ORINS-41

MASTER

OAK RIDGE INSTITUTE OF NUCLEAR STUDIES

Medical Division

Research Report for 1961

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TABLE OF CONTENTS

FOREWORD

The major program of the Medical Division has been directed in recent years toward the study of radiation effects. Patients have been given total-body irradiation in low, medium, and high dose ranges and biochemical, immunologic, and hematologic studies have been carried out. These results have been compared with findings obtained here on the five persons treated at the Medical Division after exposure in the 1958 Oak Ridge Y-12 criticality accident.

Interest has continued in the development and testing of special medical physics instruments needed for the Medical Division research program; these include the ORINS linear scanner, the ORNL gold-collimated area scanner, the cesium-137 total-body irradiation facility, and the high-level whole-body counter. Design and engineering studies have been completed recently for a new low-level, multiple-crystal, whole-body counter.

In the preclinical area the program has pursued detailed biochemical studies on an induced fatty liver lesion discovered some time ago to be an effect of cerium and related elements. Now this methodology is being applied to a study of changes in lipids, particularly bone marrow lipids, following irradiation.

The abstracts that follow represent recent activities in the Medical Division. Most of the material in this report has already been presented in a limited distribution at the April 1962 meeting in Oak Ridge of the Atomic Energy Commission Biomedical Directors. The compilation, organization, and editing of this report is the work of Elizabeth B. Anderson, technical editor, Medical Division.

Recognition should be given to the enthusiastic contributions and leadership provided by the first chairman of the Medical Division, Marshall Brucer, who retired in December 1961, for medical reasons, after 13 years of productive and stimulating activity.

Gould A. Andrews

Gould A. Andrews, M.D.

Remission in Acute Leukemia after Total-body Irradiation without Bone Marrow Infusion

(G. A. Andrews, B. W. Sitterson, D. A. White, R. M. Kniseley, and F. V. Comas)

In previous attempts to obtain successful grafts of homologous bone marrow in patients with acute leukemia after large exposures (250 to 900 r) to total-body irradiation, a remission in the disease occurred in some patients, although there was no conclusive evidence of a "take" of the infused marrow. This suggested that the marrow played no part in these remissions and led to trials of treating children with acute leukemia by total-body irradiation alone.

Experience indicated that about 300 r midline air dose was the maximum we could give with a reasonable expectation of being able to provide adequate supportive care to have the patient survive the bone marrow suppression resulting from the radiation. The radiation was given in a single dose.

Five children with acute leukemia (two primitive cell, two acute granulocyte, and one acute lymphocytic) have been treated by this means. One patient had received no previous therapy. The other four were in relapse after treatment with antileukemic drugs. If still being administered, these drugs were omitted just before irradiation. If the patient was receiving adrenocorticosteroids, this medication was continued at the same dose throughout the period of study. Transfusions of fresh whole blood and platelet-rich plasma were given freely in the preirradiation and postirradiation periods as indicated by clinical developments and blood counts. Antibiotics were not given prophylactically but were administered at the first sign of infection.

Definite and complete remissions in the leukemia occurred in two patients. A rapid fall in white blood cells to a level of 1 to 2000 per cubic millimeter occurred in the first few days. Blood platelets were very sparse before treatment. White blood cells and platelets remained at low levels for about three weeks, after which a rise in counts and evidence of remission in the disease occurred. The changes in peripheral blood elements of one of these patients is shown in Fig. 1. Both patients subsequently had relapses in their disease and died of leukemia.

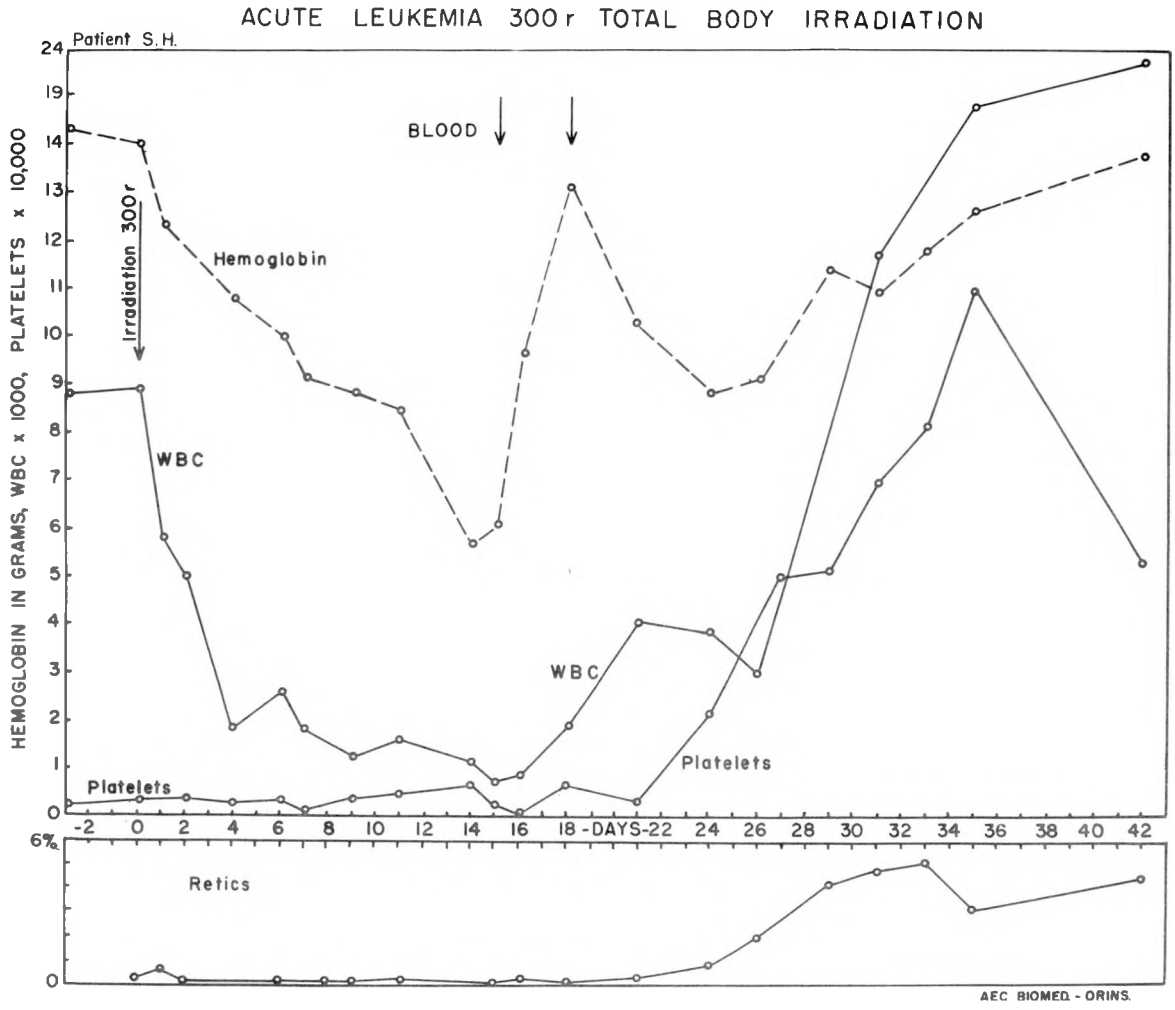

FIG. 1 HEMATOLOGIC VALUES IN AN EIGHT-YEAR-OLD BOY WITH ACUTE LEUKEMIA IN RELAPSE. A TEMPORARY HEMATOLOGIC REMISSION WAS ACHIEVED AFTER 300 R TOTAL-BODY IRRADIATION.

Two of the other three patients were in extremely critical condition before irradiation and expired on the third and eighth postirradiation days. The fifth patient ran an average course for 16 days and then developed pneumonia. In spite of antibiotics and supportive treatment, death occurred on the eighteenth postirradiation day.

The following conclusions seem warranted: 1) Remissions in acute leukemia may be produced by total-body irradiation alone; 2) postirradiation recovery of the hematopoietic system occurs earlier in leukemics than in normal persons; and 3) in leukemic patients treated by total-body irradiation and bone marrow infusion, care must be taken in interpreting a remission in the disease as evidence of a successful graft of the marrow because the remission may be the result of radiation alone.

Total-body Irradiation, 50 r and 100 r (D. A. White, B. W. Sitterson, G. A. Andrews, and R. M. Kniseley)

Since September 1959, 27 patients with chronic leukemia or malignant lymphoma have received 30 treatments of 50 r or 100 r of total-body irradiation. Two patients were lost to follow-up because of failure to return and early death; and in three the follow-up was interrupted by complications of the primary disease.

Of 13 patients with chronic lymphocytic leukemia, 10 received 50 r. One received 100 r later. Three were treated initially with 100 r. Little difference was noted in the hematologic and clinical response of the two groups. Most patients showed a significant drop in the white count, at times to leukopenic levels (Fig. 1). In general peripheral lymph nodes regressed considerably or entirely. Hepatosplenomegaly often regressed. Nearly all had definite subjective improvement.

More consistent subjective and objective results were noted in four patients with chronic myelocytic leukemia given 50 r. In all the white count lowered significantly. Splenic size regressed in all patients and hepatomegaly decreased in three. All had subjective improvement.

Perhaps our most significant results have been obtained in lymphosarcoma. Five of six have had an adequate posttreatment evaluation. Of two patients with far-advanced disease, one showed little objective or subjective improvement. The other had transitory subjective improvement with some regression of peripheral nodes. His leukocytosis disappeared, but anemia and thrombocytopenia increased. One patient, who received 50 r in December 1959, has remained asymptomatic with no recurrence of lymphadenopathy. Another patient

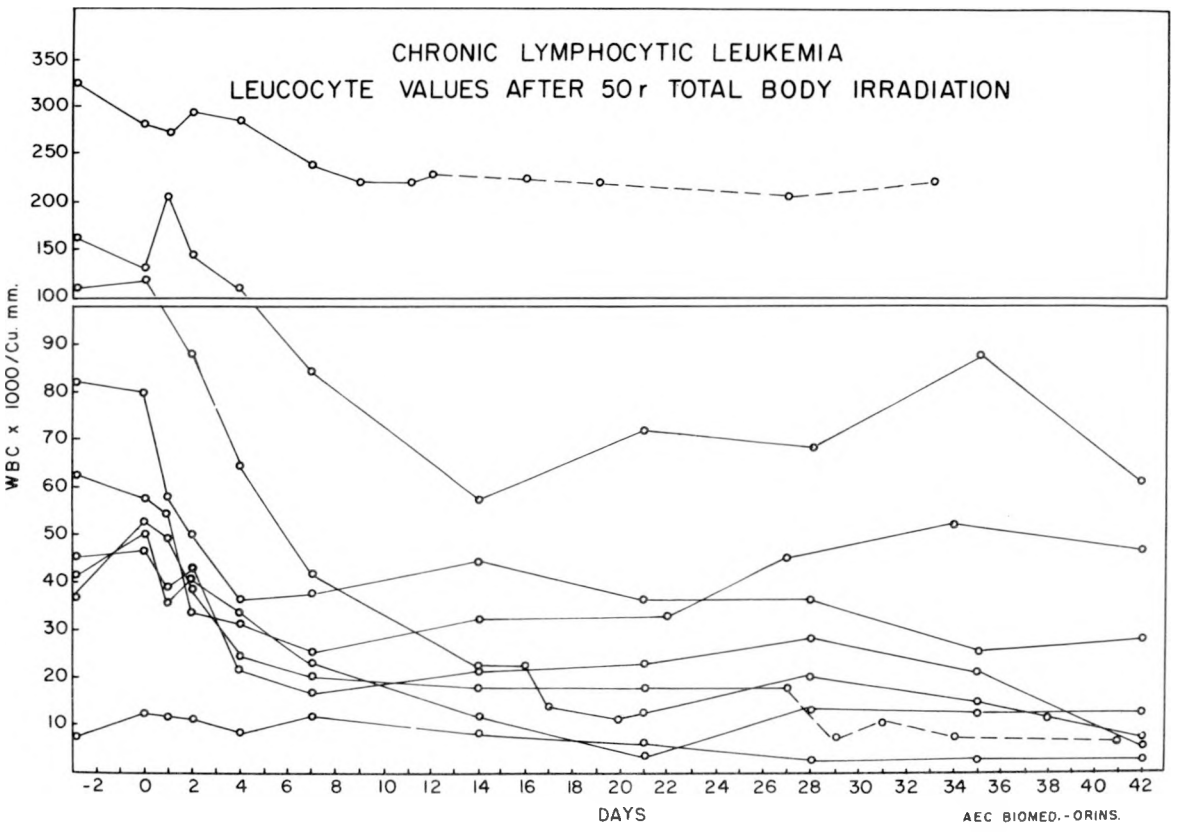

FIG. 1 LEUKOCYTE VALUES IN NINE PATIENTS AFTER A SINGLE EXPOSURE OF 50 R GIVEN IN THE ORINS TOTAL-BODY IRRADIATOR. WITHOUT EXCEPTION THERE WAS A SIGNIFICANT REDUCTION IN LEUKOCYTES. DOTTED LINES INDICATE INTERVENING THERAPY.

