium group of the series. The summaries record considerable progress toward an understanding of this prominent hepatic disturbance.

The group plan of a regular weekly conference that began a few years ago has improved further and provides more efficiency in the critical review of the latest results and is projecting experimental plans. Summaries of these work sessions are distributed weekly and accrue in a topical classification. A register of experimental animals consumed during 1959 gives another index to the scope of work; these include 1263 rats, 64 dogs, and 239 of miscellaneous species (mice, hamsters, rabbits, guinea pigs, chickens, and pigs), or a total of 1566 for all species.

NEW ISOTOPES AND METHODS

Calcium-47 and linear scanning in rats. (Granvil C. Kyker and John J. Rafter)

A small shipment of calcium-47 became available to the Medical Division during the latter part of 1959 (from Abbott Laboratories, Oak Ridge). This isotope is of greatest interest for various clinical uses. A portion of the shipment was allocated for animal studies. Calcium-47 (half life, 4.7 days; beta emissions, 0.7 and 2.0 Mev; gamma emission, 1.3 Mev) yields a daughter, scandium-47 (half life, 3.4 days; beta emission, 0.46 and 0.60 Mev; gamma emission, 0.150 Mev). Besides, this preparation was reactor-produced from enriched calcium-46, and contained calcium-45 produced from calcium-44, which was prominent in the enriched material. Other radiochemical contamination was unknown at delivery, but the producer later discovered the presence of certain rare-earth radioisotopes (lutetium-177 and probably others).

We undertook two kinds of measurements. One consisted of extensive beta counting under calibrated conditions through several half-life intervals. From this effort, one must conclude that beta counting is not adequate for definitive interpretation. A spectral gamma detector is necessary.

Our other experimental effort was by use of the small-animal linear scanner (ORINS-23, p. 23; ORINS-27, p. 13). The isotope was given to rats by intravenous, intraperitoneal, intramuscular, and subcutaneous routes. The injected animals were scanned at seven intervals between 0 and 15 days; the first four scans came within the first day to define any early changes in the linear pattern of distribution. The intravenous dose showed an early profile resembling the body projection and then gradually developed a peak over the pelvic area during the first day. The scans of animals injected by the other routes (interstitial or intracavitary) showed a sharp peak over the site of administration, which was gradually mobilized to develop a pattern resembling that of the intravenous route.

These preliminary results were obtained with relatively large doses (15 to 18 microcuries per rat; about 75 microcuries per kg) to enable scanning through several half-life intervals. The radiochemical contamination, which later came to light, imposes some limitation on

final interpretation. The present results do, however, afford another excellent example of the value of the small-animal scanner to rapidly appraise the internal pattern of a new isotope. A second shipment of calcium-47, free of other elemental contamination, is scheduled early in 1960. We plan to repeat these measurements before further interpretation.

Distribution of a radioactive colloidal yttrium in rats. (John J. Rafter and Granvil C. Kyker)

The use of nondialyzable yttrium-gelatin-phosphate by six routes of administration in rats appeared in the summary of last year (ORINS-27, p. 7). Low absorption of oral doses and poor mobility of parenteral doses characterized the pattern of results, but with more variations than we saw with inorganic yttrium preparations in earlier studies. Also, localization of intravenous doses in liver was quite high by comparison with similar doses of the element as yttrium chloride.

The studies have been continued with two preparations of yttrium-gelatin-phosphate (carrier-free, less than 10^{-11} Moles/kg; and carried-added, more than 10^{-6} Moles/kg) to compare the effect of elemental dose by the intramuscular and intrapleural routes. The resulting patterns of distribution showed some distinct differences by comparison with previous studies of inorganic preparations. The immobilizing effect of added carrier established in general for rare-earth chlorides appeared qualitatively similar for the colloid administered intramuscularly. Another effect existed for both dose levels; the lower segments of the injected leg (intramuscular, upper segment of hind leg) contained an unusually large part of the dose. Evidence of significant drainage of an interstitial dose has not appeared in the previous scope of rare-earth studies; no circulatory or metabolic factors are known to afford an explanation.

The large difference in carrier dose had little effect on intrapleural immobilization. This behavior is quite difficult to correlate with that of inorganic preparations (yttrium chloride) or with the effect of complexing with various agents, as studied elsewhere. A possible interpretation would be the assumption that yttrium-gelatin-phosphate is partially degraded *in vivo* (to a different degree by different sites) yielding a fraction of yttrium analagous to an inorganic dose and leaving a fraction in the protein-complexed aggregate. The projection of medical applications based on these results in animals is not immediately apparent, but this atypical behavior for yttrium is noteworthy.

Selective irradiation by the intralymphatic injection of radioisotopes. (James Meyer,* John J. Rafter, Gould Andrews, and Granvil C. Kyker)

Successful selective localization of a radioisotope within a specific organ or system remains unchallenged as an ideal way to deliver therapeutic radiation. This goal appeared early in the development of nuclear medicine; and the only problem remaining is that it has not been accomplished by natural processes (except for radioiodine in thyroid) or by simple procedure. Three kinds of poorly mobilized radioactive preparations (which include radioactive colloidal gold, salt solutions of rare-earth radioisotopes, and inert suspensions) have found certain special therapeutic uses, mainly within major cavities. There is, however, no way of directing the radiation to a specific site inside the cavity.

The introduction of radioactive preparations into the lymphatic drainage of an area offers intriguing possibilities. Earlier related efforts (ORINS-16, pp. 13-14; ORINS-23, p. 19) dealt with lymphatic localization of rare earths after interstitial administration (intramammary route in dogs). The present effort deals with a comparison of radioactive colloidal gold (Aurcoloid, Abbott Laboratories), radioyttrium, and radiocerium after their injection into a lymphatic vessel in the hind foot of a dog. The yttrium and cerium were used as weakly acid solutions of the chloride salt. The intralymphatic injection was made about 10 minutes after an interstitial injection of a dye (Direct Sky Blue, 4 per cent, Wyeth Laboratories, Inc.) between the toes. The dye clearly delineates the lymph vessels, which are otherwise very difficult to isolate. Sixteen dogs were studied. Twelve of these were divided into three groups and examined at 1, 3, and 7 days after injecting either radiogold, or radioyttrium with a low, medium, or high level of added carrier. Two others received radiogold with a tenfold difference in the injected volume and were examined at 5 days. The remaining two were examined after longer intervals: yttrium at 11 days and cerium at 27 days. Complete distribution was not measured but attention was largely to blood, liver, spleen, and lymph nodes, including popliteal, inguinal, mesenteric, iliac, periaortal, renal, and mediastinal nodes.

Yttrium and cerium behaved quite similarly. The ratios of radioactivity in nodes on the injected side to the opposite side were quite large, usually several hundredfold. Also, the radioactivity in the small amount of nodal tissue exceeded several times the total in the liver and spleen combined; on a basis of unit weight localization in liver greatly exceed that in spleen (9 out of 11, more than 10 times).

Gold gave similar ratios for nodal localization on the injected and opposite sides and for liver and spleen but showed some other distinct differences. For example, the combined radioactivity in liver and spleen exceeded that in the nodes.

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The two dogs examined at 5 days after receiving gold in doses that differed greatly in volume (0.5 ml and 5.0 ml) showed distinct differences; relatively, the larger dose resembled in some respects an intravenous dose with lower nodal deposition and a high blood level immediately after injection. These two dogs were among the first that were examined and this effect of volume was recognized in all subsequent injections so that the doses were between 0.05 and 0.1 ml per kg. On the other hand, the dog that received cerium was injected with 5.0 ml, similar to one of the doses of gold, and the pattern resembled those observed for yttrium.

Although several animals were examined in considerable detail, the experiment included several factors that were varied. The results are, therefore, preliminary and contribute more suggestions than conclusions. Further evaluation suggests the possibility of the selective destruction of a specified portion of the lymphatic drainage by the use of internal emitters. Photographic evidence indicates that the lymph vessels in other areas are similarly delineated by the dye, although one of the extremities may be a more convenient site for its use.

Preliminary radioisotopic studies with an artificial kidney. (Asri Rasad*, Granvil C. Kyker, Fred Snyder, and Marshall Brucer)

The purpose of this study is to determine the usefulness of the artificial kidney as a tool for studying the internal behavior of various radioisotopes and to evaluate its potentiality for internal decontamination. The first stage of this work is a determination of the response of the standard coil in the artificial kidney to various in vitro systems. In preliminary studies of this stage, the rates of removal of sodium-22, of iodine-131, and of cerium-144 have been measured. Equilibrium between the plasma compartment was reached at about 2 hours for sodium-22, at about 4 hours for iodine-131, and at about 5 hours for cerium-144. The curves representing the specific radioactivity in the plasma and the filtrate compartments were normalized to intersect when 50 per cent of the substance is removed from the plasma compartment. This intersection point for sodium-22, iodine-131, and cerium-144 was observed at 15, 30, and 50 minutes, respectively, when the dialysis was carried out in a physiological saline system. The solution in the plasma compartment and its pH affect the filtration rate, especially for cerium. Other factors receiving attention in the in vitro measurements include the effect of pumping rate, concentration of chemicals, and chelating agents. After further standardization, the hemodialysis of various normal and abnormal plasma constituents of medical interest will be evaluated with radioactive tracers and laboratory animals.

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Paper electrophoretic studies of serum (Granvil C. Kyker and Lois A. Gerst)

Extensive data have accumulated in the course of applying paper electrophoresis to the estimation of protein fractions in a variety of sera. A large part of the material has accrued during the past year. The general application of this method has been mainly that of a service to other studies to show distinct changes in sera or to provide supportive evidence in both hospital patients and experimental animals. The material is therefore heterogeneous and includes differences in the nature and state of disease, the species and strain of animals, radiation therapy, dietary stress, chemical treatment, methodology, and other factors. The accumulation, however, now affords a number of homogeneous groups adequate for comparative analysis. Much effort has gone to an organization of the data according to homogeneous groups, but conclusions cannot be drawn now.

A liquid scintillation technique for extracting and counting a labeled fatty acid in the presence of protein. (Fred Snyder and Edgar A. Cress)

A simple method for extracting a labeled fatty acid from a protein solution has been developed for a liquid scintillation counting system. The advantage of this method is that the extraction procedure is accomplished in the counting vial in the presence of the scintillation mixture. Essentially, the method consists of diluting the protein to a 1 per cent level. An aliquot of the 1 per cent protein solution is then added to a liquid scintillation counting vial, which contains 5 ml of toluene-phosphor cocktail plus 1 ml 1 N $HClO_4$. The protein is precipitated by the acid, and the labeled fatty acid is quantitatively extracted into the cocktail for counting. No quenching is observed in this system unless red blood cells are present. The method described has been applied to measuring the uptake of labeled fatty acids by liver in perfusion experiments.