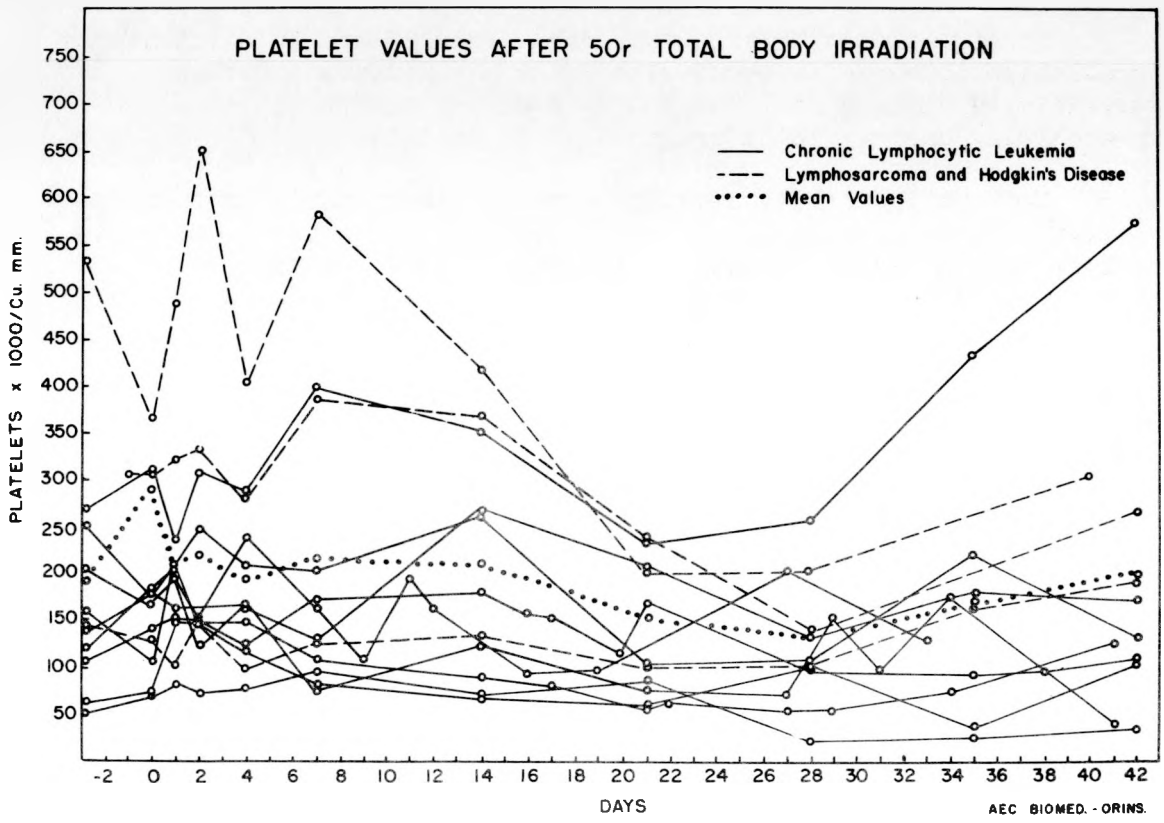

FIG. 2 PLATELET VALUES IN 11 PATIENTS AFTER 50 R TOTAL-BODY IRRADIATION. THERE IS A MILD DEPRESSION IN MEAN VALUES DURING THE FOURTH AND FIFTH WEEKS FOLLOWING IRRADIATION.

has responded twice to 100 r. A third patient with generalized adenopathy and a hypocellular marrow containing large numbers of lymphocytes improved little after 50 r. After 100 r she developed pancytopenia. Five months later, however, her blood count was normal and her marrow was cellular and histologically normal.

No significant benefit was noted after 50 or 100 r total-body irradiation to three patients with Hodgkin's disease. Rather surprisingly one who received 100 r developed pancytopenia and a hypocellular marrow, which previously was cellular.

Most patients had no early symptoms attributable to radiation therapy, but an occasional patient complained of nausea or anorexia or both on the day of therapy or the following day. With two exceptions these symptoms were limited to the 100 r group.

Except in the few patients that developed pancytopenia, little change in red cell values was noted. Most patients had some mild to moderate platelet reduction usually in the fourth or fifth week (Fig. 2).

Although this type of therapy in divided doses has been used for many years, the present series was undertaken to obtain more systematic laboratory data, with the treatment given in a single dose.

Bone Marrow Lipids in Animals Exposed to Total-body Irradiation
(F. Snyder, E. A. Cress, and N. Stephens)

An elevation in total lipids of bone marrow of irradiated rabbits was first reported by Dietz and Steinberg (1). Evidence from the clinical program at ORINS concerned with total-body irradiation effects in humans also indicates that fat fills the marrow cavity after exposure to irradiation. An inquiry into these changes has been initiated to aid in the interpretation of current problems associated with irradiation and bone marrow transplantation. The specific questions we are pursuing are 1) the chemical nature of the increased lipid content of irradiated marrow, 2) qualitative changes that might have occurred in these lipids, and 3) what physiological implications these changes might have on the metabolism of the marrow cells or on the metabolism of the whole animal.

Female CFN rats (175) received 800 r (4.08 r/min Cs^{137}) in the ORINS total-body irradiation facility. The animals were sacrificed at 1, 7, 14, and 45 days after exposure. Total lipids of the marrow were extracted and the chemical separations were made using silicic acid microcolumns, thin-layer chromatography, and gas-liquid chromatography. At 1 day after 800 r the triglycerides of the rat marrow doubled; after 7, 14, and 45 days the triglycerides had increased 4- to 5-fold.

1. Dietz, A.A. and Steinberg, B., Arch. Biochem. 23, 222 (1949).

A reciprocal relationship existed for the water and lipid content of the marrow. The major component of rat marrow was found to be triglycerides; however, five other distinct fractions (including cholesterol and phospholipids) were detectable on the thin-layer plates. The C_{16} and C_{18} fatty acids of the triglycerides isolated from the marrow of control and irradiated rats were similar to those of adipose and liver tissue.

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

Twelve patients treated for leukemia with total-body irradiation (in the range of 200 to 940 r) have now come to autopsy at ORINS. With the exception 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. 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 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. Three patients with diseases other than leukemia (Hodgkin's disease and poorly differentiated neoplasms) have also come to autopsy after total-body irradiation and are of particular interest in the study of the effects of such therapy on the "normal" marrow.

Biochemistry of Homologous Disease* (A. L. Kretchmar)

As part of the work on homologous disease, more complete study of the time course of the enlargement of the liver of irradiated animals given bone marrow indicated that the peak enlargement in mice

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

given isologous cells is at nine or ten days whereas in animals given homologous cells it is on the thirteenth or fourteenth day. Correlation between the increased liver weight and the weight of the spleen in these mice was striking. This was interpreted in terms of a relationship between the proliferating hematopoietic and immune system and the liver. Further evidence for this relationship was derived from biochemical data on the livers of these animals. Nitrogen content and the phosphorus to nitrogen ratio were increased whereas the glycogen and fat content was not much affected, although the glycogen content was reduced during the first two weeks after irradiation and bone marrow treatment. These findings ruled out a degenerative process as explanation of the enlarged liver and suggested an increase in cytoplasm, since this is in the major cellular compartment of nitrogen and phosphorus. The histology of liver of these animals showed that the major effect was in the cytoplasm of the liver cord cells. There were areas of hepatic hemopoiesis and cellular infiltration, but this effect was relatively minor and not closely correlated with the biochemical findings. The increase in aspartic acid of liver or irradiated mice given homologous cells was defined more completely. It began on about the third day after X-ray and bone marrow treatment and remained elevated for at least 35 days. The maximum levels were reached on about the seventh day. It was postulated that the homologous graft in some way disrupts the balance of synthesis and utilization of aspartic acid in the liver. This could be due to an acceleration in the rate of formation of purines and pyrimidines, which results in formation of excess glutamate. This, in the presence of the liver transaminase system, yields aspartic acid.

Nitrogen Balance Studies in Irradiation Chimeras* (Elizabeth Rupp)

An investigation of nitrogen balance in irradiation chimeras has been undertaken to determine whether impaired utilization of dietary nitrogen is present in homologous disease. In addition, further information with respect to the nitrogen intake of irradiated bone marrow treated mice would also be obtained in the nitrogen balance study.

Several experiments have been carried to 30 days postirradiation. In each of these experiments LAF_1 mice were used although the homologous bone marrow donors were (101 x C_3H)F_1 animals. The recipients were given 950 r and then 40×10^6 isologous (IBM) or homologous (HBM) bone marrow cells. There were two IBM and HBM animals and one normal and one X-ray-only control mice in each experiment.

Table 1 shows the nitrogen intake data for the most satisfactory of these experiments. There was no significant difference in the nitrogen intake of HBM mice as compared with IBM mice. Despite the similar nitrogen intake, the HBM animals averaged 1.75 g loss in body

*This work was done in collaboration with Dr. William MacArthur, Knoxville College.

weight while the IBM animals averaged a 2.15 g weight gain. Both bone marrow treated irradiated pairs ingested less nitrogen than did the normal control.

There was no significant difference in the nitrogen balance between the bone marrow treated irradiated pairs. In both, the mice were consistently in moderate positive nitrogen balance after the fourth posttreatment day.

It is concluded that storage of dietary nitrogen in mice treated with homologous bone marrow is different from that in mice given isologous marrow after irradiation. The 4 g average difference in body weight between HBM and IBM treated irradiated mice would be accounted for if 115 mg of dietary nitrogen were stored as antibody-antigen complex in the HBM animals. Most of this would presumably occur during the last 20 days of the experiment.

TABLE 1

AVERAGE DAILY MILLIGRAMS OF NITROGEN INTAKE BY IRRADIATION CHIMERAS

DAYS AFTER TREATMENT	IBM	HBM	X ray Only	NORMAL
1-10	90, 91	99, 71	84	118
11-20	129, 94	113, 97	32*	122
21-30	100, 100	90, 105		130
Final Body Weight	23.3, 25.5	19.5, 18.0	15	25.5
Initial Body Weight	23, 21.5	20, 21	23	24.0

*Died Day 15

Effects of Oxygen on Radiosensitivity of Rat Tissues (F. Comas)

Work has been continued on the influence of vascular hypoxia on the radiosensitivity of the bone marrow and of the Walker carcinosarcoma-256 in the rat. The purpose of this study is to determine whether it is possible to obtain a favorable differential effect upon the radiosensitivity of a tumor compared with that on normal tissues by occlusion of vascular supply to deprive both of oxygen. If such a difference could be obtained, this method might have important clinical applications in the treatment of cancer in humans.

This problem poses several questions: 1) degree of hypoxia by vascular occlusion; 2) direct effect of interfering with the vascular supply; and 3) quantitation of radiation effect.

Determination of the amount of oxygen in the tissues has been under study for two years. During the past year, development of a platinum microelectrode has permitted the measuring of oxygen partial pressures in the tissues of the rat. It has been found that after the iliac artery is clamped there is usually a fast drop in tissue oxygen, which falls to zero in less than a minute. The anoxia persists for the duration of the application of the clamp; normal tissue oxygen values are recovered upon removal of the clamp. This, however, is not always true; in some animals, after a period of anoxia, the tissue partial pressure of oxygen rises, presumably because of development of collateral circulation. Still, in other animals, complete anoxia is never obtained, indicating either that collateral circulation was already well established or that the occlusion of the major blood vessel was not complete.

In order to assess irradiation effects on tumor and normal tissue, the animals were given an intravenous injection of tritium-labeled thymidine one hour before sacrifice and the specific activity of the DNA of the various tissues was determined. To determine whether the operative procedure (laparotomy, dissection of the great vessels, and clamping of the left iliac artery for one hour) has any effect, a group of control animals was operated upon but not irradiated. The results indicate that DNA synthesis is initially depressed during the first six hours after operation with a rebound to values above normal in about eighteen hours. It is somewhat puzzling that the same changes occur in the bone marrow and tumor of the two sides of the animal - one side having the blood supply occluded, the other with the blood supply not interfered with. This point is being further investigated in an effort to see whether this effect is due to operative trauma, anesthesia, or reflex vasomotor changes on the nonoccluded vessels.

A protective factor of 2.5 has been found on the bone marrow temporarily deprived of blood supply during radiation at doses from 250 to 1500 r. The protective effect of anoxia on the tumor amounts to about 1.7.

Effects of Local Irradiation (Cobalt-60 Teletherapy) on the Peripheral Blood and Bone Marrow (F.A. Goswitz, G.A. Andrews, and R.M. Kniseley)

In contrast to the extensive published studies concerning the effects of total-body irradiation upon peripheral blood and bone marrow, there are few reports dealing with the changes produced by port irradiation. Eight patients, six with carcinoma of the urinary bladder and two with carcinoma of the lung, were treated with cobalt-60

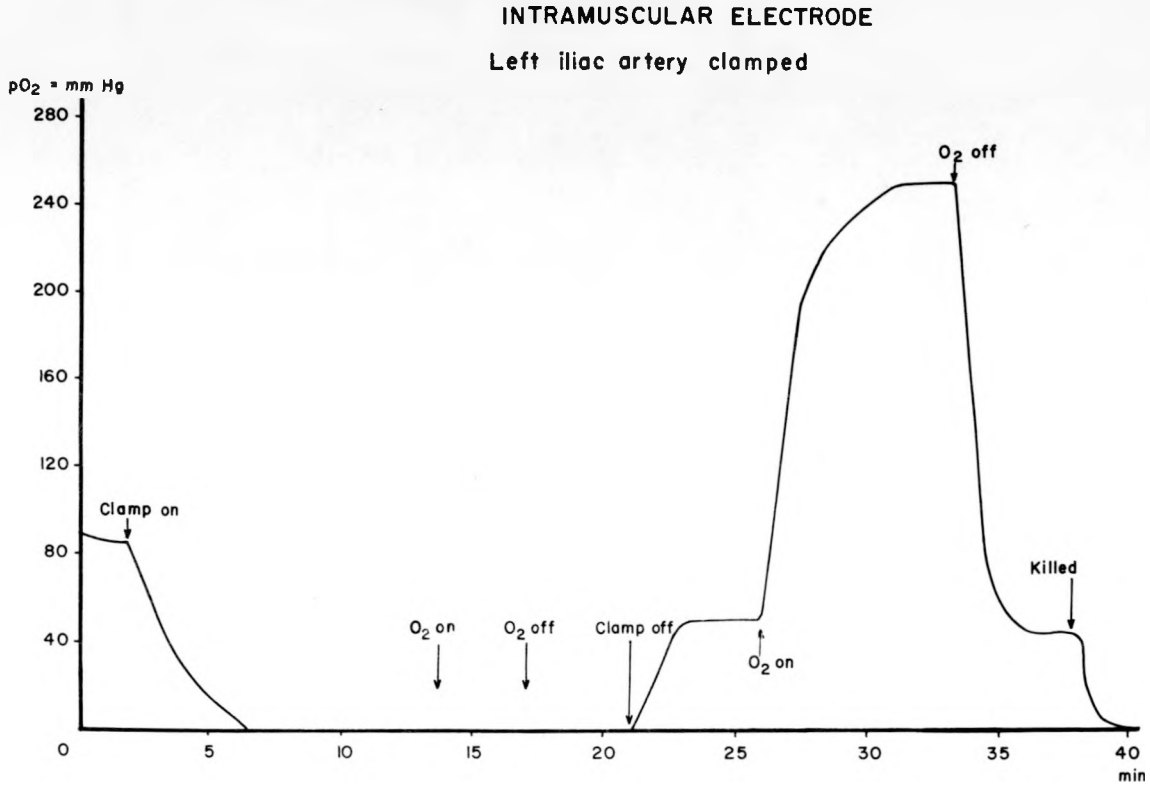

FIG. 1 EFFECT OF CLAMPING THE LEFT COMMON ILIAC ARTERY ON TISSUE OXYGEN LEVELS. INITIAL HIGH MUSCLE O_2 AND SLOW DOWNFALL AFTER CLAMPING ARE PROBABLY DUE TO DAMAGE TO A SMALL ARTERY ON INTRODUCING THE ELECTRODE. THERE IS COMPLETE ANOXIA OF THE TISSUE DEPRIVED OF ITS VASCULAR SUPPLY EVEN WHEN THE ANIMAL IS GIVEN 100% O_2 TO BREATHE. UPON REMOVAL OF THE CLAMP, TISSUE O_2 VALUES RETURN TO NORMAL; MUSCLE O_2 INCREASES RAPIDLY WHEN THE ANIMAL IS BREATHING PURE O_2.

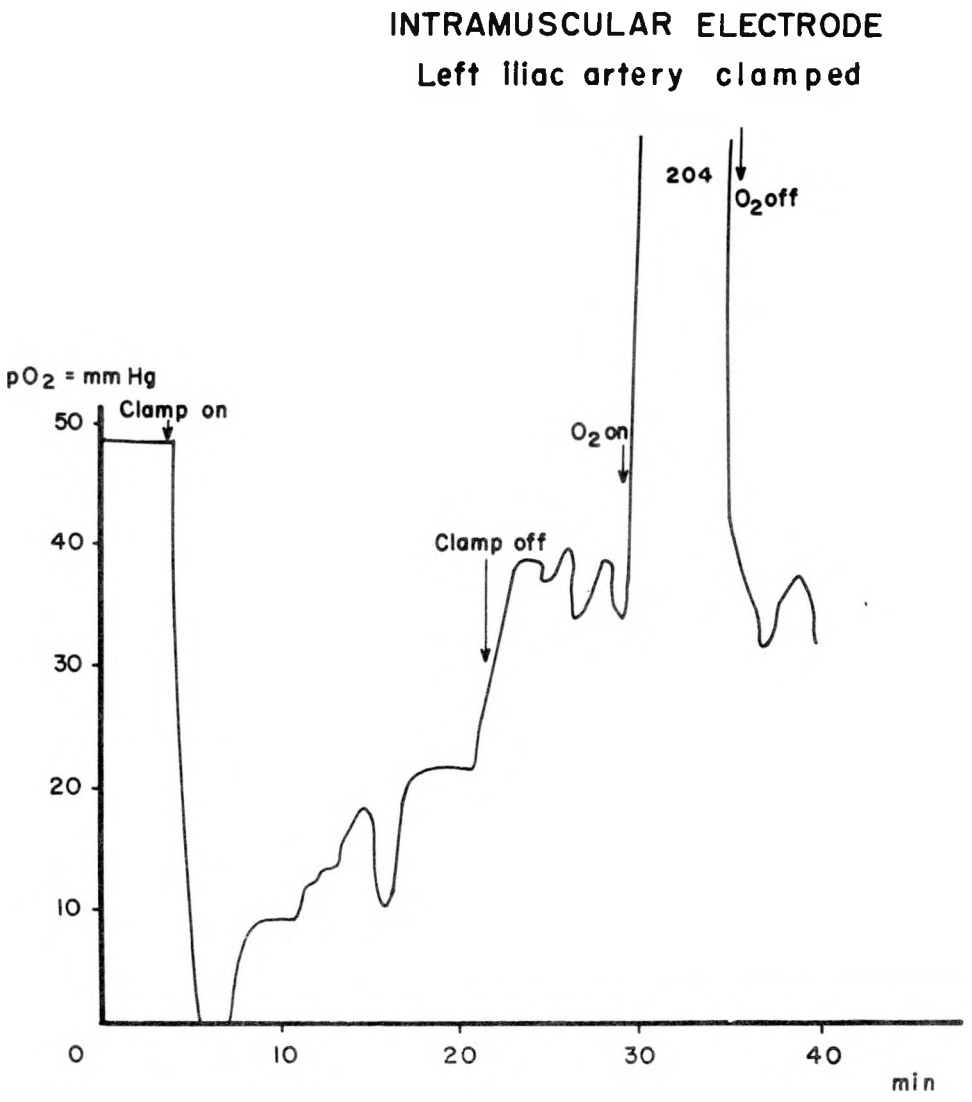

FIG. 2 EFFECT OF CLAMPING THE LEFT COMMON ILIAC ARTERY ON TISSUE OXYGEN LEVELS. ON APPLICATION OF THE ARTERIAL CLAMP, MUSCLE O_2 FALLS RAPIDLY TO COMPLETE ANOXIA, WHICH, HOWEVER, IS MAINTAINED ONLY FOR ABOUT TWO MINUTES. WAVERING RISE IN TISSUE OXYGEN IS DUE TO OPENING OF COLLATERAL BLOOD VESSELS.

teletherapy, in general a 4-week to 5-week course totaling approximately 6000 r. Bone marrow was aspirated from the irradiated sites at the following intervals: before therapy, midtherapy, at the completion of therapy, and at one, two, three, or four months postirradiation. Marrow was aspirated from control sites, not in the field of irradiation, at most of these times. Local irradiation was followed by a reduced total leukocyte and absolute lymphocyte count in the peripheral blood. The bone marrow demonstrated no morphologic changes in control sites after port irradiation. In the field of treatment, a panhypoplasia of the marrow appeared with reduction of red and white cell precursors, especially early forms, and a relative increase of plasma cells, "mononuclear cells," and lymphocytes. Qualitative marrow changes in the treated marrow sites consisted of an occasional giant-sized metamyelocyte, vacuolization of neutrophils, pyknosis of metarubricytic nuclei, and bilobulation of granulocyte nuclei. Hemosiderin increased in varying degrees in the irradiated marrow sites but showed no change in the control areas. The autoradiograms of marrow cells incubated in vitro with tritiated thymidine demonstrated a lower percentage of labeled cells in the treated than in the control sites. The marrow did not regenerate to normal cellularity in the irradiated sites during the four months after therapy; however, it is significant that hematopoietic cells are present as early as one month after completion of "cancerocidal" doses of radiation, and also that cells capable of DNA synthesis have been demonstrated at this time interval.

RADIATION PHYSICS AND INSTRUMENTS

Whole-Patient Counting (Douglas A. Ross and A. C. Morris, Jr.)

The value of measuring the total radioactive content of a patient or a normal person has been amply demonstrated. The procedure is useful in rate-of-turnover and other physiological studies, in toxicological investigations, in clinical diagnosis, in the back-calculation of accidental neutron dosage, in monitoring the intrusion of fallout - via air, food and drink - into "normal" people, and in evaluating the therapeutic problems arising from a wide variety of contaminating accidents, ranging all the way from a minor chemistry-lab spill up to a nuclear detonation.

Many of the patients who come to the ORINS hospital could serve as favorable experimental material enabling us to take full advantage of this method, and for the past three years we have been designing and planning. Special and difficult problems arise whenever one attempts to count the activity in any large-volume sample at near-background levels, and if the sample is a human subject, who cannot be expected to remain in one position for several hours, the many difficulties are multiplied. They center mainly around the nature and arrangement of the detector and its shielding enclosure, but the analysis, recording, and storage of the data also contribute their problems. In spite of ORINS's favorable position in regard to human material, we do have one serious problem: namely the huge range of activities that our patients present. They may contain anywhere from one or two hundred millicuries (of I^{131}, for example) down to a few hundredths of a microcurie, where the levels merge into the body backgrounds found nowadays in normal people. We have had to abandon hope of making any single instrument cover this 50-million-to-1 span; such an instrument could be designed, but it would be very costly. Accordingly, we plan to have three whole-patient counters: a "hot-patient counter" for the high-dose range; a "warm-patient counter" for the intermediate range; and finally the costly and challenging "cool-patient counter" for tracer-dose patients and normal people, whose count rates will be very little above ambient background.

Hot-Patient Counter. We have already built a counter of this type (see Fig. 1). Since high sensitivity is unnecessary, the detector-to-patient distance can be made large - about 2 1/2 meters as

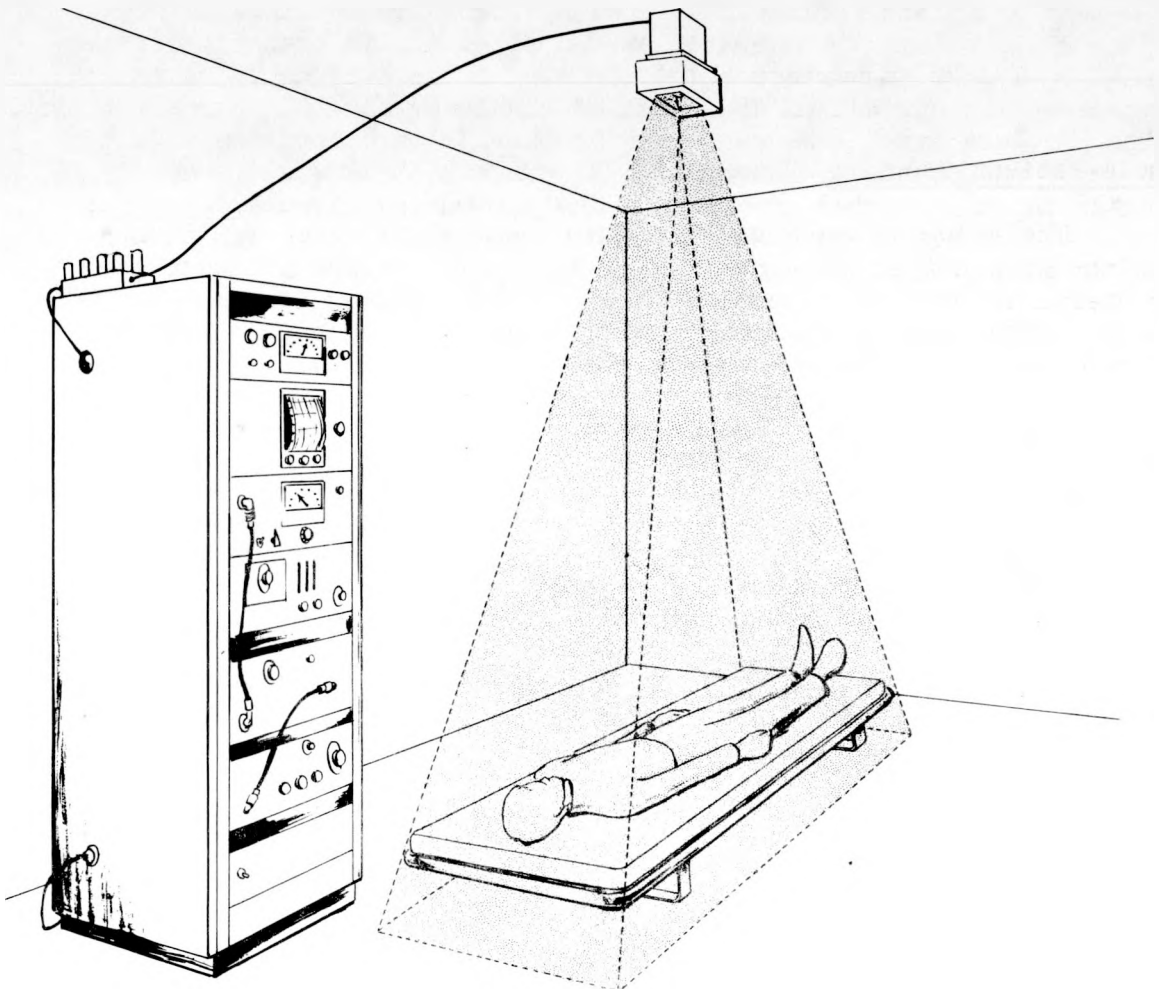

FIG. 1 "HOT-PATIENT COUNTER."
(RANGE: 100 MILLICURIES → 25 MICROCURIES)

it turns out - and this makes the detector geometry more nearly uniform, and easily repeatable. The patient lies supine on a stretcher a little above the floor; it is a basement floor, so that there is no significant radioactivity beneath him. If distribution is spotty we can count him both supine and prone, and average the two results. The detector is a 2-inch by 2-inch cylindrical, sodium iodide crystal looking down from up near the ceiling. Moderate lateral shielding is used to keep the background down to a reasonable figure, for there is no point in unnecessarily curtailing the range at the lower end. It turns out that with the bare crystal we can count iodine-131 in quantities down to about 25 microcuries. But a 100-millicurie patient would severely batter the unprotected crystal and its supporting circuitry, so for the hotter patients we cover the end of the collimator with either one or two removable slabs of half-inch lead, each of which cuts the radiation down to one-tenth of what it was, for energies in the 350-kev region. The bare crystal and the two stages of reduced sensitivity give us a working range of around 4000 to 1.

The hot-patient counter is currently in clinical service, and we are gathering data bearing on its usefulness and the improvement of the method of operation. Since a "monocular" detector, viewing the patient from one side only, is handicapped by the absorption of some radiation within the source, it becomes highly desirable to use a realistic phantom in counting the dose (or a known fraction of it) for comparison with the patient. But patients vary widely in dimensions if not in composition, and an artificial phantom is hard to devise. For the moment we are studying the feasibility of using the patient himself for the phantom by counting him shortly after administration of the dose - namely as soon as it becomes evenly distributed but before it is concentrated in the stomach, bladder, or thyroid gland.

Most of the data collected so far deal with the movement of iodine through the patient 1) when some normal thyroid tissue is present; 2) when functioning thyroid cancer is present, but no normal tissue; and 3) when neither normal nor malignant thyroid tissue is present. The plots of total patient content versus time fall roughly into three groups, corresponding to the three categories listed (see Fig. 2). The most rapid disappearance occurs, of course, when no thyroid-type tissue is present to immobilize the administered iodine, and it is clear already that thyroidectomized cancer patients in whom this rapid disappearance does not occur should be searched carefully for hidden metastases. This series of patients is still small, but it is being expanded, with improving technique, as fast as suitable clinical material becomes available.