RARE-METALS METABOLISM

Cerium and fission product uptake by various microorganisms. (G. T. Johnson* and Granvil C. Kyker)

The uptake of cerium and of mixed fission products by growing cultures of microorganisms was surveyed with selected bacteria, yeasts, and fungi. The study stemmed from questions of biological and medical interest. It is of biological interest that no evidence is known to implicate cerium or any other rare earth in a specific metabolic role. In a negative sense recent observations indicate that these elements can upset normal metabolism in an acute manner (Snyder, Cress, and Kyker, J. Lipid Research 1, 125-131, 1959). An evaluation of the response to a variety of microorganisms appeared to be the most efficient approach to compare metabolic possibilities in a scope of different cellular systems. In a medical sense, uptake of radioactivity by microorganisms relates to the practical problem of protection. The rare earths are a prominent part of fission products, ranging from one fourth to one half of the radioactivity at various stages of decay, and cerium was used as a convenient index of rare-earth behavior because of the close chemical similarity among the elements of this series.

The results were obtained by measurement of the radioactive tracer that remained in the growth medium after harvesting the organisms. The uptakes of cerium and mixed fission products by eight bacteria were compared at 24, 48, and 96 hours. Escherichia coli was the most active, removing more than 90 and 60 per cent, respectively, of cerium and fission product tracers in 24 hours. Proteus vulgaris was the least active, removing about 20 per cent of each in 96 hours. Six other bacteria ranged randomly between these extremes. Arranged according to degree of uptake, the eight bacteria appeared in the same order both for cerium and for fission products. Almost without exception the uptake of cerium was greater than the uptake of mixed fission products. This difference is opposite to that which might have been predicted because of the very complex elemental composition of fission products, some components of which are required by biological function. The observation that the uptake of cerium exceeds the combined uptake of the mixture of elements in fission products points to some specific relationship that remains to be explained.

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The general conclusions drawn from the results with bacteria are qualitatively the same as those that were also observed for four yeasts and nine fungi. A correlation appears to exist between the rate of uptake and the rate of growth, and preliminary evidence suggests that the uptake measurements reflect incorporation rather than absorption. These evidences appear indicative of metabolic involvement.

The excretion of yttrium and cerium in dogs. (Granvil C. Kyker and John J. Rafter)

Measurements have continued on the excretory pattern of yttrium and cerium in dogs included in the series that began much earlier. The method and results with various doses of yttrium were summarized previously (ORINS-23, p. 18) and described further by additional results for cerium with carrier (ORINS-27; p. 7). A delay interrupted the study until radiocerium of higher specific activity became available. The special cerium was first received from ORNL late in 1959; it was not a carrier-free preparation in the classical sense but enabled doses that differed from carrier-free yttrium by only about one order of magnitude.

The complete series in 6-hour terminal experiments confirm earlier preliminary summaries that the fecal excretion of intravenous yttrium or cerium is not by way of bile as might be expected from the prominent localization in liver and generally poor deposition in major soft tissues. This regularly occurred for the various dose levels of both elements. On the other hand, all segments of the gastrointestinal tract ligated at several levels indicated direct excretion by the prominent appearance of the tracer both in the tissues and in the contents of the tract.

A parallel relationship of increased dose and increased ratio of fecal to urinary excretion has regularly appeared in our measurement of distribution of these and other rare earths in small animals. The possible importance of biliary excretion on fecal excretion at later stages is not revealed by the 6-hour series of surgically prepared dogs. Other dogs have received similar injections and remained intact for study at 1, 7, 30, and 180 days. The study of the 180-day animals is in progress. Results from the other periods have revealed no interval of biliary excretion and continue to show gastrointestinal localization. The longest period of study should also permit some evaluation of the biological half time in liver and other main sites of early deposition.

Localization of intravenous cerium in fatty liver.* (Granvil C. Kyker, John Rafter, Nelson Stephens, and Fred Snyder)

The acute fatty liver that occurs in rats after intravenous injection of cerium, or certain other rare earths, reaches its peak in 2 to 3 days and returns to normal within a week. Also, prominent localization in liver is well established for these elements. The route of administration and the level of dose are prominent influencing factors. Published information failed, however, to define an internal pattern for cerium administered intravenously at a level that causes the acute accumulation of lipids in liver.

Using tracer radiocerium-144, we studied three levels of the element in small groups of Carworth-Farm-Nelson female rats at six experimental periods ranging from 4 hours to 12 days. Both the amount of cerium and of total lipids in liver were measured and correlated. Some groups were included in the study of distribution to compare the effect of fasting, hypophysectomy, and repeated daily doses of cerium. The measurements show distinct patterns for the content of cerium and of lipids in liver as a function of time. The prominent localization of a single dose of cerium in liver approaches its maximum during the first four hours and remains essentially unchanged through 12 days. The localization of successive daily doses of cerium increases progressively through the fourth day. The results suggest also that the cycle of fatty liver response regularly seen after a single dose is affected little by the subsequent doses.

The conditions for maximal production of fatty livers after a single dose were repeated weekly through five successive weeks. According to the time function study, the liver would have returned to normal before giving the next successive weekly dose. The tabulated summary shows that the localization of the cerium continues to about the same degree week after week. The fatty livers continue to recur also, but to a slightly reduced degree.

* Presented at Annual Meeting of Southeastern Section of Society for Experimental Biology and Medicine, Charleston, South Carolina, October 29-31, 1959.

Localization of Repeated Weekly Doses of Cerium in Liver and the Level of Total Liver Lipids (4 CFN Females/Group - Fasted 24 hours) 2 mg Ce/kg rat.

Weekly procedure:

Tu	W	Th	F	S	Su	M	Tu	W	Th
0	1	2	3	4	5	6	7	8	9
x							*	f	k

Number of Doses	Experimental Weeks					
	0	1	2	3	4	5
1	*(72)[a] (13.5)[b]					
2	x	*(70.2)[a] (10.9)[b]				
3	x	x	*(66.5)[a] (12.5)[b]			
4	x	x	x	*(77.8)[a] (8.2)[b]		
5	x	x	x	x	*(75.6)[a] (7.54)[b]	
6	x	x	x	x	x	*(72.3)[a] (10.0)[b]

x, Intravenous cerium (as chloride), 2 mg/kg body weight.
*, Radiocerium tracer included in dose, x.
f, Began fasting 24 hours before killing.
k, Kill and collect liver specimens for [a]cerium in liver, percentage of dose; [b]total lipids in liver, percentage of fresh weight.

The reactivity of rare earths with biochemicals. (Granvil C. Kyker, Lois A. Gerst, and Fred Snyder)

A definite explanation of the internal behavior of rare earths is now largely a matter of speculation. Knowledge of the similar chemical properties of these elements and of the physicochemical nature of blood enables a safe prediction that an initial hydrolysis will occur to yield an insoluble product when, for example, a solution of a rare-earth salt is injected intravenously. On the other hand, only meager information is available to describe equilibria that must operate to explain the gross pattern of internal behavior and localization that develops.

We could find only one report in the literature dealing with the interaction of heavy metals and biochemicals that included examples of the rare earths in a qualitative survey of considerable biochemical scope (Neuberg and Grauer, Biochim. Biophys. Acta, 12, 265-72, 1953.) The results depend on precipitation of metal salt solutions by numerous biochemical acids prepared in general as 0.1 or 0.01 M solutions of the sodium or potassium salt. Practically all of fifty or more reagents gave precipitation. A question arose concerning the interpretation of the authors that precipitation signifies reaction. The reagent salts hydrolyze to give alkaline solutions, which in turn should yield insoluble metal hydroxides. Our experience in various animal studies suggests conversely that the internal environment stabilizes rare earths under conditions of instability for aqueous solutions.

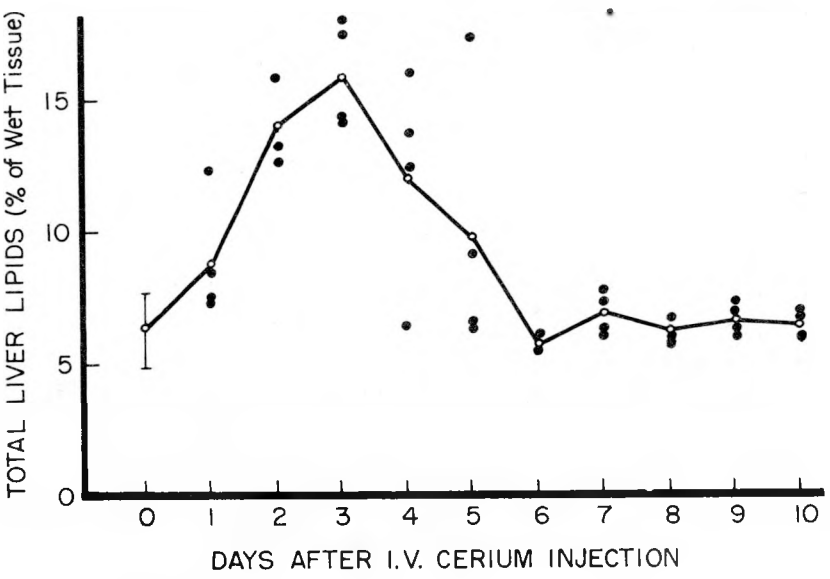
We have undertaken to design procedures to measure the extent of aggregation or stabilization of cerium by selected biochemical reagents. Conditions of concentration, temperature, pH, equilibration, and centrifugation are standardized and the fraction of tracer cerium that remains in the supernatant is measured. One unstable system (acetate) in which the metal precipitates at pH 7.5 is used to measure soluble complex formation and the other stable system (citrate) is used to measure precipitation; the latter will, however, detect only those precipitates that are sufficiently low in solubility to compete with the stability of cerium-citrate complex.