Warm-Patient Counter. We prefer to postpone the design of a permanent warm-patient counter until later, after we know the overload point of the future cool-patient counter, but in the meantime we are

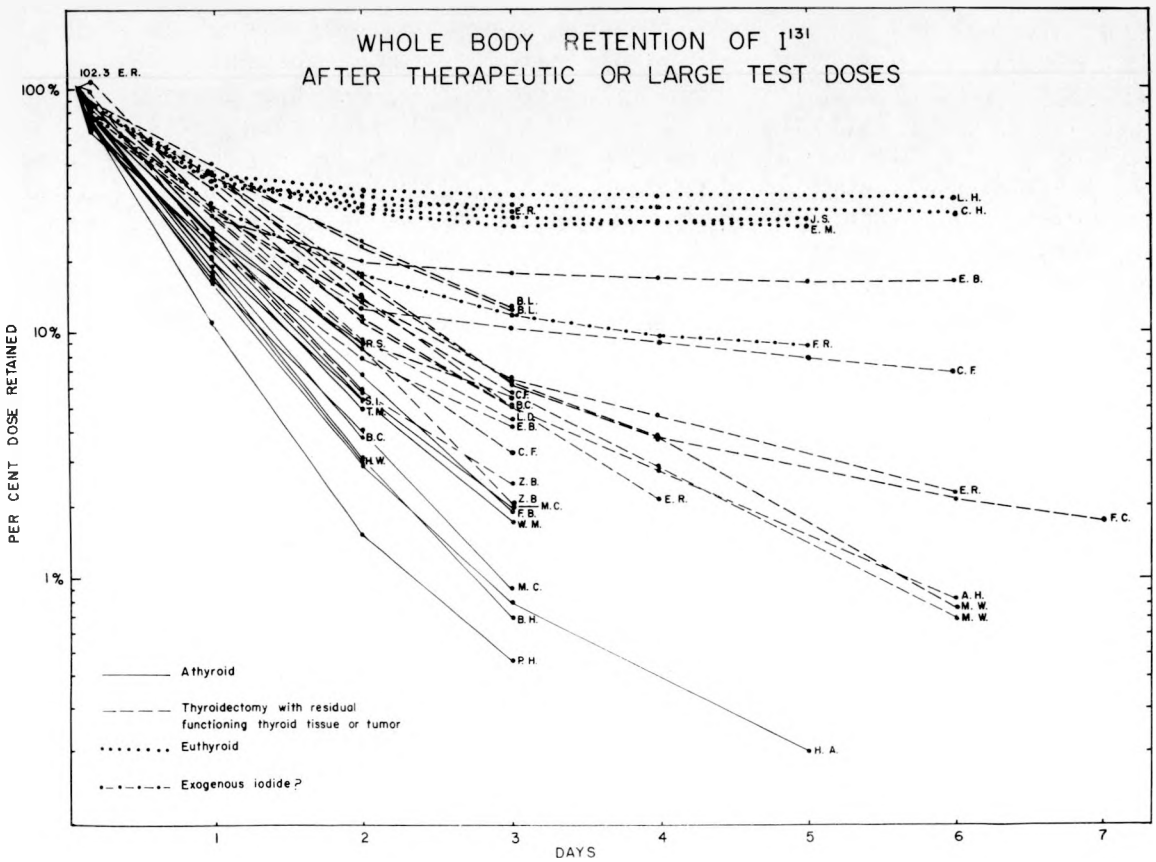

FIG. 2 THE RADIOIODINE RETENTION IN PATIENTS COUNTED WITH THE "HOT-PATIENT COUNTER" SHOWS DISTINCT GROUPINGS OF THE CURVES ACCORDING TO THE AMOUNT OF FUNCTIONING THYROID TISSUE.

exploring the use of our present linear scanner for intermediate activity levels. The linear scanner's normal job is to sweep the patient through a detecting gap for the purpose of plotting out a contour of count rate in terms of position on the long axis of the body. This tells us in what cross-section(s) of the patient the administered activity is concentrated. But one can put a scaler on the detectors and add up the pulses as the sweep proceeds, in which case the total gives a measure of the whole-body count. There are 12 crystals (NaI, 2" x 2") in this detector system, and the working distance (average) is around 50 cm, so although each plane of the patient is visible to the detectors for only a rather short time, there is, nevertheless, considerably more sensitivity available than with the monocular, hot-patient counter. Moreover, the crystals view the patient, who lies on his back, from both above and below, so that sensitivity of detection is made reasonably uniform. There are still some electronic troubles associated with this system, but current indications are that it will be usable down to activity levels of around one microcurie. We are using this system to follow the course of excretion of an administered radionuclide in patients who have become too lukewarm to stand out above background in the hot-patient counter.

Cool-Patient Counter. This is the whole-body counter in the usual sense, designed to provide a high-sensitivity detector located in a special counting room where enormous pains are taken to reduce ambient background to an extremely low level. To provide a fallout-proof, contamination-proof, teletherapy-proof, and hot-patient-proof location for this counter we are building a new, underground extension from the north end of ORINS hospital's middle floor. This annex we propose to call the "low-background facility," to remind everybody to keep even moderate amounts of radioactivity well away from it. Patients about to be counted will leave their clothing in an undressing room, take a shower to wash off any surface contamination, and put on a clean nightgown in which to be counted. They will lie on a stretcher supported on a track system so that they can be pushed through a circular opening into a steel-and-concrete-shielded counting room called the "Cave." Analyzing, processing and recording instruments will be located in the adjoining Clean Laboratory, and there is to be a small office where the data can be examined, recorded, and filed. After being counted, the patient is pulled out into the Clean Laboratory and returns to the office, where he recovers his clothing.

Since two or more radionuclides are always present even in normal people, the detector system will be designed to permit spectral analysis, so that the various components of the emitted radiation can be sorted out. The new facility is scheduled for completion around the end of 1962.

Total-Body Irradiation Dosimetry (R.L. Hayes and W.R. Butler, Jr.)

Aqueous chemical dosimetry is a convenient method for the measurement of absorbed dose, for such dosimeter systems can act as their own phantom media. The well-known ferrous sulfate system has been used to determine the effect of body size on absorbed dose in the ORINS cesium-137 total-body irradiation facility. Compartmentalized phantoms simulating an adult, an adolescent (12 years old), and a child (3 years old) have been used for this purpose. The compartmentalization made possible the determination of the average absorbed dose to the different parts of the anatomy as well as the average dose to the total body. The ratios of the adolescent and child average total-body absorbed dose to that for the adult were 1.05 and 1.07, respectively. Similar ratios for the various phantom compartments did not exceed 1.12. In previous depth-dose measurements in these same phantoms, the lowest percentage of the air dose encountered was 46, 52, and 65% for the adult, adolescent, and child. These measurements indicate that the design of the total-body irradiation facility effectively overcomes dose variations due to differences in body size.

ORINS-ORNL Scanner Evaluation Program (C. C. Harris, Oak Ridge National Laboratory)

In November 1959, the prototype of a new radioisotope scanner developed by the Medical Nuclear Instrumentation group at the Oak Ridge National Laboratory was placed in clinical service at the ORINS Medical Division. A program of evaluation of the scanner in clinical service was begun in order to test the success of improvements in collimation, electronics, and recording embodied in the scanner.

The scanner was expected to be used in studies on thyroid disease, and in localization of metastatic thyroid carcinoma. To broaden the clinical use, a small program of localization of intracranial neoplasia was started with the cooperation of regional neurosurgeons. In addition, some liver scanning was done.

In the physical evaluation of the system, the expected reliability was found. The system has operated more than 22,000 hours, with less than 50 hours of lost time for maintenance and repairs. Several important modifications to the control system, suggested by Ruth Black, were made. The results are that scanner use is now simpler and operator errors are less likely. With the help of A. C. Morris, Jr., the digital photorecording system was improved and shows promise of improving the clinical interpretation of scan records.

Clinical results in the brain-tumor program have been gratifying. Several tracer compounds have been used, with radioiodinated human serum albumin serving as a "standard." Arsenic-74 (sodium radioarsenate)

and Hg^{203}-Neohydrin were used. Of approximately 40 patients handled, positive evidence of space-occupying lesions were found in 17. Confirmation at surgery or autopsy was obtained in 10. Two were considered confirmed by conventional radiographic techniques. In four no confirmation has been obtained. The remaining positive scan was a doubtful localization; surgery showed a diffuse hemorrhagic condition in the region of the indicated "lesion." One subdural hematoma, known to exist, was not visualized. Some of the negative scans, particularly of the "doubtful-negative" class may yet be shown to be lesions that were missed. No false positives were obtained with the exception noted. In those confirmed surgically the scan record appearance of the tumors agreed extremely well with actual tumor outlines.

Several of the brain scans have been used as test cases in the development of a data-extraction method, in which a scan record itself is scanned to produce a more easily interpretable record.

Clinical results in scanning of other organs are given elsewhere in this report.

MEDICAL NUCLIDES AND LIPID METABOLISM

Internal Dose in Man (R.L. Hayes, W.R. Butler, Jr., and J.E. Carlton)

Present estimates of the radiation dose received by the human gastrointestinal tract as the result of the ingestion of various radionuclides are based on a "standard-man" model of the GI tract. The actual dose received by individuals will, of course, vary greatly from person to person. Previous studies in animals, where *in vivo* dose measurements were made with glass microdosimeter rods showed extreme variations in dose from animal to animal. These observations led to the initiation of a tracer study in man for the purpose of estimating the dose to the lower large intestine on an individual basis. The lower large intestine would normally be the critical portion of the tract for nonabsorbed radionuclides having appreciable half-lives. Direct dose measurements in man are, of course, impractical; however, by using this tracer technique to determine the concentration-time behavior for passage of radioactivity, it is possible to estimate the maximum dose that an individual could have received. The ratio of the person's estimated dose to that for the standard man then constitutes a measure of that person's variation from standard-man behavior. By making such measurements on a sufficient number of people it should be possible to make some assessment of the appropriateness of the "standard-man" model of the GI tract. Figures 1 and 2 show the results of this study to date. The subjects used were patients having normal gastrointestinal tracts, with an age range from 7 to 74. No obvious relationship between age and dose index is yet apparent. As indicated in Fig. 2, a decrease in half-life tends in general to decrease the dose index. The average dose index for a long-lived radioisotope is 2.0 while that for a 1-day half-life is at present 1.3.

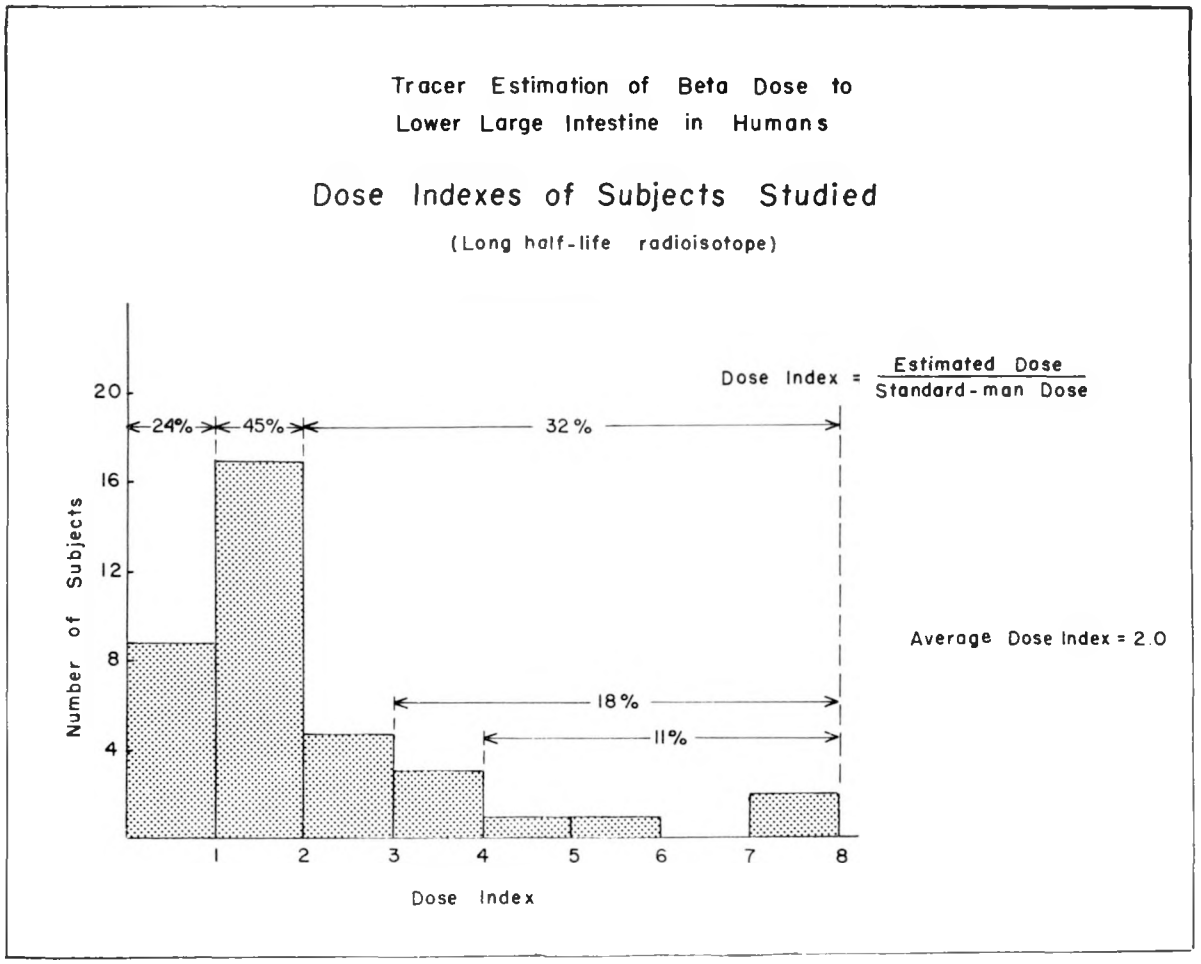

FIG. 1 INTESTINAL DOSE IN MAN FROM INGESTED RADIOACTIVITY.

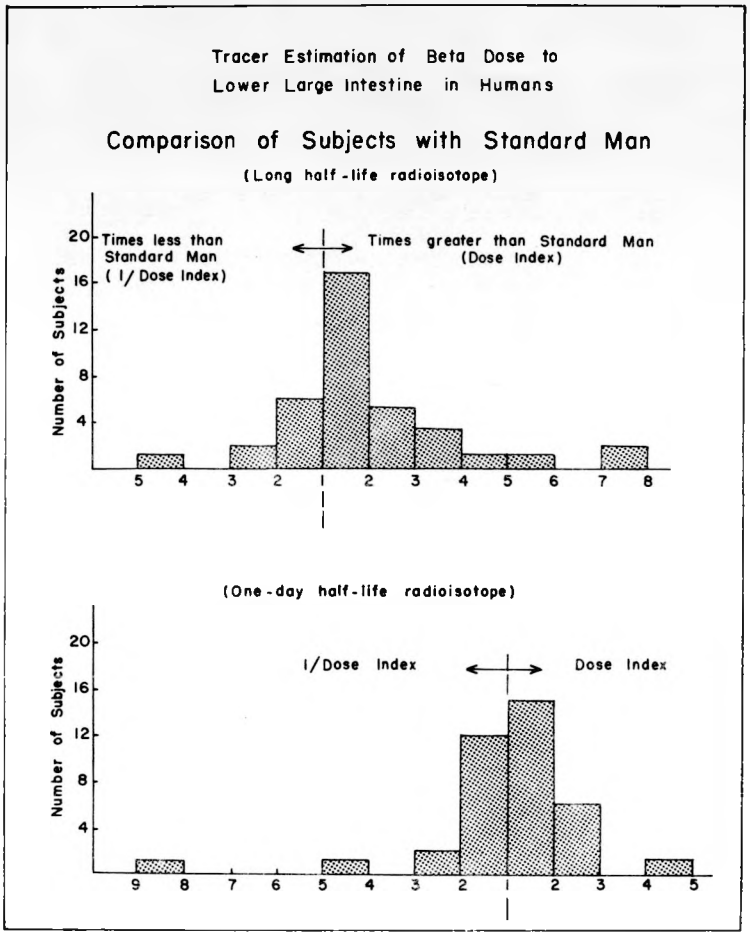

FIG. 2 EFFECT OF HALF-LIFE ON DOSE TO LOWER LARGE INTESTINE IN MAN.