Results of the survey now include the reaction of cerium with phosphate, oxalate, sera, and various serum-protein fractions, fatty acids, and several miscellaneous compounds at pH 7.5; both phosphate and oxalate were studied in acid solutions also (pH 3, pH 5). In brief, all tests with sera of various species and certain standard fractions of serum proteins have shown that cerium is stabilized. When the molar ratio of cerium to reagent greatly exceeds that of a large intravenous dose, stabilization is partial. By contrast, phosphate and oxalate are efficient precipitants for cerium. Other miscellaneous biochemicals now completed show intermediate or no evidence of reaction (fatty acids, secondary proteins, plant acids, sugars). Preliminary evidence suggests that sucrose interacts mildly. The survey and also methodology are being continued. A single system at the pH of blood that offers a base line of partial stability to reflect either a decrease or an increase is the method of choice; some evidence for tartrate, which has a weaker complexing action than citrate, suggests that it may afford this improvement.

BIOCHEMICAL STUDIES

Liver lipids 1 to 10 days after intravenous cerium. (Edgar Cress, Fred Snyder and G. C. Kyker)

Forty Carworth-Farm-Nelson female rats representing 10 experimental groups were killed at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days after an intravenous injection of cerium (2 mg/kg). All rats were fasted 24 hours before death. The total liver lipids were determined and the results are shown in the figure below. The liver lipids reached the highest level at 3 days after administration of the cerium, and 6 days later they returned to normal. The greatest biological variation was observed during the onset and recovery phases of the fatty change.



I = range for the control group (0 days)
● = individual rat value
O = mean value

The liver-lipid response due to cerium in various species. (Fred Snyder, Edgar A. Cress, and G. C. Kyker)

Because cerium appears to be representative for studying the behavior of the first part of the rare-earth series, this element has been used to survey the lipid response in both males and females of mice, hamsters, rabbits, adult dogs, puppies, guinea pigs, and chickens under the same conditions that had caused the fat disturbance in rats. The data show a significant elevation in liver lipids for rats (at 3.5 mg/kg for females and 14.0 mg/kg for males), mice (at 7.0 and 14.0 mg/kg for females), and hamsters (at 7.0 and 14.0 mg/kg for males). No fatty livers were seen in any of the other species except in two male rabbits that had 14.7 and 18.2 per cent liver lipids at 48 hours after a 3.5-mg dose of cerium. This variation in response among the six species studied may be due to biochemical species differences or to differences in time of response.

Effect of intravenous cerium on liver composition in fasted and nonfasted rats. (A. W. Lawrence,* Fred Snyder, and G. C. Kyker)

In view of the dramatic effect upon liver lipids when certain rare earths are administered intravenously to female rats, it was important to clarify the effect of cerium upon other liver components, i.e. protein and glycogen. We had previously found that cerium had no significant effect upon the water content of liver. The initial investigation was also designed to measure any effect of a 24-hour fast before death upon liver components and to correlate all observations to the nitrogen balance of the animals. The work also includes analyses of kidneys and spleens.

Twenty-four female CFN rats ranging from 140 to 220 grams were paired on a weight basis and assigned to 4 groups of 6 rats. Each group had about the same average weight. Group 1 served as a saline control for group 2, which received cerium chloride intravenously (2 mg/kg body weight). These groups were fasted the last 24 hours before death. Group 3 was saline control for group 4, which also received cerium chloride (2 mg/kg). Groups 3 and 4 were fed ad libitum until the time of death. All animals were individually housed in stainless steel metabolism cages designed for easy and accurate collection of urine and feces. Records of food and water consumption were kept for all of the groups. Urine and feces were collected daily and analyzed by Kjeldahl method for total nitrogen. Weight records of each animal were kept during the experiment. The animals were killed by exsanguination under light ether anesthesia, the liver was rapidly removed, weighed and a portion was removed for glycogen determination. The remaining portion of the liver was weighed again and retained for H_2O, lipid, and protein analysis. The kidneys and spleens were also removed for further measurements.

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Glycogen was determined by rapidly adding a weighed portion of the liver to 30 per cent KOH solution preheated in a boiling water bath. After the tissue had dissolved, the glycogen was precipitated with 95 per cent C_2H_5OH and removed by centrifugation. This centrifugate was dissolved in hot water and filtered to remove insoluble impurities and the filtrate was reprecipitated with alcohol and redissolved two additional times for purification and weighed directly. The tissue lipids were extracted in Soxhlet extractors with a 2:1 (v/v) alcohol-ether mixture. The lipid extract was weighed directly after filtering the chloroform solution and the solvent was evaporated under an infrared heat lamp. The water content of the liver was determined by drying a weighed portion of liver to a constant weight at 110° in a drying oven.

The cerium-treated rats showed (1) an increase in the liver protein expressed as percentage of body-weight, but a decrease in the liver protein when expressed as percentage of liver; (2) a marked increase in the liver as percentage of the body weight; (3) a decreased glycogen content that could not be attributed to a reduction in the food intake; and (4) no difference in the water content of the liver. Liver lipids increased in cerium-treated animals fasted 24 hours before death, as compared to the liver lipids of cerium-treated rats fed ad libitum until death. The spleen of cerium-treated rats showed a slight decrease in lipid content, with essentially no change in the water or protein fractions. The cerium caused no change in the protein, lipid, and water content of the kidneys.

The effect of cerium on lipid mobilization from adipose sites. (Fred Snyder, Nelson Stephens and Edgar Cress)

The rapid accumulation of liver lipids after the intravenous injection of rare earths raises the question of their source. Experiments during 1959 have indicated that mobilization of lipids and a reduced liver-fat oxidation are two important factors in this connection. Attempts of measuring the mobilization of lipids from adipose tissues have been done in several ways: 1) Measurement of plasma-free fatty acids, 2) measurement of the lipid content of fat depots, and 3) use of labeled fat depots (intact and transplanted).

Plasma-Free Fatty Acids: Measurement of the plasma-free fatty acids (FFA) according to the method described by Dole, shows that a marked increase in FFA occurs at 24, 30, and 48 hours after administration of the rare-earth element. This increase occurs in both fasted and ad libitum-fed rats injected with cerium. The total plasma protein is decreased in the cerium rats, whereas the albumin fraction is elevated at 48 hours after the cerium injection. The data suggest that at least part of the increased liver lipids caused by rare earths is from adipose sites.

Lipid Content of Fat Depots: Measurements of depot fat have indicated that a decrease in total lipids from these areas does occur after the administration of cerium. The response, however, is not too uniform. The variability in fat depots in animals even of the same age or weight is considerable; therefore, any small change is difficult to determine. In one experiment the perirenal-genital fat decreased progressively at 6, 12, 24, and 48 hours after the injection of cerium, during which time the liver lipids were progressively increasing. Because males do not show the marked fatty changes caused by cerium, the technique of using the fat content of the epididymal body as an index of lipid movement has not been used in these studies.

Labeled Fat Depots (intact and transplanted): The technique of measuring lipid transport by determining changes in the specific activity of labeled lipids from intact C^{14}-fat depots and C^{14}-fat pads transplanted in the abdominal cavity has been under development during the latter part of the year. The results from these experiments are too preliminary for any definite conclusions.

Liver protein-S^{35} turnover in cerium-treated rats. (A. W. Lawrence,* Fred Snyder, and G. C. Kyker)

The effect of cerium on the turnover of liver-protein sulfur was measured in thirty selected CFN female rats, ranging from 160-220 grams. The animals were paired according to weight and assigned to six groups of five rats per group. Groups I, II, and III received a tracer dose of sulfur-35 methionine and Groups IV, V, and VI received a tracer dose of sulfur-35 cystine intravenously (20 microcuries per 250 grams of body weight.) Two animals from Group I (methionine injected) and two from Group IV (cystine injected) were killed at 1 hour to determine the level of sulfur-35 incorporation in the liver at this early time interval. The remaining three rats of these two groups were fasted 24 hours and then killed. Groups II and V and groups III and VI each received saline and cerium injections, respectively, 24 hours after the methionine and cystine. The rats from these groups were killed 48 hours later. Urine for sulfur-35 assay was collected from all animals except those killed at 1 hour.

All livers were divided into 4 portions for determinations of H_2O, lipid, protein, and sulfur-35. The sulfur-35 liver sample was homogenized and the protein precipitated with 10 per cent trichloracetic acid, centrifuged and washed twice with dilute trichloracetic acid. The protein precipitate was transferred to a 100-ml Kjeldahl flask and digested to a colorless solution with 5 ml 60 per cent $HClO_4$ and 1 ml concentrated HNO_3. This solution was brought up to 100 ml and an aliquot was used for counting. A 2-ml aliquot of the daily urine output was also oxidized with $HClO_4$-HNO_3. All samples were counted in the Packard Automatic Tri-Carb Spectrometer

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using a PPO-POPOP phosphor scintillation system with napthaline, xylene, dioxine, and ethanol as solvents. All counts were corrected for quenching by adding an internal standard.

The data show that cerium causes a significant retention of the sulfur-35 label in liver protein of rats injected with methionine-S^{35} or cystine-S^{35}. This agrees with earlier data, which demonstrated an increase in the total liver protein compartment. There was no difference in the excretion patterns for sulfur-35 in any of the groups of rats. The water, protein, and lipid content of the control and cerium-treated livers were essentially the same as that observed in a previous experiment. The results are essentially the same as data obtained in studies of protein-S^{35} turnover in fatty livers caused in rats by a dietary methyl deficiency. From this one might speculate that cerium is interfering with transmethylation, although our earlier work on the relationship of methionine and choline to the prevention of the cerium fatty liver indicated that these lipotropic agents could not reverse the effect of cerium.

Fatty acid oxidation by fatty livers. (F. D. Baker* and Fred Snyder)

Cerium, when administered intravenously to rats, produces a fatty liver. The purpose of the present work was to determine the relationship of fatty acid oxidation, if any, to the onset and disappearance of the fatty liver after the cerium injection. The oxidation of octanoic acid by rat liver homogenates and mitochondria was measured by the system of Lehninger, J. Biol. Chem. 164, 291 (1946). The oxidative rate was studied in both control and cerium-treated (2 mg/kg) rats in vivo and in vitro. The data show practically a complete inhibition of fatty acid oxidation by homogenates and mitochondria from rats killed 48 hours after intravenous cerium. This inhibition appears to be related to the increase in liver lipids resulting from the cerium administration and not to the cerium per se. Additional evidence for the latter statement was obtained when no difference from control was found in the fatty acid oxidase system to which cerium was added in vitro.

(Abstract of a paper given at the annual meeting of the Southeastern Section of the Society for Experimental Biology and Medicine, October 31, Charleston, South Carolina.)