The Effect of Cerium on Lipid Metabolism (F. Snyder, N. Stephens, E.A. Cress, and G.C. Kyker)

Earlier reports from this laboratory concerning biochemical studies of the fatty liver that occur after intravenous administration of some of the stable rare earths were summarized in the Report for 1960 (1). The evidence suggests that the initial effects of cerium on lipid metabolism are probably mediated through a hormonal imbalance. Work during 1961 has dealt with two facets of the problem: 1) the chemical composition of the triglycerides that were found to accumulate in the cerium liver; and 2) the secretory capacity of that liver to release triglycerides for extrahepatic purposes.

The use of thin-layer chromatography has revealed that the fatty acid composition of triglycerides from adipose and liver tissues is identical in both control and cerium-treated rats (Fig. 1). However, since the increase in liver lipids caused by cerium is entirely due to triglycerides, the total fatty acid composition of those liver lipids becomes similar to that of adipose fat. The fact that triglycerides are not qualitatively affected by cerium indicates that the esterification of fatty acids in the liver is not selectively altered by cerium. The use of C^{14}-labeled fatty acids to assess the relative turnover of the fatty acids that occurs in triglycerides is expected to yield additional evidence on our concept (2) that an increased mobilization rate of adipose fat to the liver occurs in cerium rats. The effect of cerium on two liver enzyme systems (3,4) does not appear important as a triggering mechanism for the fatty infiltration.

The proposition used for explaining the fatty accumulation in the CCl_4 fatty liver (5) can be rejected as an explanation for the rare-earth fatty liver. In the CCl_4 fatty liver, Recknagel et al. (5) have shown that the secretion of triglycerides is markedly inhibited. Such a block in the normal secretion of triglycerides would result in the abnormal accumulation of liver triglycerides. We have used the procedure of Recknagel and co-workers to test this possibility. Triton, a surface-active agent, was used to prevent the escape of triglycerides from plasma; under these conditions it has been shown that about 90% of the plasma triglycerides come from the liver (6). Any agent, such as CCl_4, that inhibits the release of liver triglycerides also decreases the plasma triglycerides that accumulate after the administration of Triton. Table 1 shows that a lowered release of glycerides from the liver is not an important factor in accounting for the fatty liver induced by rare earths.

1. ORINS-39, Report for 1960.
2. Snyder, F. and Stephens, N., Proc. Soc. Exp. Biol. Med. 106,202 (1961)
3. Snyder, F., Baker, F., Rafter, J., and Kyker, G.C., Biochim. et Biophys. Acta 43, 554 (1960).

4. Snyder, F. and Cress, E., Experientia 17, 303 (1961).
5. Recknagel, R. O., Lombardi, B., and Schotz, M.C., Proc. Soc. Exp. Biol. Med. 104, 608 (1960).
6. Byers, S.O. and Friedman, J., Am. J. Physiol. 198, 629 (1960).

TABLE I

EFFECT OF CERIUM AND ETHIONINE ON THE RELEASE OF ESTERIFIED FATTY ACIDS FROM LIVER

Treatment	μEq Esterified Fatty Acid in Neutral Lipids/100 ml Plasma
Control	138
6-hr Triton	6108
1-day cerium, 6-hr Triton	5256
2-day cerium, 6-hr Triton	3167
3-day cerium, 6-hr Triton	4444
1-day ethionine, 6-hr Triton	1547
2-hr Triton	1838
1-day cerium, 2-hr Triton	2125
3-day cerium, 2-hr Triton	1719
1-day ethionine, 2-hr Triton	576

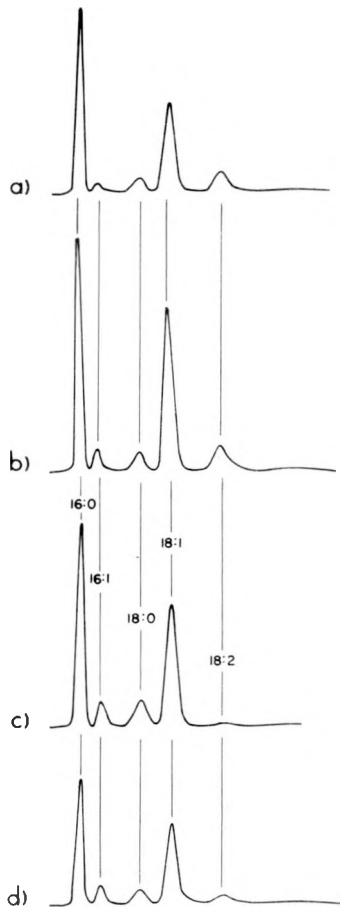

FIG. 1 GAS CHROMATOGRAMS OF TRIGLYCERIDES ISOLATED BY THIN-LAYER CHROMATOGRAPHY OF (A) CONTROL LIVER, (B) CERIUM LIVER, (C) CONTROL ADIPOSE TISSUE, AND (D) CERIUM ADIPOSE TISSUE.

Physiological Factors and Fatty Liver Due to Cerium (G. C. Kyker, J. J. Rafter, and F. Snyder)

Hormones. Earlier characterization of rare-earth fatty liver (Fed. Proc. 16, 207, 1957; J. Lipid Res. 1, 125, 1959) emphasized the prominent influence of sex, adrenalectomy, and hypophysectomy in affecting or preventing this metabolic disorder. These studies were extended to include other endocrine factors. Thyroidectomy has also proved regularly effective in protecting against fatty liver due to cerium. The gland was removed surgically in a group of animals and destroyed by a therapeutic dose (0.5 millicurie per rat) of iodine-131 in another group. The removal of thyroid function was verified in each experimental animal by linear scanning after a test dose of radioiodine. Both groups had full protection, and replacement therapy by thyroid hormone reversed the protection. This reversal and the coincident parathyroidectomy that occurred during surgical thyroidectomy allows the interpretation that the parathyroids do not affect this fatty infiltration. Demedullectomized rats responded to cerium like intact animals so that the earlier protection of male rats by adrenalectomy can be attributed to some cortical hormonal factor or factors. More experimental effort with at times equivocal results has occurred in the study of partially pancreatectomized rats (alloxan diabetes). Variations in the response to this treatment now appear explicable by varying responses to alloxan. Rats with severe alloxan diabetes verified by blood sugar levels are usually protected against cerium fatty liver.

Vitamins. Several groups of female CFN rats have been tested for cerium fatty liver after receiving a massive intramuscular dose of a multiple vitamin B-complex preparation (Folbesyn, Lederle). The fatty liver was prevented in most, but not all, of the rats. This possible relationship was also tested from the opposite direction. Since weanling rats require much larger doses of cerium (3 to 5 x) to cause fatty liver, young rats with severe B-complex avitaminosis were tested for an increased susceptibility to cerium; here no effect was observed.

Caloric Intake. Various earlier observations showed a general toxic effect of intravenous cerium indicated by reduced intake of food, reduced activity, reduced body temperature, and reduced metabolic rate. Rats conditioned to 5% and 10% glucose in place of ordinary drinking water will continue to consume a large fraction of their normal average caloric requirements even after cerium treatment. The degree of fatty infiltration appears as an inverse function of the glucose consumed. The relationship also appeared to hold for the total calories consumed (reduced intake of colony ration plus glucose in drinking water).

Intravenous Cerium in Rat Liver (G. C. Kyker, J. J. Rafter, and F. Snyder)

Measurements of the localization in liver of intravenous doses of cerium were made previously to correlate its presence with the acute transient fatty infiltration caused by this element. It was soon apparent that there was no correlation between the liver content of cerium and the fatty infiltration, that the metabolic disorder was due to an effect immediately after injection, that localization proceeded rapidly for 10 to 12 hours, and that cerium deposited in the liver was quite static. About 60% of the dose deposits in liver within four hours and 70 to 75% within 10 to 12 hours.

These measurements have been continued and now include experimental periods through 434 days. The biological half-time in liver exhibits three phases: 35 days during the first month after treatment, 130 days during the next five months, and more than 1200 days for results beyond six months. These results are obtained on the adult female CFN rat, which is most susceptible to the metabolic disorder. Weanling rats require larger doses of cerium to cause fatty liver. Their rate and extent of localization of the dose in liver is the same, but their loss of liver cerium is much more rapid, showing a half-time of one week for the first phase; extended time functions were not continued on the weanling rats.

Other measurements on animals treated in a variety of ways that prevent the development of acute fatty liver show similar localization of cerium. Also, in a series of numerous repeated doses at weekly intervals, the same degree of localization occurs for each individual dose.

DIAGNOSTIC AND THERAPEUTIC RADIOISOTOPES

Scanning Program (B. W. Sitterson, D. A. Ross, and G. A. Andrews)

A research scanner designed and built at the Oak Ridge National Laboratory was put into clinical use at the ORINS Medical Division in January 1960. The outstanding (but not the only) improvement offered by this scanner is a 37-hole, honeycomb collimator fabricated in gold, with surrounding lateral shielding of tungsten. The gold provides greatly increased opacity between the focusing holes, while the tungsten gives enhanced suppression of the patient's body background. These features are of special value during the scanning of intracranial tumors, where the target/nontarget ratio is often low.

Liver Scanning. For the study of liver disease we have used mainly colloidal gold-198 and iodine-131-labeled rose bengal, both administered intravenously. In patients with metastatic liver disease we have run a few scans using iodinated human serum albumin, but these have not been rewarding, in spite of favorable reports in the literature. Rose bengal is much more informative. Following the policy developed at Roswell Park, we first give the patient 150 to 300 mg of stable rose bengal, and (about 10 minutes later) 0.3 to 0.5 millicuries of the I^{131} preparation. Displacements of the liver from the normal position, changes in size or shape, and the presence of any reasonably large tumor or cyst, are usually well shown. In a few cases of lymphoma the patchiness of the liver scan has suggested irregular liver involvement. Failure of the gallbladder shadow to materialize after half an hour or so is informative, and emptying of the gallbladder into the intestine is signaled by the appearance of dark patches in the upper intestinal region. Rose bengal does not provide a spleen shadow; gold-198 does so, although only weakly.

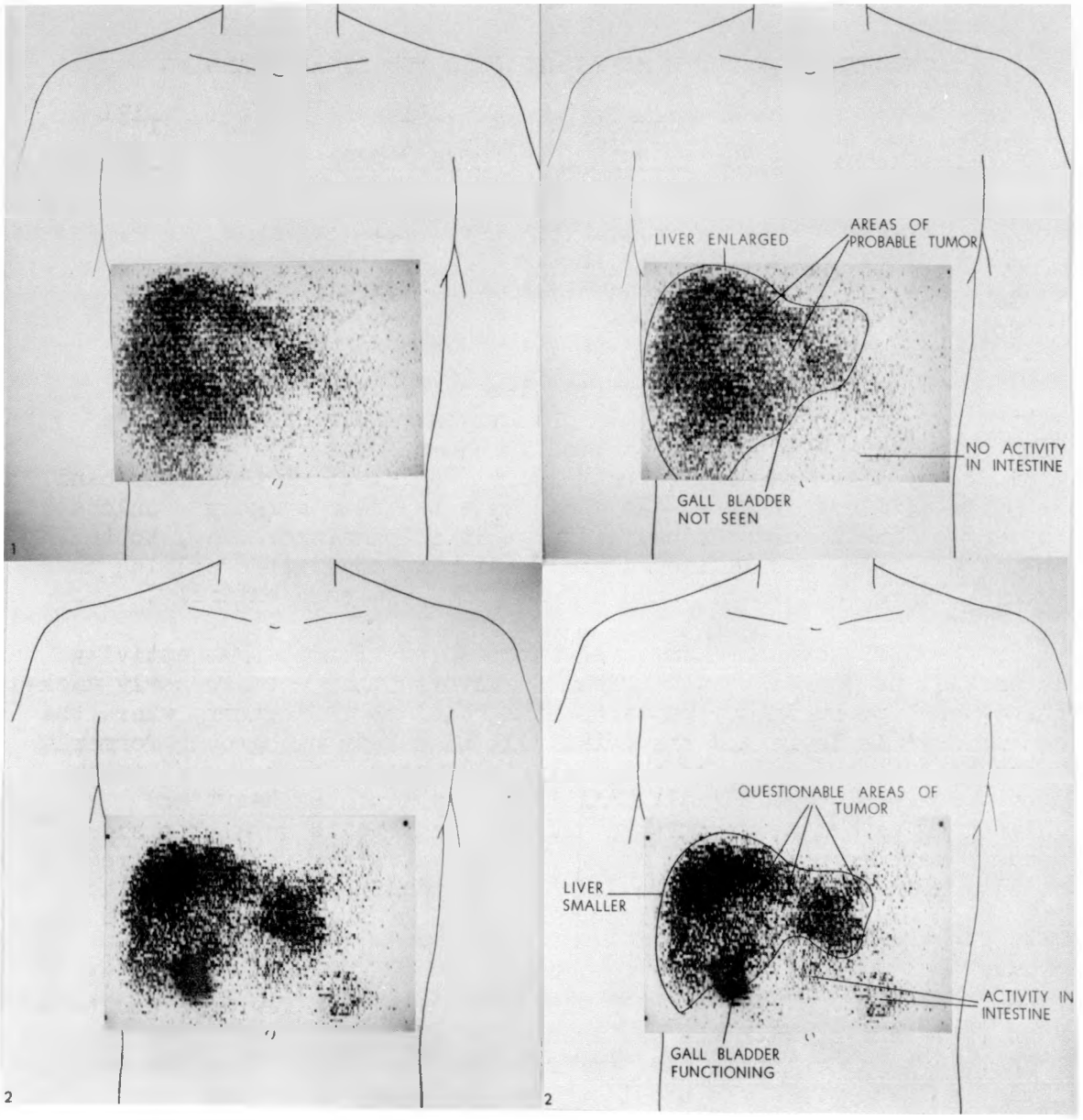

FIG. 1 TWO PAIRS OF SCANS ON A PATIENT WITH CANCER INVOLVING THE LIVER. ALL SCANS WERE PREPARED AFTER AN INTRAVENOUS DOSE OF ROSE BENGAL LABELED WITH IODINE-131. THE TWO UPPER PICTURES ARE OF THE SAME SCAN: THE ONE ON THE RIGHT HAS HAD ADDITIONAL MARKINGS TO HELP WITH INTERPRETATION. IT SHOWS THE FINDINGS DURING A PERIOD OF OBSTRUCTIVE JAUNDICE CAUSED BY METASTATIC CANCER. ON THE BASIS OF THIS SCAN, THE PATIENT WAS GIVEN EXTERNAL THERAPY WITH COBALT-60 TO THE AREAS OF APPARENT TUMORS IN THE MID-PART OF THE LIVER. SUBSEQUENTLY THE JAUNDICE CLEARED, THE PATIENT IMPROVED, AND THE SECOND SCAN WAS OBTAINED (SHOWN IN THE LOWER HALF OF THE PICTURE). AGAIN THE RIGHT-HAND SCAN IS A COPY OF THE LEFT ONE AND HAS HAD ADDITIONAL MARKINGS TO HELP WITH INTERPRETATION.