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The effect of two different diets on liver choline oxidase in rats. (Fred Snyder and Edgar Cress)

Earlier work (ORINS-27) has shown that cerium can cause a decrease in the liver choline oxidase. These experiments were continued after we had made a change in the commercial feed used as a stock colony ration; the changeover in diets was from Rockland Rat Diet to a diet prepared by Dietrich and Gambrill, Inc. (D&G). The results obtained for animals fed the D&G diet were somewhat confusing since the choline oxidase activity in controls showed little or no difference between endogenous samples and samples with choline present as substrate during the first 30 minutes of incubation in the Warburg. Longer experimental periods showed the expected effect of added substrate. The animals fed the D&G food appeared to have a higher endogenous choline level than rats fed the Rockland diet. Confirmation of this dietary effect on the choline oxidase system occurred in an experiment using female Carworth-Farm-Nelson rats of the same age and weight fed either Rockland or D&G commercially available diets for three weeks before the determination of liver choline oxidase.

MEDICAL PHYSICS

Total-body irradiator. (M. Brucer, D. A. Ross, R. L. Hayes and A. C. Morris, Jr.)

The total-body irradiator tends to be listed as a device for treatment of leukemia. This is true in the sense that leukemia patients will be the first to be investigated with total-body irradiation. It is not true, however, that the machine is a device only for treating leukemia. Total-body irradiation has a widespread potential usefulness. The human radiation dosimetry problem is so complex that it can be approached only with a simplified system. One of the first and obvious uses of the total-body irradiator will be to study dosimetry in compartmented manikins. The entire field of the treatment of all widespread tumors, in which localized surgery or irradiation is impossible, is a field for investigation by total-body irradiation.

The early history of total-body irradiation in the 1920's indicates a potential usefulness in many of the radiosensitive cancers, some of which are widespread. Unverified statements indicate that total-body irradiation is also useful in what we now call radioresistant tumors. If an immunological change occurs in man after irradiation, a situation that is probable and has good animal-research background, then the problem of controlled immunological damage is even more important than any single radiation therapy. Also, with total-body irradiation we have the only situation in which it is possible to investigate the consequences of radiation accidents.

During 1959 the total-body irradiator was constructed and installed, and the first calibrations were started. During 1960, after the calibration of the instrument, all the potential usefulness of the total-body irradiator will be brought into play.

The ORINS total-body irradiator is one of three that now exist in the country. One is at the Naval Medical Research Laboratories in Bethesda. This is a close-in cobalt-60 device. Another machine is at the City of Hope Hospital in Los Angeles, California. This is a close-in cesium-37 device. The ORINS device is a long-distance cesium device. There are at the present time a number of ordinary teletherapy devices used for total-body irradiation. These unfortunately are of only limited adequacy in the problem.

Probably the first real problem that came up in the construction of the ORINS device was the manufacture of a system of eight units that would give approximately 300 r uniform exposure over a 2' x 2' x 6' volume of air. It would be in a room that is totally protected but still has an open entry and passageway between nurse and patient. A system of eight "teletherapy machines" was devised that will produce a uniform exposure. Housing for these eight teletherapy machines and a treatment bed in the center of the array has been built. The room entry is arranged in a maze that will allow a wheeled stretcher to travel through. A system of mirrors allows the nurse to see the patient and the patient to see the nurse at all times. A gate at the entrance to the passageway also acts as the emergency turnoff mechanism. The machine cannot be turned on while the gate is open.

Early check runs for health-physics purposes indicate that the radiation exposure measured with the usual equipment is substantially zero at all points in and out of the machine when the sources are turned off. When the sources are on and the machine is in operation, the radiation exposure falls very rapidly as the meter is moved out through the maze until, at the gate, the level is approximately 10 mr per hour. The radiation exposure at all places other than in the passageway seems to fall rapidly to unmeasurable levels.

A series of models, both of the radiation device itself and the loading device in full size, was built to test the loading and scatter hazards. These have been highly informative, with one exception to be mentioned later. The models are now available for teaching purposes.

The favorable health-physics situation did not prevent trouble when special scintillation equipment was used to measure the background. This sensitive equipment had shown no change in the total-body irradiation room when the large machines in the nearby therapy rooms were pointed at the total-body irradiation room and turned on. However, after one of the eight housings had been fully loaded, the "source-off" background was found high enough to compromise some of the measurements that had been planned. After a great deal of work we discovered that this background seems to emanate from two places in the source housing and can be reduced to about a sixth of its level with two-inch lead-brick shielding. This has been done for all eight sources.

The measurement of spectra will not be possible with the large sources. Therefore, a series of eight small sources made from the same batch of cesium as the large sources had to duplicate the spectrum of the device itself. A sufficient number of spectra were run with the small sources to indicate that our spectra can be matched with microcurie sources. Eight of these are being made and a

position has been made in each machine to mount them. These sources, however, will be inadequate for spectra deep within the phantom. Therefore, an additional series of sources will have to be made with hectomicrocurie cesium sources.

The radiation dose from all eight machines comes from eight 500-curie cesium sources. These sources were matched with an accuracy of approximately one curie, a chemical feat in itself. The original calculations took into account only the high-energy radiation and indicated that about 250 r would be available at a center point of all eight machines from the high-energy emission only. The preliminary spectra indicate that approximately half the radiation is at a lower energy. The preliminary radiation exposure measured in the center of the room is about 450 r per hour.

Probably the most important system to be set up (from a clinical point of view) was the filter system. These filters will adjust the exposure to achieve any exposure rate from background radiation to a maximum of 300 r per hour. A system of five filters is in each device, each filter being a multiple of a tenth of a tenth-value-layer of lead. The filters range from 0.1 continuously to 3.1 tenth-value-layers. This makes 31 possible treatment situations. For clinical purposes a system has been set up that allows the treatment to be given on one-hour intervals with any filter system between 0 and 31.

The acoustics appear to be better than adequate; the cleaning is simple, but probably will cause some trouble in the future; the lighting is excellent; a TV system has not yet been tried.

Clinical dosimetry is just beginning and the physical dosimetry of the device will probably take years to complete. Preliminary work for carrying out depth-dose and absorbed-dose measurements is in progress. Using phantoms and low-level mock-up sources to simulate the therapy sources, we have begun the measurement of the gamma-ray spectra. Since the interposition of the attenuating filters will inevitably change the characteristics of the spectrum, an extensive series of spectral measurements is planned so that the irradiating spectrum will be known for any desired attenuation setting. This will occupy the single-channel analyzer for some time.

A set of "pure" background spectra was obtained in the absence of all but natural sources. These measurements were made to determine the characteristics of radiation that might be expected from an underground low-level counting situation enclosed with ordinary construction materials. The recordings indicate that most of the activity above 50 kev seems to be carried in a broad spectral hump (centered about 125 kev), which falls off to very low levels at higher energies. Study of this background has been facilitated by use of a special low-background encapsulated 5 x 3 inch NaI(Tl) crystal-photomultiplier-tube assembly purchased from the Harshaw Company.

Additional spectral work has been done to determine the proper way to mount small sources in or on the source wheel within the source shields. The reason for this series of measurements is that any attempt to do spectral work in the presence of eight 500-curie sources would hopelessly jam the crystal-spectrometer system, so that this spectral information must be obtained by using smaller sources. To do this, the spectra of the small measuring sources must match those of the large therapy sources, but the count rate must be much lower. It was found that large spectral errors existed when the measuring source was simply taped to the outside edge of the source wheel. To correct for these errors it was necessary to mill a cavity in the source wheel to accept the small source. Insertion of the small source into this cavity now gives a result almost identical to that of the same source in the therapy opening.

Another set of data has been taken on the leakage background obtained with one 500 curie source loaded into the source wheel and turned to the "OFF" position. A broad scatter peak centered about 120 kev was found with negligible primary 660 kev leakage noted from the cesium-137 source.

The scanning program. (M. Brucer, D. A. Ross, A. C. Morris, Jr., Ruth Black, and Janice Byrd)

In addition to the continuing work on the linear scanner and the multiplication of additional area scans, a new research scanner has been developed at ORNL (by P. R. Bell, J. E. Francis, C. C. Harris, and D. P. Hamblen) and has been put in operation at the Medical Division. This research scanner at the present time is using three simultaneous methods of displaying the information from any scan. In addition, a tape recording system is available. With these three scans, coupled with additional scans, both on the older area scanner and on the linear scanner, it is possible to open up the problem of radioactive isotope localization and deposition into a much wider field. Most important in the new research scanner are numerous other devices -- an improved howler, a scope monitor, an improved spectrometer-scaler system, improved lateral shielding for the detecting crystal, and most important of all, a new focusing insert made of gold. The combination of all of these has placed a tremendous load on the clinical interpretation of the scans. It appears that we can now scan high-energy as well as low-energy isotopes; but it also appears that with some of the isotopes, particularly radioactive iodine, we are at a level of sensitivity that gives us almost too much information. The problem has now shifted to one of interpretation rather than one of machine design.

The ORNL scanner was designed particularly with the aim of providing for brain-tumor scans, but this is by no means its only function. However, since it is presumably good for brain scanning, a new program has been instigated in cooperation with several of the

neurosurgeons in Knoxville, whereby they will send us from time to time patients with suspected intracranial neoplasms, so that we may test the new scanner in this field. This project is still in its early stages.

The linear scanner. (A. C. Morris, Jr.)

In the course of radioisotope research at the Medical Division, the need for equipment to provide a means of determining radioisotope distribution has become apparent. In answer to this need, a linear scanner has been designed and constructed, providing an excellent means of recording longitudinal distribution, as well as furnishing somewhat more general lateral information.

In principle, the scanner uses two opposing collimating heads to give a plane of detection-sensitivity that is perpendicular to the longitudinal axis of the body. The subject is then slowly driven on a movable table between the two detecting heads; the count rate, representing the activity profile, is registered through an external recording system. Each detecting head contains six 2 x 2 inch NaI(Tl) scintillation crystals mounted in a line. A sharp linear response characteristic is obtained through the use of long, movable, lead collimating lips, which allow adjustment of the entrance slit from zero to four inches wide on each head.

Each of the twelve detecting crystals is connected to its own photomultiplier tube. The gain of each of these tubes is individually adjustable so that all may be calibrated to the same gamma-energy photopeak, as desired.

The electrical pulses from the photomultiplier tubes are then fed into medical spectrometers that provide for as many as 8 recording channels. The electrical connections at this point are made quite flexible. If the subject activity is very low, all the detected counts may be pooled into one recorded trace. If the activity present is higher, opposing crystal pairs may be connected to give six simultaneous recordings, providing some information on lateral location of isotope concentrations.

The entire apparatus, including the motor-driven bed, measures 7 x 15 x 4 ft. Lead shielding contributes a major portion of the unit's weight, which approaches three tons.

The linear scanner has been in clinical use since February 1959. Initial clinical work with the scanner started under the direction of Dr. Felix Pircher, a visiting physician, and a summary of his work is nearing publication form.

As this scanner has been used, the need for certain operational and physical changes and additions has come to light. One such addition arose from the difficulty in accurately setting index tabs opposite anatomical landmarks for indication of positional information on the recorder chart. This difficulty has been corrected through the installation of an optical source, which projects a line of light across the subject and scanner table so that the index tabs may be accurately positioned.

The linear scanner serves its greatest usefulness when all twelve detecting crystal outputs combine into one spectrometer, with a single recording trace. The mode of operation where the six opposed pairs of crystals are recorded separately on six traces does not give much lateral information. The reduction of total count rate per trace is another adverse effect of this six-pair connection.

Operationally, one of the largest advantages this equipment has demonstrated to date is the ability to make repeated surveys (at 4-minute intervals if necessary) of isotopic distributions in the body after administration of a dose of radioisotope. Other interesting results include the activity profiles taken from the neutron-bombarded victims of the Y-12 accident in Oak Ridge in 1958. With this new equipment, surveys (taken at regular intervals over a period of many hours or even weeks) may give very useful information in many important fields of investigation.

Pulse-height spectrometry. (D. A. Ross)

Single-channel studies - The single-channel analyzer has been in frequent use during the past year to plot gamma-ray spectra for various purposes. One of its jobs has been the plotting of serial spectra obtained from a sample of calcium-47, other portions of which were used in clinical and animal studies. On the basis of the spectral data, a method of counting the calcium-47 was devised, the arrangements being such that chromium-51 and scandium-47 would be kept out of the picture. The other studies with this sample of calcium-47 are described in the clinical and preclinical sections of this report.

2" x 2" detectors - Late in 1958 the crystal-photube assembly that had been used until then with the single-channel analyzer developed a serious fault and had to be shelved. Since the detecting head containing this ORNL crystal was nonstandard in many respects, we thought it advisable to redesign a detector that would be able to use commercially procurable sodium iodide crystals. The difficulty here was that the commercial crystals are somewhat larger in diameter than the one canned at the Oak Ridge National Laboratory, so that the containing sleeve and the collimator assembly required some modification. While the changes were being made, care was taken to include a magnetic shield for the photomultiplier, which had not been present in the

original setup. The new detecting system is now in operation, and its resolution on the cesium-137 gamma ray is around 9 or 10 per cent.

3" x 3" detectors - The struggles with the new 2" x 2" detector brought up the question of whether or not the Medical Division should explore the virtues and vices of 3" x 3" sodium iodide crystals for medical work, and after considerable discussion, correspondence, and negotiation, two new detecting heads with 3" x 3" crystals were ordered. They arrived late in 1959 and will be tested as soon as an analyzer becomes available. (It has been used in the total-body irradiation project.) We shall be obliged to design new collimating devices for the 3" x 3" crystals and have them made in the ORINS shops, since proper collimators for this crystal size do not seem to be available commercially.

Stability - Before the single-channel analyzer was turned over for studies in the new irradiating facility, it was carefully checked over and readjusted, and a number of runs were made to investigate its stability. These included changes occurring during warmup, or immediately after readjustment of the high voltage, or immediately after the initial presentation of a new source to the detector, as compared with the steady state achieved after the detector has been looking at the source for a while. Drift was found to occur in all of these situations, and this will be borne in mind as the machine is used in future projects.

Large crystal - In anticipation of the time when we may be able to install a low-level counting facility, we began to study the Medical Division's old 5" x 3" sodium iodide crystal. Its can was found to be leaking. After considerable consultation with the people at the ORINS shop and also with the very cooperative engineers at Harshaw Chemical Company (the manufacturers of the crystal), we decided that the crystal should be recanned at the Harshaw plant. They are using the new policy whereby a 3-inch photomultiplier (not larger) would be glued to the rear end of the crystal, while the white, reflecting powder is applied around the unoccupied part of the rear end of the crystal as well as elsewhere. The crystal is now back at the Medical Division where it can be used for exploratory studies as needed.

100-Channel analyzer - Again looking forward to the eventual availability of a low-level counting facility, considerable effort has been put into a study of the various multiple-channel analyzers that are now commercially available. Last February Morris and Ross attended a one-week instruction course given by the Radiation Instrument Development Laboratories (RIDL), of Chicago, the purpose of the course being to familiarize the participants with the features, peculiarities, habits, and diseases of RIDL's 200-channel analyzer. It is clear that these instruments are not yet all that one could desire, so it is hoped that pending the acquisition of a low-level counting setup, the analyzer design will progress to a point where it is less temperamental. Some

time was also spent in studying a competing type of analyzer manufactured by the Pacific Electro-Nuclear Company of California. The Radiation Counter Laboratories (RCL) and RIDL analyzers stem from a design that was originally worked out by Schumann and McMahon at the Argonne National Laboratory; the Pacific Electro-Nuclear (PENCO) analyzer, on the other hand, is the commercial descendent of a Los Alamos design. Currently we have less information about the PENCO analyzer than about those of the Argonne type, but all the reports about the PENCO machine have been favorable, so that we must at least consider it carefully when, or if, the time for purchase arrives. A number of transistorized analyzers are available commercially now; these offer certain advantages, but their arrival in the field is relatively recent and verbal reports indicate that they still present numerous electronic hazards. For this reason we are still inclined to think that the hard-tube analyzers, for all their faults, represent less risk.

Low-level counting facility. (D.A. Ross and M. Brucer)

Preliminary plans - Since June 1958, it has been necessary that a low-level, human counting facility be eventually set up at the Medical Division. A great deal of thought and study has been devoted to the design of a facility that will incorporate as many as possible of the virtues of similar systems already in operation, but will at the same time avoid the mistakes that earlier workers may have made. This planning project has involved visiting and discussing many low-level facilities already in operation, in this country and in England, coupled with detailed discussions of the various design possibilities among the members of the Medical Division's senior staff. A general outline of a proposed approach has been drawn up and mimeographed; it is being sent to many well-informed workers in this field, with the request that they should comment, favorably or otherwise, upon the proposed design. The basic problems involve a decision whether a human counter should be of the tank (Los Alamos) or scintillation-crystal (Argonne) type, or whether both of these types are needed. If reasonably accurate spectral analysis is to be undertaken, a further decision will have to be made between the Argonne arrangement using a single large crystal, and other schemes using multiple-crystal arrays. Numerous other problems will also have to be tackled, prominent among which is the choice of a suitable multiple-channel analyzer for spectral work. This project is still in the discussion stage.

Therapeutic equipment. (D.A.Ross, A.C.Morris, Jr., R.L.Hayes)

High-energy thimble chamber - Early in 1959 we procured from Victoreen Instrument Company a high-energy measuring chamber of the condenser type, designed specifically to handle radiation ranging in energy between 400 to 1300 kev, and thus covering nicely the cesium and cobalt gamma rays. When the new chamber arrived, it was tested against the older chambers (an X-ray thimble device with an added 4-millimeter

plastic cap), and a considerable discrepancy was observed. For this reason the new high-energy chamber was sent to the National Bureau of Standards for calibration, and it was returned with a correction factor of 0.99. The old and new chambers were therefore compared again, more carefully, and it was established that in order to convert the "old roentgens" to "NBS roentgens" the earlier measurements would all need to be multiplied by a factor of 1.2. This has necessitated the refiguring of the doses given to the first eleven patients who received total-body cobalt irradiation. A description of the irradiation procedure, with the revised figures for the doses, is now in preparation.

Surgical probe - The ORNL surgical probe is still in demand for thyroid operations. Early in 1959 we were loaned a considerably modified commercial version of this surgical probe for clinical trial, but the device has not worked out very happily. We plan to return it to the manufacturer with appropriate suggestions regarding its redesign. It does have the virtue of high sensitivity, but this is obtained along with other features, some of which are rather undesirable.

The design of a new surgical probe and associated electronics has been completed. This instrument will use a small diameter thin end-window GM tube as a beta detector, which may prove useful in human or animal operations involving isotopic tracers having beta emissions, for example iodine-131. Construction of the probe unit has been completed. It is made from stainless steel about the size of a standard cigar with a long, thin collimating nozzle on the front end. The probe unit may be immersed in Zephiran or other antiseptic solution for sterilization purposes.

An electronic system for use with this probe is nearing completion. The electronic speaker output from this probe and system will be in the form of audible clicks, peeps, or variable-pitched howls, to be selected at the surgeon's desire or distraction.

Spectra in water phantoms - Pending further developments with the total-body irradiation facility, Dr. Yoichiro Umegaki, a Japanese radiologist working at the Medical Division, has irradiated a large water phantom with gamma rays from cesium and cobalt sources, and has measured the spectra of the scattered irradiation emanating from the interior of the phantom as a function of the angle subtended between the incoming beam and the outgoing scatter. This should provide the deep-therapy radiologist with information bearing on the essential processes that go on during the irradiation of a deeply located neoplasm. The study also provides some information concerning total-body irradiation.

Chemical dosimeter systems - Aqueous chemical dosimeter systems are quite useful in measurements of total energy absorption since they are integrating detectors that can be substituted for phantom media. In general, however, they lack sensitivity. Attempts to develop aqueous chemical systems of radiation detection that are more sensitive and stable than those now available have continued. A survey of absorption spectra changes in aqueous dye systems produced by ionizing radiation is being made. Sixty dye systems have been investigated so far. The most sensitive dye uncovered in this survey is still Alphazurine 2 G (Patent Blue V). Although this dye is the most sensitive so far encountered, its radiation sensitivity is dose-rate dependent and subject to variations because of the presence of minor impurities. Other potential dye systems are being investigated. Many of the dye systems surveyed, although apparently less sensitive than Alphazurine 2G, are nevertheless promising. David B. Camp, summer participant from the University of the South, Sewanee, Tennessee, has carried on these studies.

Electroscope board - A new electroscope board has been built and installed in the radiation storage vault for clinical dose measurements. Removing the site of dose measurement from the instrument room provides for more convenience in making up doses. This will also eliminate some of the hall traffic of hot sources.

Clinical uptake machine - The clinical uptake equipment has been modified for mobile operation by installing a spectrometer, decade scaler, and scintillation detector head on a modified Picker stand. This innovation permits the uptake counter to be moved about the building at will, and it can be moved to the bedside of a patient when necessary.