COMPARISON OF THREE MATERIALS USED FOR LIVER SCANNING

	Colloidal Au^{198}	I^{131} Rose Bengal	I^{131} Serum Albumin
Liver	+++	+++	+
Tumor in liver	Cold spot	Cold spot	±
Gallbladder	0	+++	0
Spleen	+	0	0

Thyroid Disease. In thyroid disease our scans have been particularly valuable, especially in thyroid carcinoma. Scans are helpful in the estimation of thyroid volume in hyperthyroidism, particularly after partial thyroidectomy. Nodules, on the other hand, are often difficult to see - as shown by subsequent surgery - unless they are of the hyperfunctioning type. It is hard, moreover, to tell a hypofunctioning nodule from a nonfunctioning one with overlying thyroid tissue, and small, cold nodules may be missed entirely.

The ORNL scanner's improved suppression of nontarget activity now enables us to see physiological structures that were formerly masked. This often happens in our patients with total thyroidectomy, where the dose of I^{131} is large and the relatively high body background formerly penetrated the shielding of the leaky collimator. We see now, in an uninvolved chest, the normal, slight darkening of the heart and mediastinum as compared with the "clear" lung fields on either side. We can sometimes note, in an athyroid neck, a faint, vertical streak of activity lying a little to the left of the midline and extending an inch or so above and below the jugular notch of the sternum. This might represent iodine held in the mucous secretions of the esophagus or trachea, but we do not really know where it comes from. We have no reason, however, to link it with disease; it lasts a few days and then fades out. Again, with the new scanner we almost routinely see oral iodine, perhaps caught in the tartar on the teeth; we often see salivary glands; and sometimes the nasal or lachrymal glands (we may suppose), or both, will darken the area of the nose.

In the abdomen we regularly see the stomach shadow, as heretofore, but now we may also see a weak shadow contributed by the airless and metabolically active liver, which will sometimes outline the right leaf of the diaphragm for us. We find vague areas of darkening here and there throughout the abdomen; these are usually due to intraintestinal iodine, but we must remain suspicious about them until we see them move, either spontaneously or in response to manual manipulation or to a cathartic or an enema. In one cancer patient an area of this kind remained stationary on repeated scanning, and it was ultimately traced to a bony metastasis in the ilium. Radioiodine in the intestinal tract

is a serious problem in a linear scan, for there it is much harder to be sure what it is.

What the foregoing boils down to is that while we can now see weak thyroid metastases, brain tumors, etc., better than before, we also see a number of normal shadows that call for considerable care in interpretation. As so often happens, the insrumental improvement has demanded a readjustment of standards.

Collimation for in vivo Counts of Cr^{51} and Fe^{59} in Spleen and Other Organs (Bill M. Nelson and Vichai Poshyachinda)

The conditions required for an assay of I^{131} in the thyroid gland in situ have been elucidated, but for other organs and isotopes external counting is only semiquantitative. Nevertheless, in vivo studies of the distribution of Cr^{51} and Fe^{59} have had rewarding clinical applications. We have tried to enhance the value of these studies by investigating the physical factors influencing external counts over such organs as the spleen, with primary attention to the effect of collimation. Isoresponse curves were plotted for a commercial collimator extending 86 mm from the front face of a 2-inch by 2-inch crystal, with a bore flaring to 80 mm distally. The collimated detector was applied to the side of a large plastic tank filled with water. A small Cr^{51} source was placed at multiple locations in the water and counts were recorded for each location. This was repeated with an Fe^{59} source, counting first the 1.1- and 1.29-Mev gammas, and then the scatter at the 270- to 370-kev Cr^{51} band. Similar curves were plotted for the same detector without the collimator, so that the crystal could be applied directly to the water tank. Subsequently, spleen and liver phantoms containing Cr^{51} and Fe^{59} solutions were counted in the water tank. The data show that shorter collimators not only increase the sensitivity of external counting over an organ near the surface but reduce the error due to faulty positioning and minimize body background.

Development of Radioisotope Diagnostic Tests (R. L. Hayes and W. M. Lauter)

Several iodine-131 labeled compounds have been prepared and studied as possible analytical aids in simplifying Segal's screening test for achlorhydria. Segal's test is based upon the acid release of the dye, Azure A, or the alkaloid quinine from a cation exchange resin when such materials are administered orally. The released compounds are absorbed and excreted in the urine. Determination of the urinary output then constitutes a measure of the secretion of hydrochloric acid. Substitution of a labeled compound would greatly simplify the analytical technique. Iodine-131 labeled iodo-azure A, iodo-chloro-quinine, and iodo-chloro-codeine have been prepared and subjected to in vivo animal

tests for iodine-bond stability. All three compounds were partially degraded as indicated by appreciable uptakes of iodine-131 in the thyroid gland. They are consequently not considered as suitable for use in the Segal test. These initial attempts to prepare a suitable labeled substance for use in the test were, with the exception of codeine, obvious first efforts to utilize the materials employed by Segal. The resin-bound substance does not have to be a dye or alkaloid but only a base with the proper affinity for cation exchange resin.

The measurement of urinary hippuric acid excretion after oral or intravenous administration of sodium benzoate is Quick's well-known liver function test. The analytical technique normally used in the test is time-consuming and subject to certain inaccuracies. Here again use of a labeled benzoate would considerably simplify the analytical procedure. Iodine-131 labeled o-iodo-benzoic acid has been prepared by direct synthesis and by an exchange reaction. In vivo experiments in animals have indicated, by the absence of any appreciable iodine-131 uptake in the thyroid gland, that the iodine bond is stable. Further studies will be carried out to determine whether this radioisotopic technique warrants trials in man.

Benzoic acid is detoxified and excreted in urine mainly as hippuric acid; a small portion is also excreted as benzoyl glucuronate. Other workers have reported that the benzoyl glucuronate excretion may be a more sensitive indicator of liver function than hippuric acid excretion. Preliminary work indicates that labeled hippuric acid and benzoyl glucuronate may be readily determined in the presence of each other through the use of counting and solvent extraction techniques.

TRACER AND BASIC BIOLOGICAL STUDIES

Antibody Synthesis by Human Cells (N. Gengozian)

Emphasis of study was placed on the development of an *in vivo* tissue culture system for propagation of human cells in the study of their antibody-forming ability. An *in vivo* diffusion chamber technique developed in 1954 by Dr. G. Algire (*J. Nat. Cancer Inst.* 15, 493, 1954) was used with slight modifications in the construction of the chambers. The materials needed to construct a diffusion chamber are a "donut"-shaped lucite ring and two millipore filters. One side of the lucite ring is covered with a millipore filter, which is sealed in place with a lucite-acetone solution. Human cells are then placed inside the small cylindrical space and the second millipore filter is used to seal the open end of the "donut," resulting in a diffusion chamber. The average pore size of the filters is 0.1 micron, which thus permits free diffusion of liquid nutrients between the chamber and its external environment but prevents passing of cells in or out of the chamber. Figure 1 shows a sealed diffusion chamber containing human cells about to be placed in the peritoneal cavity of a recipient mouse.

Irradiated mice and hamsters were used initially as recipients of the diffusion chambers. Of the two hosts studied, mice appear to be more suitable for longer periods of cell cultivation and also less troublesome relative to bacterial infections, which are apt to occur after radiation and the operative procedures. Results have shown the possibility of maintaining human lymph-node, spleen, and bone-marrow cells with this technique. Using commercial TAB vaccine as an antigen, which is mixed with the cells in the chambers, it has been possible to obtain antibody formation by the human cells. This antibody is detected in the chamber fluid collected from the diffusion chambers at varying intervals after cultivation in the recipient mouse. If a sufficient amount of antibody synthesis occurred, one could also detect antibody in the serum of the host, indicating the passing of antibody through the filters into the host environment. Table I shows the results of one experiment with normal human lymph-node tissue. In addition to the bacterial agglutinating antibody produced by the cells, verification of human protein synthesis was obtained by the detection and identification (by serum-agar diffusion) of human gamma globulin in the fluid collected from the chambers. This protein could not be detected in chamber fluids that were negative for bacterial agglutinating antibody.

Initial studies with titrated thymidine have shown a marked premitotic activity of the human cells cultivated in the chambers up to a period of 14 days postincubation in the hosts.

TABLE I

ANTIBODY RESPONSE BY HUMAN LYMPH NODE CELLS
CULTURED WITH S. TYPHI (T) ANTIGEN IN DIFFUSION CHAMBERS

Tissue Source:	Cells	Antigen	H Agglutinin Titer After Eleven Days in Culture	
Patient	x 10^6	x 10^6	Chamber Fluid	Mouse Serum
			Log_2	
	10	30	9	4
			8	4
			6	2
			4	0
			0	0
J. L.				
	10	20	6	3
			7	4
			8	5
			3	0
			3	0

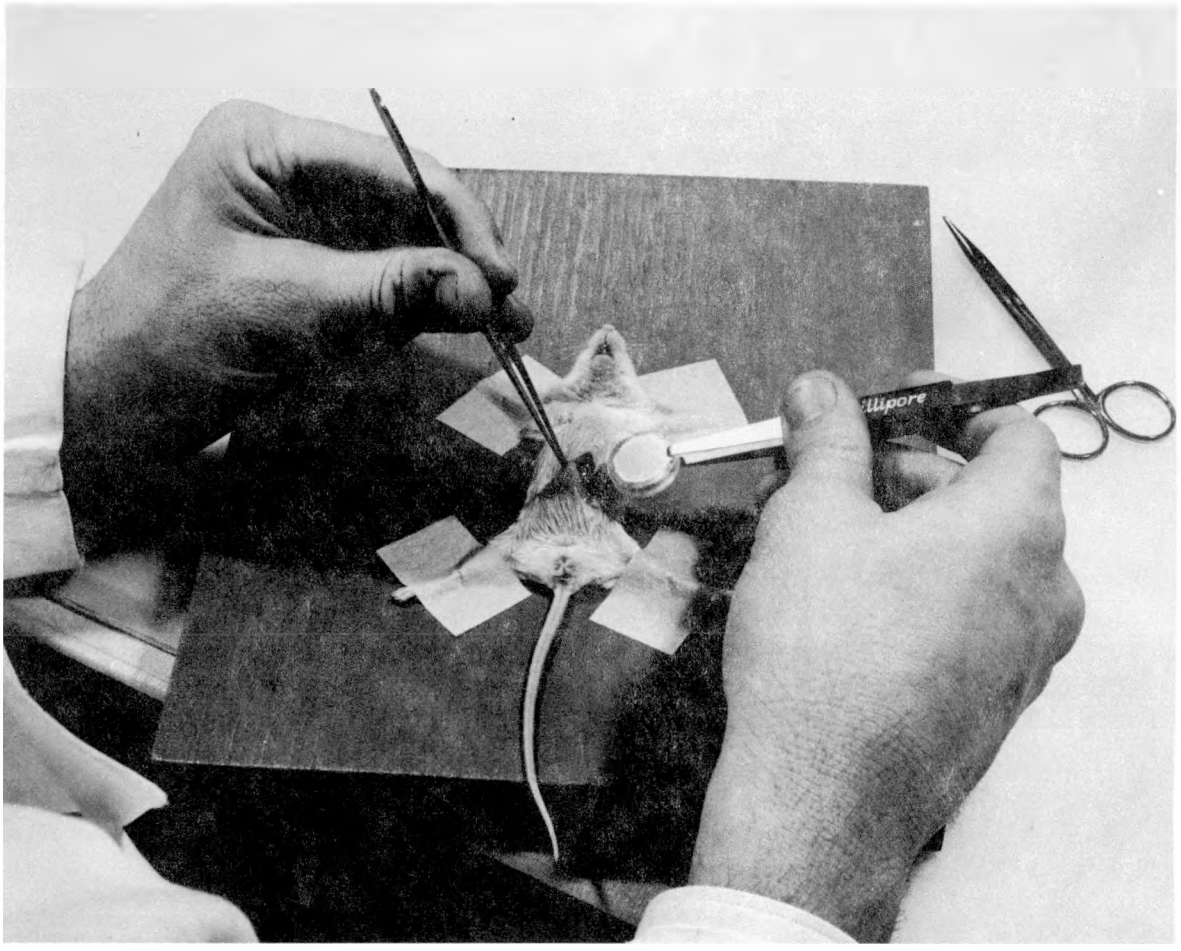

FIG. 1 DIFFUSION CHAMBER CONTAINING HUMAN CELLS BEING PLACED INTO PERITONEAL CAVITY OF IRRADIATED MOUSE THAT SERVES AS AN IN VIVO TISSUE CULTURE MEDIUM.