Oximeter. (F. Comas and A. C. Morris, Jr.)

An oximeter for measurement of oxygen in normal and tumor tissue in connection with therapeutic radiation is in the process of development. The preliminary design of this equipment was made by Dr. Umegaki. Dr. F. Comas has continued this work since Dr. Umegaki's return to Japan.

Several rather difficult problems have arisen in this measurement of oxygen content in tissue. One of these problems concerns the delicate fabrication of probes small enough to be inserted into the tissue site and, at the same time, have the very high electrical insulation qualities required to make reliable measurements possible. This problem has been overcome through the use of small diameter glass tubing heated and drawn over the 0.003-inch platinum electrode wire. Another serious problem associated with a workable oximeter is the necessity of DC amplification between the probe and recorder. Several standard amplifiers to fit this need have been built, but none has

given satisfactory performance. The main obstacle here comes from the fact that less than 1×10^{-9} amperes from a high impedance source is to be measured and, at this level, amplifier drift and interference pickup present unusual difficulties. A special amplifier to operate at high impedance with 80-db line interference rejection has been ordered for this project.

Several other modifications are contemplated for the oximeter after procurement of a workable amplifier. The first of these changes will provide for the switchable selection of one of eight implanted probes, thus providing for the measurement of oxygen content at any probe site during the course of an experiment. Also, a bank of precision, switchable resistors will be installed for each probe so that probe current may be accurately measured.

Preliminary results indicate that the oxygen content of bone marrow, muscle, and subcutaneous tissue varies within large limits according to the physiological state of the animal, such as depth of anesthesia, and respiration rate. Oxygen content in the tumor is highly variable from one area to another and is influenced by oxygen inhalation in an unpredictable manner, whereas normal tissues always show an increase in tissular oxygen when the animal is made to breathe it. In one set of experiments, anesthetized rats were given pure oxygen to breathe, and with the electrodes inserted into the transplanted tumor and neighboring subcutaneous tissue, a steep increase in the subcutaneous tissue oxygen was demonstrated. The tumor, however, showed a very slight increase in available oxygen. This may be an indication that an unfavorable differential effect would take place when a tumor is irradiated under high oxygen tension.

As part of a study on the comparative radiosensitivity of rat bone marrow and a transplanted tumor under vascular hypoxia, a method has been developed to obtain a reliable index of radiation effect. It consists of assessing the time delay for the cells of those tissues to recover normal values of DNA synthesis after irradiation. Synthesis of DNA is gauged by the uptake of thymidine labeled with tritium. A technique has been worked out to prepare the tissue samples so that they can be incorporated in the phosphor of a liquid scintillation counter.

Irradiation source for oximeter experiments. (F. Comas, M. Brucer, R. L. Hayes, and A. C. Morris, Jr.)

A "portable" teletherapy machine, provided with a cesium-137 source of about 300 curies, was moved to temporary quarters in the Animal Laboratories, where it is used to irradiate small animals. Because it is intended to work at short source-animal distances (10 centimeters) the measuring of its output and radiation distribution

is somewhat critical. Measurements were taken in free air and inside a small water phantom similar to a cage designed to keep rats restrained during the irradiation time. The determinations were made with a high-energy Victoreen r-meter and with discreet volumes of the ferrous-ferric sulfate chemical dosimeter placed inside 1 ml lucite containers, which could be positioned accurately in the phantom. Even with exposure times sufficient to deliver about 18,000 rads, the chemical-dosimeter reproducibility was rather poor. By and large, however, both sets of measurements agreed fairly well with one another.

Spectrometer units for the animal laboratories. (A. C. Morris, Jr., G. C. Kyker, and J. J. Rafter)

Much tribulation in counting different isotopic sources has been experienced in the past at the ORINS animal laboratories when we used a threshold-type scintillation counter. The threshold setting for a high-energy isotope would give very poor results on a low-energy isotope. Often the threshold settings were neither stable nor reproducible. We decided to replace these threshold counters with equivalent spectrometer types. Completion of this change enables the operator to set the spectrometer window on the proper energy range for the isotope in question, allowing a more accurate count to be made.

One detecting probe used with the old threshold systems was directly applicable to the new spectrometer system. The other probe was reworked and rewired to make it also compatible to spectrometry.

Radiation leukemogenesis. (N. Simon, R. L. Hayes, and M. Brucer)

Much interest has recently been generated concerning radiation leukemogenesis. Studies of medical records of patients who have received radiation therapy may be of help in assessing the leukemogenic nature of radiation. A study of the incidence of leukemia in a total of 71,000 cases of carcinoma of the cervix treated with radium has been completed. The final evaluation indicates that such treatment does not produce an increase in the incidence of leukemia among the survivors, although the dose to the bone marrow adjacent to the pelvic compartment is quite high (100 to 500 rads depending on body height.) Data on the cases were compiled with the cooperation of medical institutions in the United States and Europe.

Autoradiography. (W. D. Gibbs)

A study of the response of 17 different types of X-ray films to various radioisotopes was continued during 1959. A constant-temperature developing tank was obtained to provide uniform processing of the films. This unit has proved to be very satisfactory. Results can now be reproduced within ± 2 per cent. To date, the relative sensitivity of these 17 types of films has been obtained for exposure to phosphorus-32 and sulfur-35. Similar studies are planned for iodine-131, mercury-203 and chromium-51. This will cover the range of energy and types of radiation of all isotopes that the Medical Division uses for gross autoradiography. Experiments to determine the response curves of these films exposed to these radioisotopes are planned.

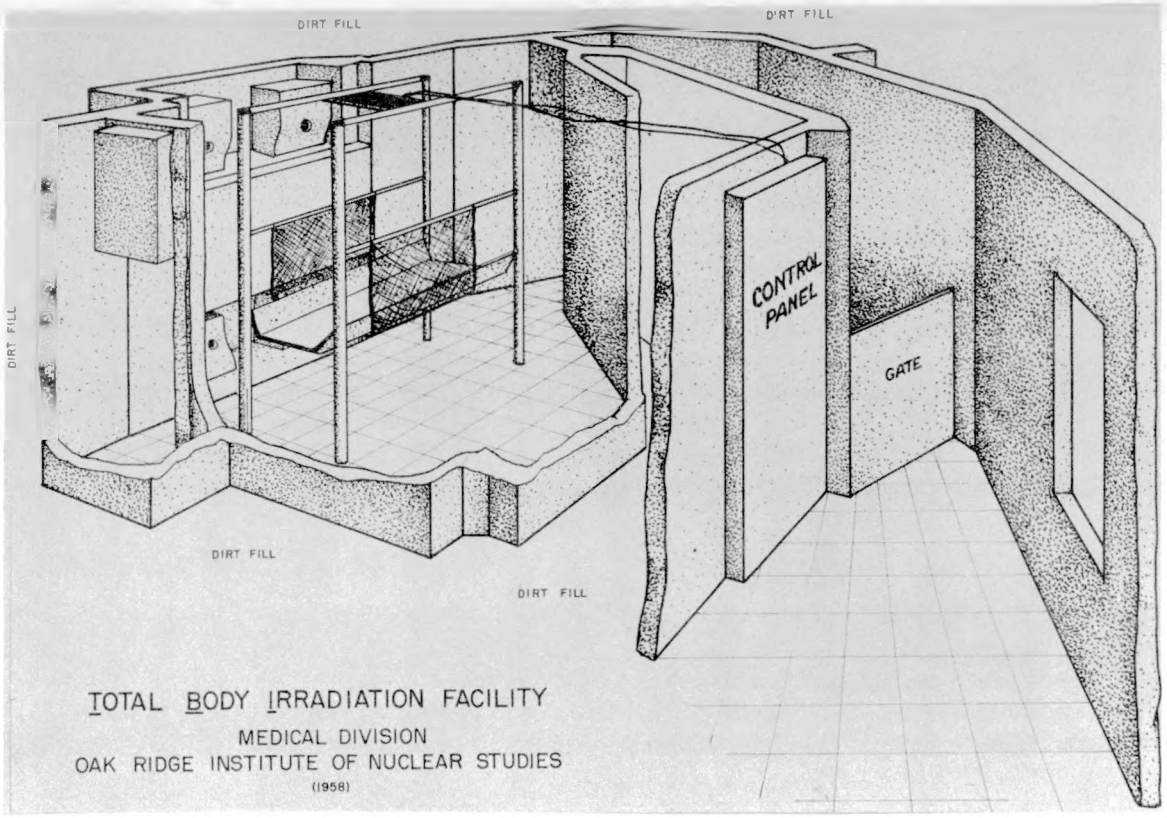
During 1959 considerable effort was put into the development of a simple technique for preparing autoradiograms of blood cells. A technique was devised that gives good results. A number of autoradiograms were made of blood from leukemic patients after it was incubated with tritium-labeled thymidine. Results obtained thus far show that the thymidine is incorporated almost exclusively in the nucleus of myelocytes.

In the first part of 1959 a freeze-dry apparatus was tested thoroughly. The temperature-control system of this equipment was not reliable and modifications have been planned.

A machine for preparing microscopic sections of undecalcified bone was obtained. Several weeks were devoted to developing techniques for obtaining good sections and autoradiograms of bone. The equipment requires some modifications, which will be made as soon as possible.