Sulfur-35 Studies in Man. I. Plasma levels and urinary excretion of inorganic sulfate (Helen A. Vodopick)

With the development of more refined techniques and instruments, radioactive detection of low-level beta-emitting isotopes has become easier. The counting of sulfur-35 in urine and plasma in a beta liquid-scintillation spectrometer was studied. The plasma levels and removal rate by urinary excretion were followed after a single dose of isotope and after multiple daily doses in five patients. In the single dose method, used in two patients, plasma levels of S^{35} showed a rapid decline, which, when plotted on semilog paper, gave a composite exponential curve indicating a multiphasic removal of the isotope into more than one compartment. Urine radioactivity in these patients showed parallel excretion curves. Within the first 24 hours, 65 and 70% of the total administered dose was recovered. The total recovery was 79 and 81% after five and nine days. In patients given multiple daily constant doses of S^{35}, the plasma level showed a slight gradual increase. Urinary excretion showed great variation in consecutive 24-hour urinary collections. With multiple daily injections over periods of two to three weeks, urinary recovery ranged between 83 and 91%. The urinary excretion varied considerably in consecutive 24-hour urinary collections. Total recovery of administered radioisotope was never achieved. Presumably the unrecovered portion is incorporated in tissues rich in sulfated mucopolysaccharides.

Sulfur-35 Studies in Man. II. Platelet survival (Helen A. Vodopick and R. M. Kniseley)

To augment the present knowledge of human platelet survival in pathologic states, carrier-free $Na_2S^{35}O_4$ was used to label platelets in vivo. Six patients were studied. One patient with no hematopoietic disease, two patients with polycythemia vera, and two patients with chronic granulocytic leukemia (and pronounced splenomegaly) had platelet survival times in the neighborhood of ten days. One patient with chronic granulocytic leukemia appeared to have a shortened survival time of seven days, the exact mechanism of which is unknown. All patients except the one with no hematopoietic disease had thrombocytosis. Because of a rather wide spread in values for radioactive platelet levels, precise life spans cannot be plotted.

Specific Activity in Radioisotopic Measurements (G. C. Kyker and Barbara Chastain)

In a laboratory procedure designed earlier for nuclear medical teaching, the goldfish proved convenient for illustrating the effect of specific activity on radioisotopic uptake. The model consisted of a series of dilutions of physiological saline that contained equal amounts of tracer sodium-22 and widely differing amounts of total sodium. After

placing a small goldfish in 200 ml of each dilution, the tracer remaining in solution was measured at intervals during two days. We have further studied and refined this useful procedure.

The present model uses ten 3- to 4-inch goldfish in a 4-liter volume placed in a small aquarium designed for radioactive solutions. In comparative measurements with sodium-22 and chlorine-36, the sodium and chlorine tracers were used in similar series of sodium chloride dilutions, and radiophosphate (P^{32}) was used in analogous dilutions of phosphate buffer, pH 7. The sodium and the chloride tracers gave similar qualitative results, but sodium exchanged faster. In a series of 1/5, 1/50, 1/200, 1/500, 1/1000 physiological saline, the movement of tracer sodium into the tissues of the fish increased with decreasing concentration. For example, in one 3-day study the uptake was less than 2% of the original tracer in the 1/5 dilution and more than 60% for the 1/1000 dilution. Expressed as millimoles of total radioactive and stable ion per kilogram of fish, the order of uptake was reversed. For example, the fish removed more than 30 millimoles of sodium per milligram in 2 days from the most concentrated pool, and less than 1 millimole in 5 days from the least concentrated pool. By comparison, the tracer phosphate gave quite variable results. Autoradiography, however, showed similar gradations for typical series with each of the 3 tracers.

METHODS

Autoradiography (W. D. Gibbs)

One major effort of the Medical Division has been an attempt to determine the distribution of radioisotopes in normal and neoplastic tissue. External counting of radioactivity (local area or whole body), external scanning, radioassay of aliquots of tissue, and autoradiography are the chief methods used. Since 1950 more than 19,000 gross and microscopic autoradiograms of human and animal specimens have been made. The techniques of autoradiography enable more precise demonstration of distribution than can be obtained by any of the other methods.

Gross autoradiograms have been made of whole organs and large bones obtained at surgery and autopsy from patients who have been given isotopes. Examples of isotopes studied are I^{131}, radioiodine labeled compounds, Cr^{51}, Fe^{59}, Rb^{86}, $Sb^{122,124}$, Ca^{45}, Ca^{47}, P^{32}, Au^{198}, Ga^{67}, Ga^{72}, Hf^{181}, Y^{90}, S^{35}, and Sr^{85}. Careful correlation of radioassay data and autoradiographic data is valuable in determining isotope concentration and distribution. This method of close correlation also minimizes the possibility of making completely erroneous interpretations of radioassay data. Few, if any, radioisotopes are distributed uniformly in tissues. If only radioassay data are collected, it is possible to make large errors in estimation of total organ content. The study of bones and bone lesions can be particularly difficult because of the variations in different parts of the skeleton, the alterations related to type of bone and growth centers, and the presence of varying amounts of bone marrow. Careful labeling of the site of the assay specimen and correlation with autoradiograms make it possible to more accurately determine the exact pattern of distribution and total organ content than can be done by radioassay alone.

It is unfortunate that, as yet, a convenient technique of quantitation of autoradiography has not been devised. One can only estimate differences in isotope concentration in a relative way between areas of one specimen. One should not make this kind of interpretation between specimens exposed on different films.

Although gross autoradiography has had the greater emphasis in the Medical Division program, various microscopic techniques have been used extensively and have contributed valuable information in specific research areas.

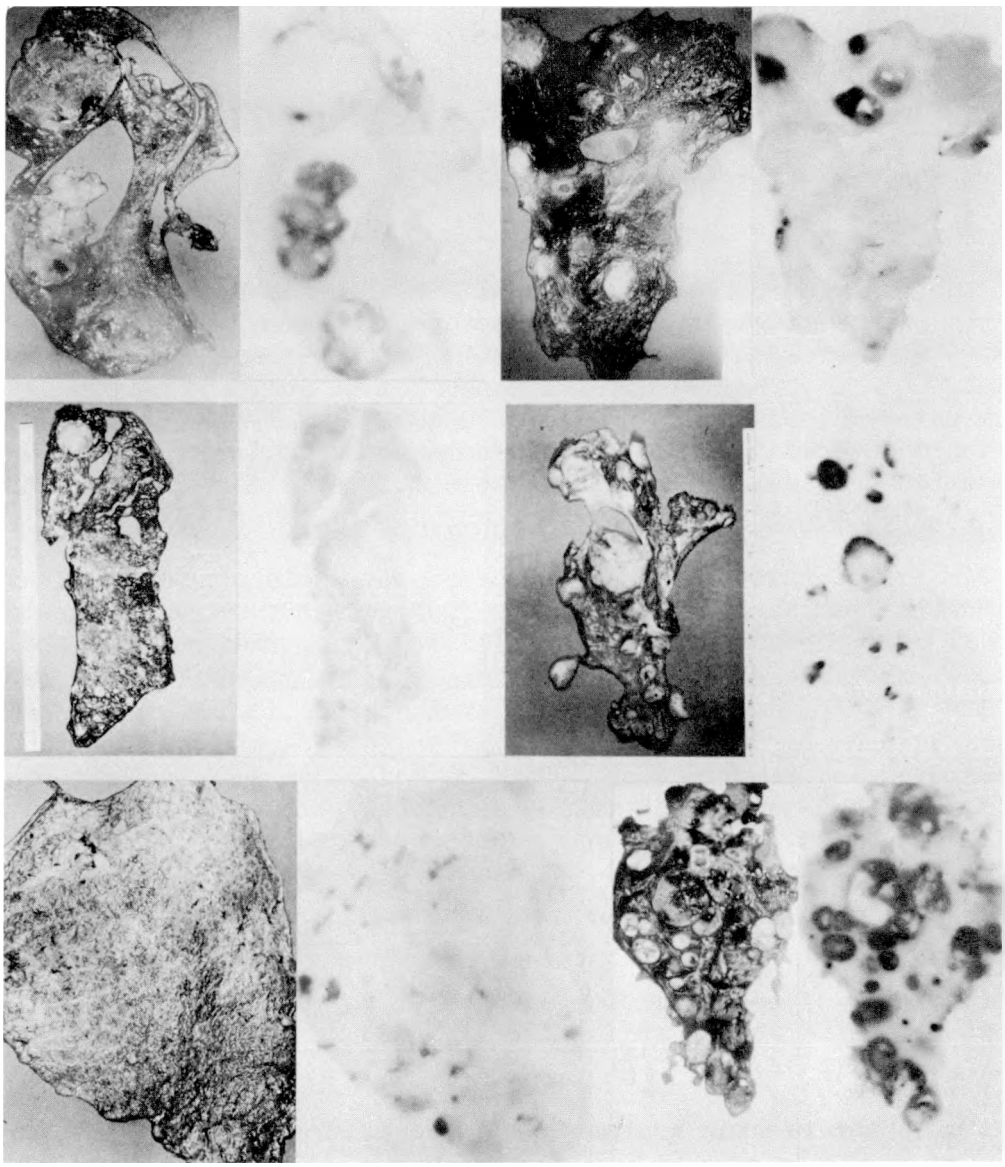

FIG. 1 PHOTOGRAPHS AND GROSS AUTORADIOGRAMS OF SECTIONS OF LUNG CONTAINING PHOSPHORUS-32.

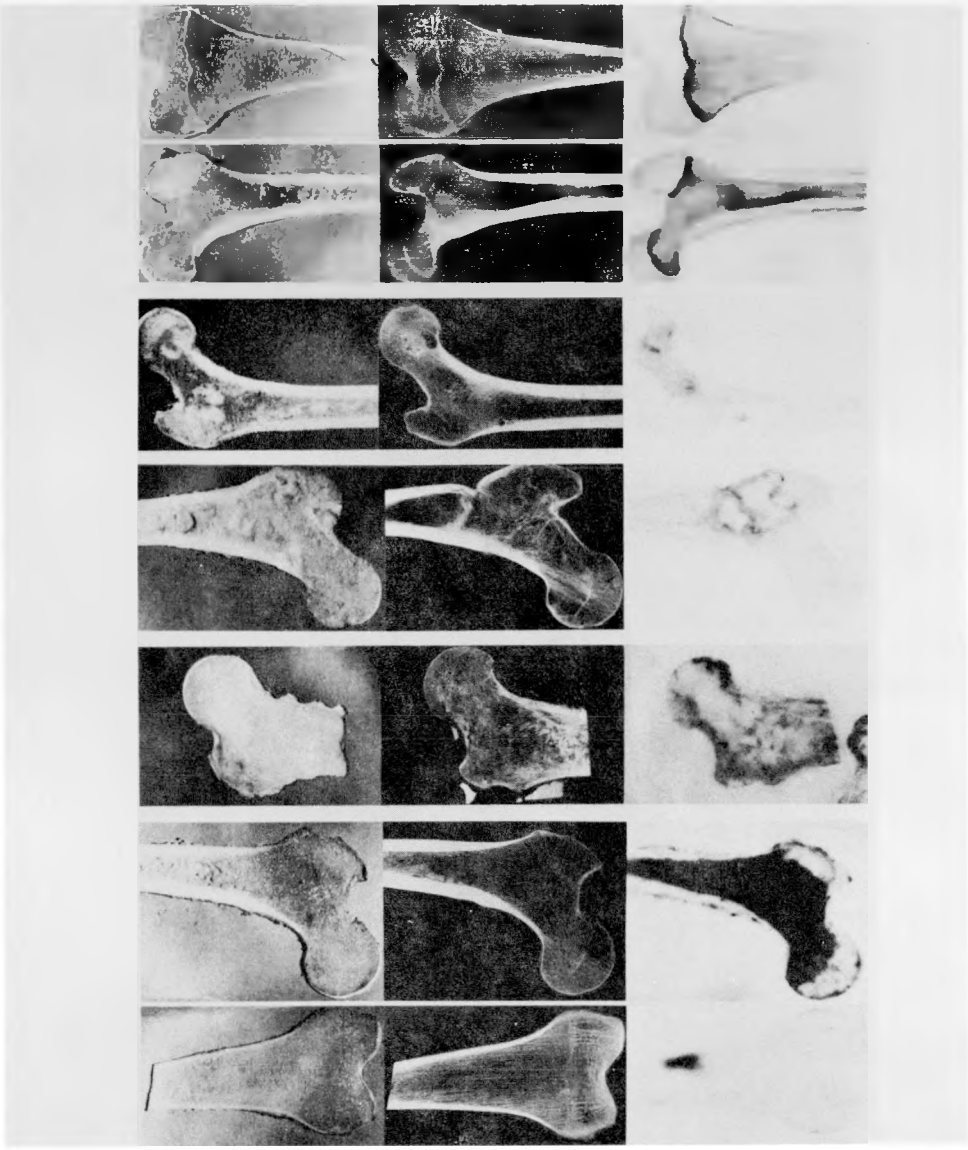

FIG. 2 PHOTOGRAPHS, X RAYS, AND GROSS AUTORADIOGRAMS OF FEMUR CONTAINING PHOSPHORUS-32.

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

Numerous radioisotopes of possible diagnostic and therapeutic use have not yet been tried in patients. Such trial calls for preliminary study in animals, and to determine distribution patterns and excretion rates is laborious. External measurement of radioactivity in cross-sectional segments of an animal provides data on the internal behavior of a radioisotope. Although the information is incomplete, rapid screening for distribution, and repeated measurements on the same animal offer advantages that are unavailable with destructive testing. We have calibrated the ORINS small-animal scanner for radioiodine-131 in the rat and applied it to verify thyroidectomy in animals used for metabolic studies. It has also proved useful with other selected radioisotopes, injected by various routes, including Na^{24}, K^{42}, Sc^{46}, Nb^{95}, I^{131}, and Ce^{144}. Repeated scans at increasing time intervals enable rapid estimation of the changing distribution.

The linear scanner, designed for rapidly screening radioisotopic distribution in small animals, uses a 2-inch by 2-inch NaI crystal with a medical spectrometer, and a chart recorder (Varian Model G-11) to plot externally detectable radioactivity as a function of longitudinal localization in the animal. The adjustable slit between 4-inch lead collimators defines the range of isoresponse patterns. The table speed for the animal is variable from 0 to 20 inches per minute.

Calibration included the isoresponse measurements for iodine-131 under various conditions. Three slit widths (1/8", 1/4", and 1/2") were studied in air, in large and small water phantoms, and in average-sized rats with implanted I^{131} sources. The isoresponse directly above the slit was symmetrical laterally; also the isoresponse curve was essentially flat for lateral displacement of the sources beyond the cross-sectional dimension of a large rat. Collimation longitudinal to the scanner table is inverse to slit width. Preliminary efforts toward quantitative interpretation are based on planimetric analysis of sections of the profile curve corresponding to arbitrary segments of the animal.