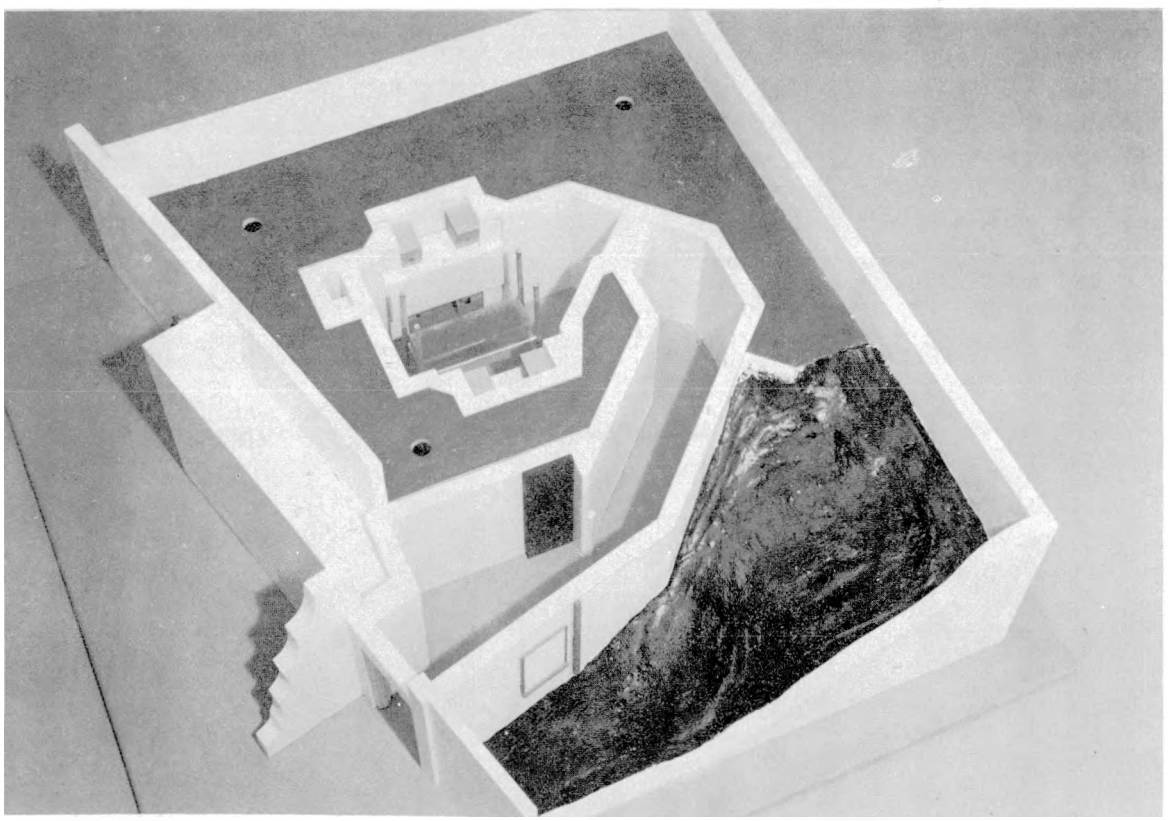
Considerable work was done on the autoradiography punch-card file. About 75 papers were read and data were punched on cards. This file now contains about 800 references.

A considerable amount of work on densitometry of gross autoradiograms has been done. Densitometry studies agree closely with data obtained by radioassay, if the autoradiogram is not overexposed.



TOTAL BODY IRRADIATION FACILITY

MEDICAL DIVISION
OAK RIDGE INSTITUTE OF NUCLEAR STUDIES
(1958)



TRAINING

Training for residents. (R. M. Kniseley)

During the year twenty short-term residents from a number of institutions averaged three to four months in training at the Medical Division. One postresident-assistant in radiology, four visiting guests from other countries, and two residents in clinical research were here for longer periods.

The resident training has consisted of informal teaching sessions, formal ward rounds, participation in study and treatment of patients with radioisotopes, the ORINS Special Training Division's basic course in radioisotope techniques, auditing of the shorter instructional seminars of the Medical Division, use of special training aids, and participation in our scientific conferences. For those in residence longer than four months, we encourage involvement in a research project bearing on the Medical Division's objectives. This gives experience in clinical and basic investigative work, and the value to our program (as well as to the trainee) has been significant.

Instructional seminars and qualification courses.

Two kinds of short-term courses were given during the year. One accommodating 20 students is actually a three-week laboratory course in diagnostic uses of radioisotopes, in separate one-week sessions as Preclinical I, Preclinical II, and Pathology. Initially, demand on staff time to devise and test the optimum experiments, training aids, and lecture sessions was heavy. Now, except for refinements and evolutionary changes, the schedule has become stable and requires less of the person responsible for each session. Preclinical I introduced language and tools. The principal topics given in half-day or one-day sessions were basic physics, statistics, decay and interaction, sensitivity, energy and spectrometry, specific activity and isotope dilution, electronics, autoradiography, and biological applications. Preclinical II presented problems in instrumentation and measurement, and introduced principles used in clinical methods. The topics were sample counting of beta emitters, sample counting of gamma emitters, survey and monitor instruments, external counting, field of vision, scanning, biological procedures and measurements, radiological safety and autopsy technique. During the third week, clinical diagnostic

procedures were performed to meet AEC license requirements. The topics included thyroid function tests, red-cell labeling and survival techniques, blood-volume procedures, fat-absorption studies, vitamin B_{12} excretion, iron kinetics, and scanning. These courses, presented three times during the year, were sponsored jointly with the Council on Radioisotopes of the American Society of Clinical Pathologists.

The second kind of short course is an instructional seminar in a specific topic, a more advanced presentation not designed for AEC licensure requirements. In January, a three-day program on scintiscanning stressed clinical applications with lectures, demonstrations, and laboratory sessions. There were 10 participants. In November, the one-week course "Radioisotopes in Hematology" was presented with 40 participants. The course consisted of lectures and demonstrations. Nineteen guest speakers, expert in their topics, served as the faculty. The audience was enthusiastic. Requests for repetition of the course were received; a number of applicants were not accommodated because of space limitations. Topics included the acute and chronic hematologic effects of irradiation; theory, clinical application, and techniques for chromium-51 to red cell labeling; iron-59 studies; theory, clinical application, and technique; vitamin B_{12}, cobalt-60 in the diagnosis of anemia; white cell labeling; tritium-labeling thymidine; platelet survival and clinical platelet studies; DFP labeling; animal studies on total-body irradiation and marrow transplantation; clinical studies on total-body irradiation and marrow transplantations; external counting in hematologic studies; therapy of leukemias, lymphomas, and polycythemia; and autoradiographic techniques in hematology.

Training aids.

One concern of the staff has been the importance of presenting sometimes complicated ideas in a clear, simple fashion. Every staff member who has responsibility for one or another of the course sessions has found it necessary to design and test heretofore unavailable training aids. In addition, equipment that has been built for other purposes is quite useful; for example, the manikins originally made for the thyroid uptake calibration study have proved valuable in laboratory exercises. Similarly, the models built to show the angular scattering spectra, and isoresponse models for various collimators graphically demonstrate concepts that are otherwise difficult to grasp.

Electronic breadboard. (A. C. Morris, Jr.) As a part of the Preclinical I course the participating physicians are introduced to basic electronics because they will eventually set up radioisotope laboratories with moderately complex counting equipment. The object of the session on electronics is not to produce one-day-wonder engineers or even technicians, but to teach something about the language of this technical field.

To aid this instruction six electronic breadboards have been built. The term "electronic breadboard" comes from the early days of radio when amateurs and experimenters built their receivers and transmitters on a breadboard base, easily obtainable from the local dime store. Now the term means a type of construction in which the diagram to an electronic circuit is printed on a board and all important electric components are mounted adjacent to the circuit symbol. Wiring is completed below the board. In this way you can see the physical component and, at the same time, observe its electrical function in the circuit. Some breadboards (including the ORINS model) take another step by inserting jacks in various portions of the wiring to allow the student to connect experimental circuits.

The first section of the procedure manual deals with definitions, such as voltage, current, resistance, capacitance, impedance, and amplification. The next section introduces the various physical components going into electronic construction. Moreover, actual components (resistors, transformers, vacuum tubes, transistors, etc.) are passed around the classroom to give students a close look at these parts.

The third part of the manual deals with a set of sixteen electronic experiments. Detailed instructions are given about the proper way to insert the plugs of jumper cords into jacks on the breadboard to give the proper hookup for a certain circuit function. Starting with the construction of a power supply, the series of experiments continues through filter, amplifier, and binary scaler stages and finally culminates in a working GM detector system, driving an output register.

The breadboard and procedure are set up to keep the operator safe from electrical shocks if he follows instructions faithfully. So far, in three class sessions, no students have had accidents.

Other electronic training aids - The students use five commercial scintillation counters (Mediac) in laboratory counting techniques. These instruments have been extensively modified so that external Geiger-Muller probes are also operated, fitted with thin mica windows and aluminum shields for beta and gamma counting. The detector tubes are halogen filled and are designed to be inserted into small shelved plexiglass stands for geometry and absorption studies. The scalers have been modified to include high and low threshold settings for the scintillation crystal.

Half-life demonstrator. (R. L. Hayes). This device permits in one laboratory session a dramatic demonstration of radioisotope decay. The apparatus uses an ion exchange column labeled with cesium-137. With alkaline-versene, the 2.6-minute barium-137 can be stripped from the cesium-137 (half life, about 30 years), in about 30 seconds time with no risk of contamination problems.

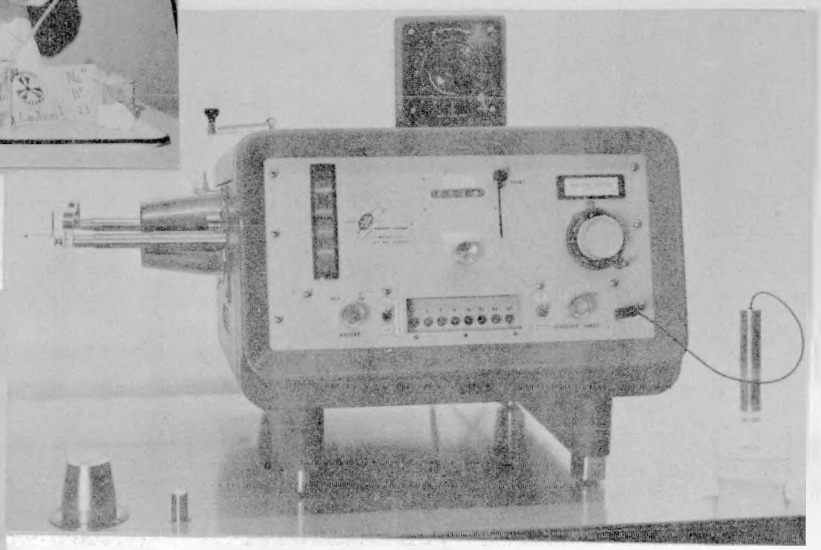
Statistics board. (M. Brucer). This precisely constructed apparatus contains a reservoir for small steel beads at the top and the student can introduce any chosen number of beads into the binomial distribution. He can see vividly the effects of blunders and skewed distributions and is oriented to the problems of statistics that he will encounter in radioisotope measurements.

Goldfish and yeast. (G. C. Kyker and F. L. Snyder). The biological problems of specific activity, isotope dilution, and biological effects have been exceedingly difficult procedures to simplify in the laboratory. A goldfish experiment, which combines autoradiography with counting, and a yeast experiment have been set up as laboratory procedures.

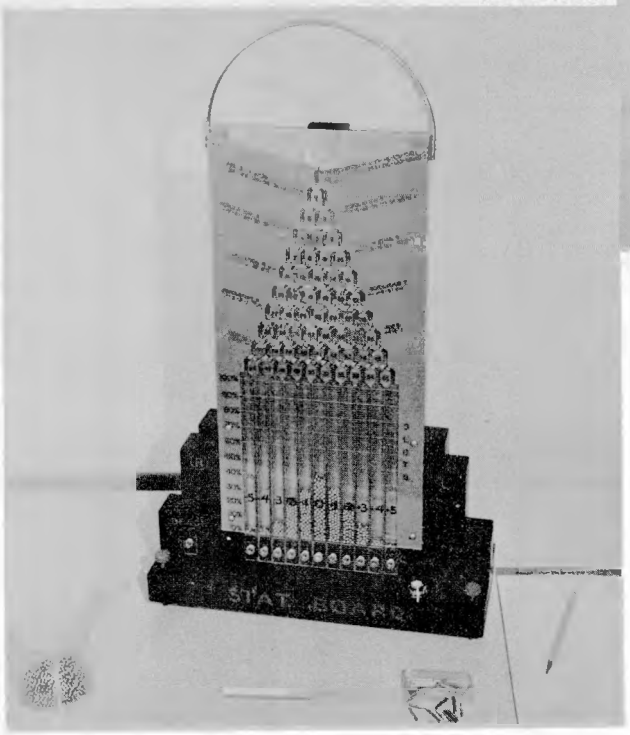
Laboratory manual. Instruction sheets for these various laboratory sessions are being revised as their use shows their strength and weaknesses. Some are more nearly finished than others. The set should eventually be combined to form a laboratory manual. Also under construction is an annotated bibliography of the significant articles in the literature. This is being done to aid the physician (new to nuclear medicine) in becoming acquainted with the field.

Training Aids

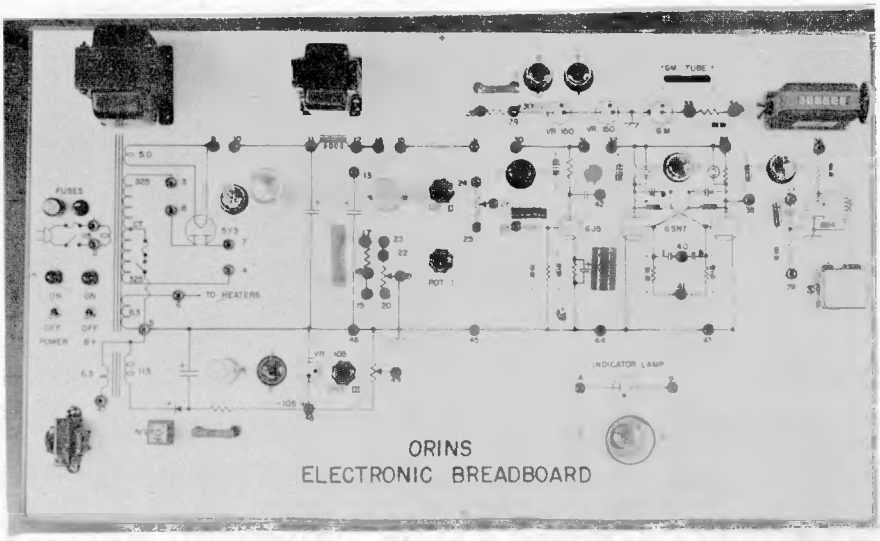
Goldfish experiment

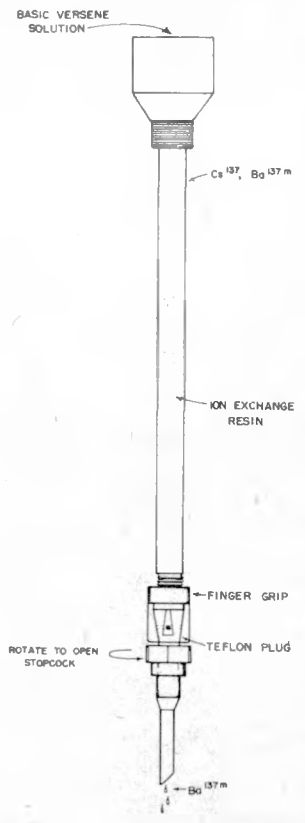


Training counter



Statistics board





Half-life demonstrator

PARTICIPANTS IN THE 1959 COURSES

Scanning (January)	10
First Preclinical I (February)	20
First Preclinical II (April)	20
Pathologists (May)	17
Second Preclinical I (June)	21
Second Preclinical II (August)	12
Pathologists (September)	14
Third Preclinical I (October)	19
Hematology (November)	40
Third Preclinical II (December)	21

Short-term residents	18
Postresident in radiology	1
Resident in clinical research	2
Research Associate (Clinical)	2
Visiting scientists from Japan (clinical)	2

TABULATION OF HOSPITAL STATISTICS

Average patient census:

January	15.7
February	17.6
March	16.6
April	19.2
May	18.9
June	19
July	18.4
August	13.4
September	18.4
October	17.9
November	17.7
December	10.2
For the year	16.7

Outpatient visits ---------- 1587

DISEASES TREATED	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Acute leukemia	5	4	1	1	2	3	8	5	5	3	4	
Effects of total-body irradiation in acute leukemia										2		
Chronic leukemia			2		5	6	4	3	3	2	2	3
Polycythemia				1	3	1	2		2	3	1	
Secondary polycythemia								1				
Myelofibrosis	2		1	1	1		2	1	1		1	1
Hodgkin's disease	1	1	3	1				1	1	1		
Lymphosarcoma	1						2	1	2	1	1	
Malignant lymphoma												3
Thrombocytopenic purpura			1			1		1				
Hemolytic anemia			1								1	
Multiple myeloma				1	1		1	1	1	1	1	1
Hypersplenic syndrome								1		1		
Hyperthyroidism	2	4	9	6	9	7	9	2	6	3	5	2
Iodine-131-induced hypothyroidism (for chronic pulm. insuff.)	1											
Carcinoma of the thyroid	6	6	8	8	2	5	6	8	6	3	7	3
Metastatic thyroid carcinoma treated with exogenous thyroid		2		1	1							
Carcinoma of the breast			1	1		2						
Carcinoma of the buccal mucosa											1	
Carcinoma of the cervix										1		
Carcinoma of the esophagus					1	1		1				
Carcinoma of the kidney								1				
Carcinoma of the larynx									1			
Carcinoma of the lung					1			1		2		
Carcinoma of the ovary			3			3	1	4	2	1	2	1
Carcinoma of the urinary bladder					2				1	1	1	1
Brain tumor (scan)				1								
Pituitary tumor								1				
Effusions - Pleural (Y-90)			1			2						
- Ascites (gold-198				1								
Autologous bone-marrow transplants after nitrogen mustard			1									
Surgical resection of discrete pulmonary metastases		1		1								
Misc. studies on various types of malignancy	5	10	6	2		5						

The following examinations and procedures have been carried out:

Blood counts	2335
Coagulation times	43
Sedimentation rates	134
Hemoglobins	2247
Hematocrits	2253
Platelet counts	779
Reticulocyte counts	55
Bleeding times	43
Lupus erythematosus preparations	4
Differential counts	2308
Urines	567
Fluids	69
Serology	80
Basal metabolism tests	116
Bone-marrow aspirations	127
Electrocardiograms	170
X-ray examinations	4291
Fluoroscopies	102
Area scans	421
Linear scans	392
Thyroid uptakes	188
Cobalt-60 treatments	903
Tissue reports	382
Autopsies	31
Surgical specimens	92
Papanicolaou tests (cervical)	28
Sputum examinations	24
Chest fluids	73
Abdominal fluids	8
Sent-in blocks and slides	62
Animal tissues	49
Gastric washings	6
Bronchial drainage and washings	6
Urine preparations for iron	2
Autoradiograms	1295

LABORATORY ANIMALS USED DURING 1959

I.	Rats		1263
	A. Preclinical research	851	
	1. Cerium-144 distribution	107	
	2. Linear scans, calcium-47, potassium-42	15	
	3. The distribution of yttrium-90-gelatin phosphate	14	
	4. The effect of rare earths on total liver lipids.	273	
	5. Sulfur-35 turnover in cerium-treated rats	30	
	6. Phosphorus-32 turnover in cerium-treated rats	28	
	7. Carbon-14 turnover in cerium-treated rats	12	
	8. Palmitic acid-C^{14} studies	35	
	9. Liver perfusion with sodium octanoate-1-C^{14}	54	
	10. Titration of plasma-free fatty acids	96	
	11. Effect of cerium on liver choline oxidase	8	
	12. Effect of cerium on liver octanoic acid oxidase	18	
	13. Miscellaneous small experiments	21	
	14. Intravenous methodology (ethyl alcohol, corn oil, coconut oil, and triethylamine)	24	
	15. Animals to provide tissues (blood)	116	
	B. Clinical staff projects served at preclinical laboratories	232	
	1. Oxygen protection in irradiated S-256 tumor rats	135	
	2. Distribution of gold-198 and iodine-131 in rats with intrahepatic S-256 tumors	97	
	C. Autoradiographic liver distribution of gold-198 (for isotopes course)	38	
	D. Provided for use in basic radioisotope courses	42	
	E. Donated during periods of surplus production	100	
II.	Dogs		64
	A. Preclinical research	54	
	1. Intralymphatic studies of gold-198, yttrium-90, and cerium-144	20	
	2. The effect of rare earths on liver lipids	26	
	3. The excretion of yttrium-91 and cerium-144	4	
	4. Hepatectomy (methodology)	4	

B. Medical Division Training Courses		10
1. Pathology courses (various isotopes)	10	
III. Miscellaneous Species		239
A. The effect of intravenous cerium on the total liver lipids:		
1. Mice	63	
2. Guinea pigs	58	
3. Hamsters	52	
4. Rabbits	39	
5. Chickens	26	
6. Pigs	1	
Grand Total: I, II, and III		1566

PUBLICATIONS

1959

For reprints from the open literature and any questions on publications, write to Mrs. Edward H. Anderson, Technical Editor, ORINS Medical Division, Box 117, Oak Ridge, Tennessee. Complete lists of Medical Division publications are available.

The Medical Division does not handle the sale and distribution of government documents, but they may be ordered from Washington as indicated in the references to them.

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