Quantitative Carbon-14 Assay of Thin-layer Chromatography Plates (F. Snyder and N. Stephens)

Thin-layer chromatography (1) with silica Gel G as an adsorbent has been used in our laboratories for the isolation of lipids from animal tissues. Its many advantages for qualitative, quantitative, and preparative work in accomplishing difficult separations in minutes has been reviewed recently by Mangold (2). Since we have been interested in studying the incorporation of C^{14}-labeled metabolites into lipid fractions, the following useful technique has been used to recover

essentially 100% of the total carbon-14-labeled mixture applied to a thin-layer plate.

Specific areas of the thin-layer of adsorbent containing the lipid components were carefully scraped with a plexiglass spatula into a Tri-Carb counting vial. The scrapings were suspended in 15 ml of a 4% Cab-O-Sil (3) toluene cocktail (5 grams PPO, 0.3 gram dimethyl POPOP, in 1 liter toluene) and counted in a Packard Tri-Carb liquid scintillation spectrometer. The Silica Gel G had no quenching characteristics and can be used in other types of scintillation liquids providing the C^{14} material is eluted from the scrapings.

Scintillation liquids without Cab-O-Sil can be used, providing the radioactive material is soluble in the solvents. The advantage of the Cab-O-Sil system, however, is that the scrapings are suspended, which makes it immaterial whether the labeled materials (except the fatty acids) are eluted or remain on the silica particles. The acidic conditions of the silica as used in the procedure are absolutely essential for counting fatty acids; when acetic acid is not used in the TLC separation, 50 lambda of acid can be added directly to the counting vial. If adsorption of long-chain fatty acids on silica gel occurs in the vial, a decrease in the counting efficiency due to self-absorption of energy will result; on the other hand, the counting of mixed C^{14}-labeled phospholipids (from Euglena) or of the unidentified polar lipids present in the impure C^{14}-tripalmitin shows the same counting efficiencies whether the radioactivity is adsorbed on particles or remains in solution.

A possible error in working with gels thickened further by the addition of silica is that some of the radioactivity can be trapped underneath the cap of the counting vial after shaking. This can be avoided by capping the vials with Scotch tape trimmed to the circumference of the opening before shaking and assay. If phosphatides are assayed according to this method, polyethylene vials should be used, for adsorption of some polar lipids on the glass surface can occur.

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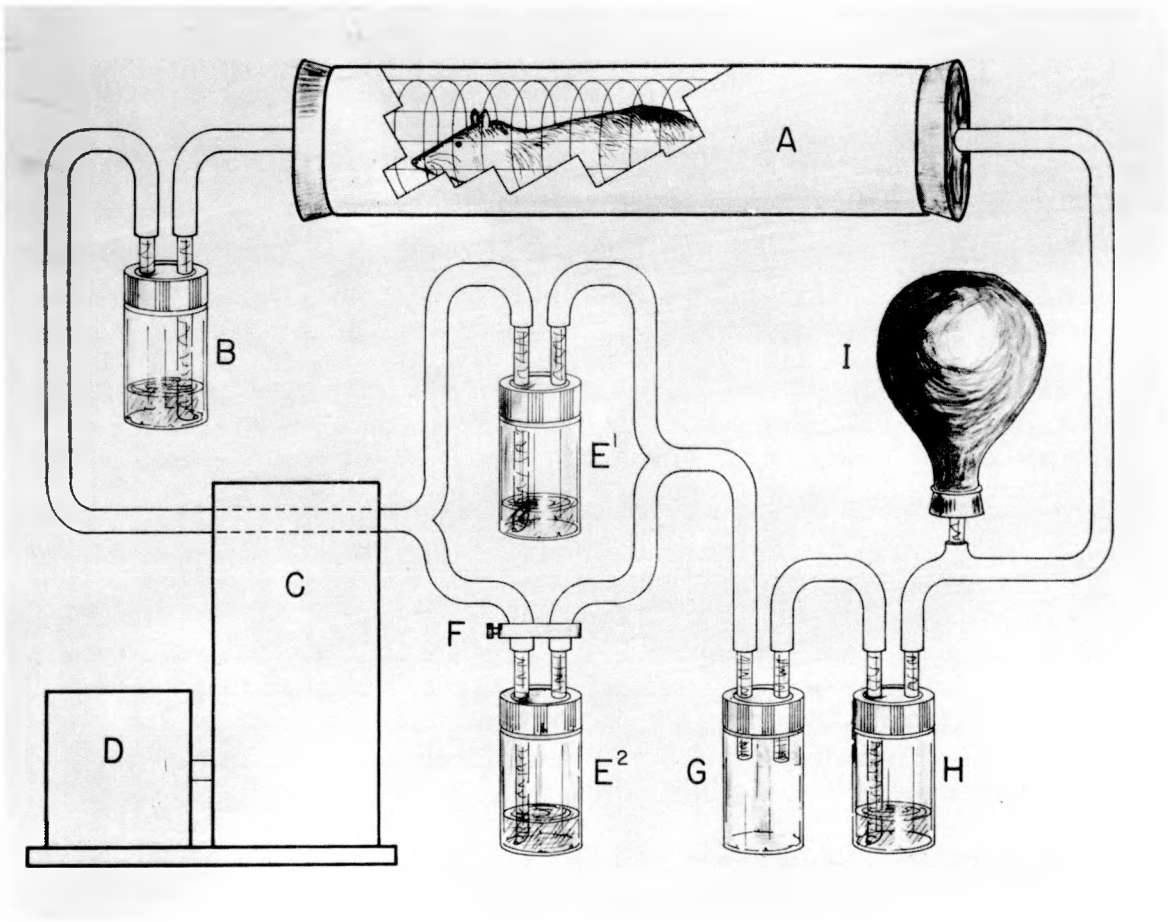

FIG. 1 APPARATUS FOR COLLECTING $C^{14}O_2$ (IN VIVO) FOR DIRECT SCINTILLATION COUNTING.

An Apparatus for *in vivo* $C^{14}O_2$ Collection and Subsequent Scintillation Counting (F. Snyder and P. R. Godfrey)

A simple apparatus has been developed at the Medical Division for collecting $C^{14}O_2$ (liberated *in vivo*) in a counting vial for liquid scintillation counting. The method involves no transfer of $C^{14}O_2$; the radioactivity can be counted immediately after the collection period. A diagram of the apparatus is shown in Fig. 1. After a C^{14}-labeled metabolite was administered intravenously to a rat, the animal was immediately placed in the metabolism tube (A). The exit tube from the chamber was connected to a scrubber (B) containing 5 ml concentrated H_2SO_4 for removal of exhaled water. The exhaled gases were circulated through the system by the kneading action of a sigma motor pump (C, D) at the rate of 185 ml per minute. The carbon dioxide was collected in a liquid scintillation vial containing 4 ml of hydroxide of Hyamine 10 X. Two such vials were connected to a Y tube (E^1 and E^2) with a pinch clamp (F) used for diverting the gas flow into only one of the vials. A filter paper disc was inserted around the delivery tube going into the Hyamine to prevent spattering. The return system to the metabolism tube consisted of an empty vial (G) (to serve as a trap for preventing the entry of H_2SO_4 into the counting system), a vial containing concentrated H_2SO_4 (H) (to absorb any methanol evaporated from the Hyamine), and a balloon (I) containing about one liter of oxygen.

After the expired gas was allowed to flow through a given vial for 15 minutes, the flow to that vial was turned off and diverted to the second vial and the process was repeated. Ten minutes after cessation of flow to a vial, the filter-paper disc was slid off the delivery tube into the counting vial and 15 ml of scintillation cocktail (4 grams PPO, 0.1 gram POPOP in 1 liter toluene) were added to the vial. The delivery tip was always rinsed with a portion of the cocktail. The contents of the vial were mixed and the radioactivity was measured with a Packard Tri-Carb liquid scintillation spectrometer (high voltage tap 5, 10-70-100 windows). Recovery of radioactivity was tested by liberating a known quantity of $C^{14}O_3$ from $Na_2C^{14}O_3$.

The apparatus offers the advantage of permitting a large-scale monitoring of the oxidation of carbon-14-labeled metabolites administered to small animals.

RADIATION IMMUNOLOGY IN THE PYGMY MARMOSET

Research supported by United States Airforce
Contract No. AF 41(657) - 398

Monitoring Laboratory:
School of Aerospace Medicine
Aerospace Medical Division (AFSC)
Brooks Air Force Base, Texas

A Study of Tamarinus nigricollis as a Laboratory Primate (N. Gengozian and J. S. Batson)

A new project now being undertaken at ORINS under sponsorship of the U. S. Air Force is the investigation of the feasibility of using a small South American primate, Tamarinus nigricollis (Fig. 1) as a laboratory animal for radiation studies. Several features of this primate recommending that some attempt be made to use it in biological research are 1) its small size, ranging from 250 to 350 grams; 2) low cost in procurement and maintenance; and 3) the occurrence of twins in the litters, a consistent feature in this species.

During the first year, emphasis was placed on the study of conditions requisite for maintaining these small animals in the laboratory. Problems such as feeding, caging, handling of the animals, treatment for parasites, and general care were studied. Caging may be done individually or in groups of two to four, although the greater number per cage tends to increase possibility of any transmission of disease among members of the colony. The food given the animals consists of a variety of substances, such as a breadlike substance rich in protein and fats, fresh fruits and vegetables, and a multivitamin supplement. Handling of the animals and restraining them while injecting or bleeding is readily accomplished. A small fishnet is used in removing them from their cages and heavy leather gloves are worn by the caretaker in freeing them from the netting. The animal is subsequently restrained on a small operating board having rubber bands with which to immobilize its lower appendages (Fig. 2). Anesthetics are thus not necessary for any routine laboratory work. Primary difficulties were experienced in receiving animals having a heavy infestation of Acanthocephalans, spiny-headed worms that tend to localize in the lower ileum. The seriousness of such parasitism is manifest, particularly in animals experiencing malnutrition, the effects of which, when compounded upon intestinal damage by the worms, often lead to death of the animal. As yet, there is no known effective treatment in eradicating these parasites.

Preliminary studies on total-body irradiation effects have indicated these small primates to be quite radiosensitive. Thus, mortality has been obtained with radiation doses as low as 100 r and 200 r, as well as 400 r, 500 r, and 600 r. Although death appears to be due to a primary failure of the hematopoietic system, it is not yet clear what role the parasitism present in the animals may play in compounding any radiation injury. Additional studies are required to clarify the apparent radiation sensitivity of this species of primates.

Breeding of the animals has been accomplished successfully in this laboratory. Of four males and females paired for this purpose, two of the females became pregnant and delivered twins. In the first litter,

one twin was a stillborn. The other young is still alive and healthy, now being approximately 8 weeks old. The second female delivered prematurely by about two weeks and unfortunately both twins died shortly after birth. The success of this small-scale experiment definitely suggests the feasibility of breeding these animals in captivity for experimental purposes.

FIG. 1. TAMARINUS NIGRICOLLIS.

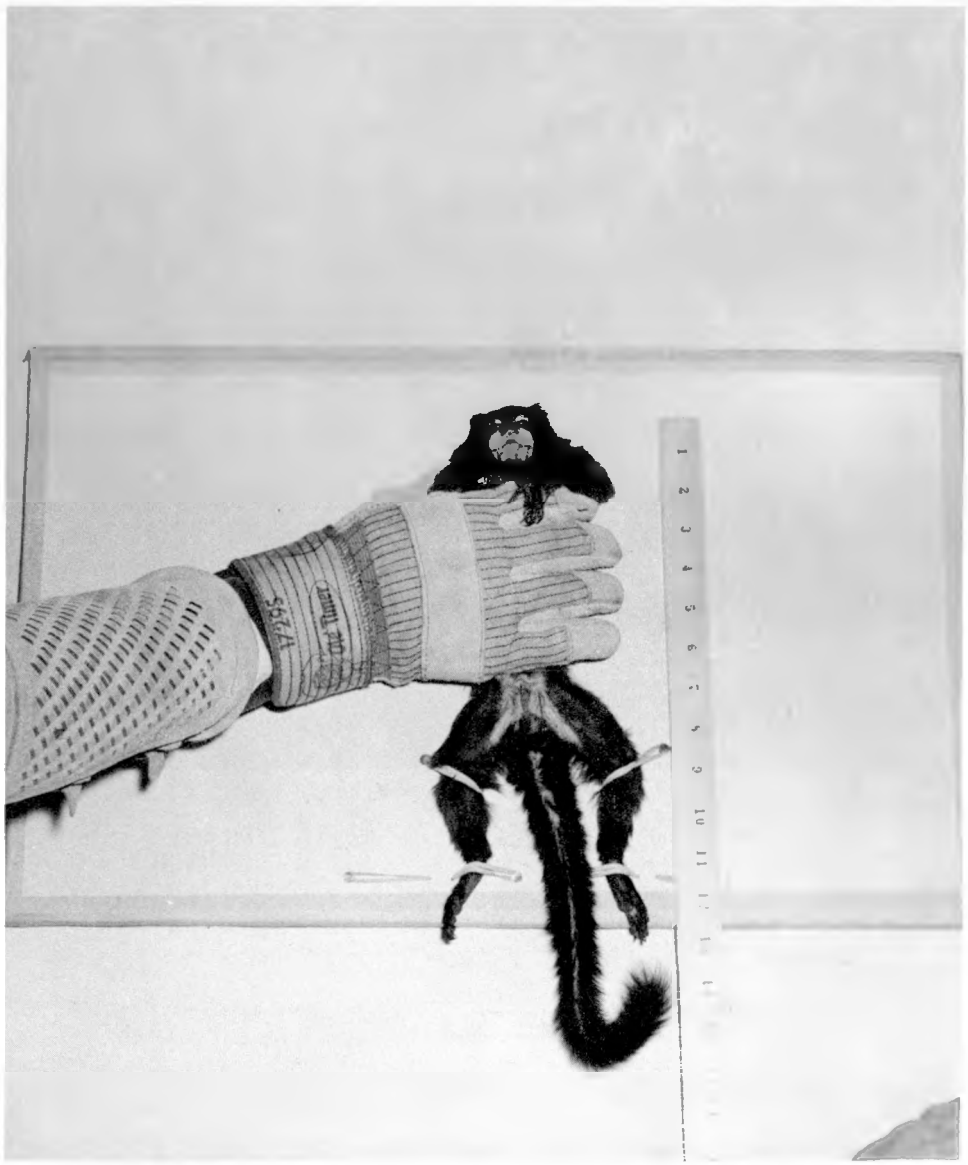

FIG. 2 THE MONKEYS ARE HELD IN PLACE FOR LABORATORY WORK WITHOUT THE USE OF ANESTHETICS. THE LOWER LEGS ARE PINNED DOWN WITH RUBBER BANDS ATTACHED TO THUMB TACKS.

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