OAK RIDGE INSTITUTE OF NUCLEAR STUDIES

MEDICAL DIVISION RESEARCH REPORT FOR 1963

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ORINS 43

OPINIONS

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FOREWORD

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

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

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

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

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

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

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

Gould A. Andrews, M.D.
Chairman

STUDIES OF RADIATION EFFECTS

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

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

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

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

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

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

50 r in Chronic Lymphocytic Leukemia

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

50 r in Chronic Myelocytic Leukemia

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

100 r in Lymphosarcoma

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

Serum Uric Acid Values

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

Clinical Effects

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

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

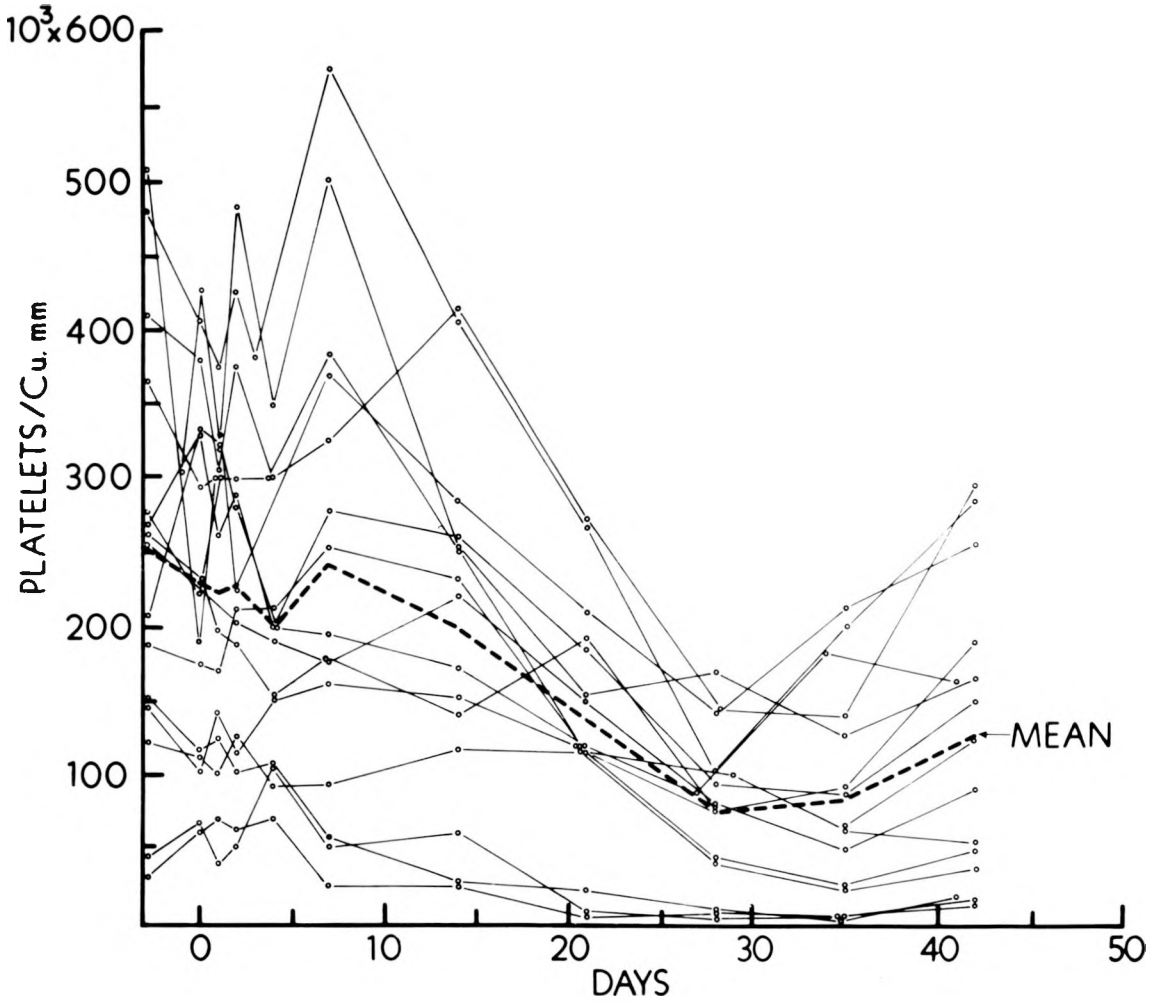

Fig. 1. Platelet values after 100 r total-body irradiation for lymphosarcoma.

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

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

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

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

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

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

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

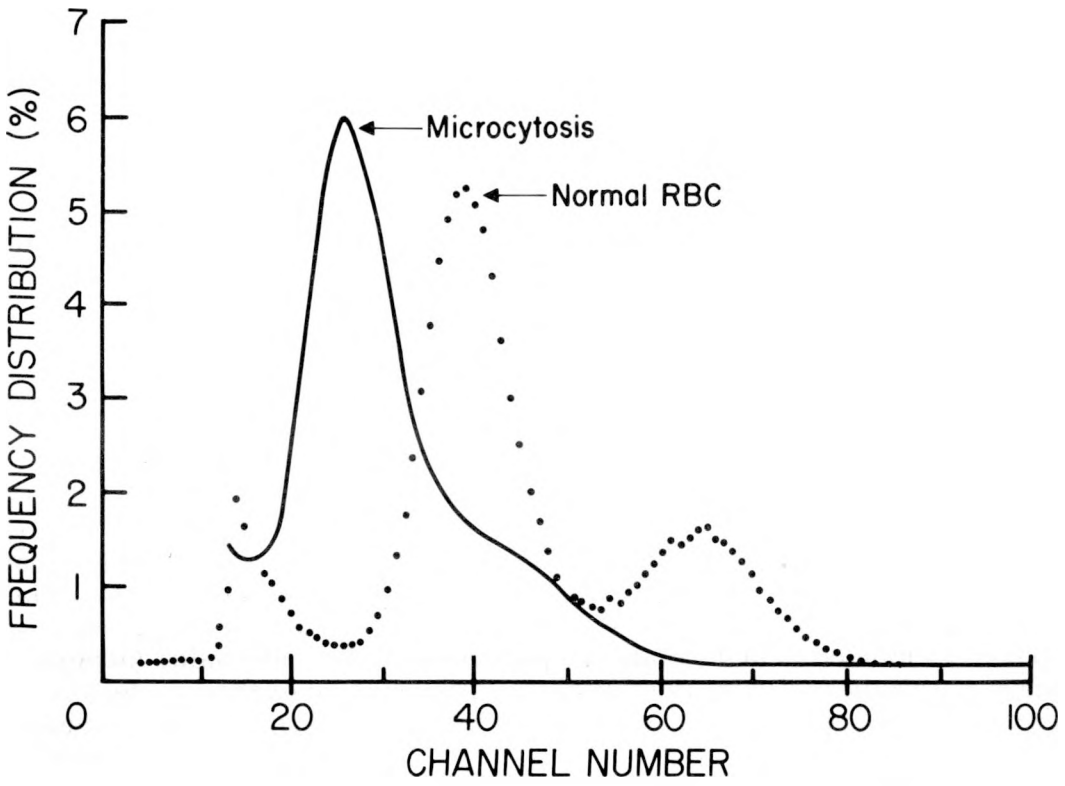

Fig. 1. Frequency distribution profiles of RBC volumes of normal man (MCV 97) and microcytosis in polycythemic patient (solid line).

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

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

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

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

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

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

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

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

2) A sizable proportion of the population may experience doses many times in excess of that assumed for the average or Standard Man. The measurements indicate that about 15% of the general population may experience a dose three times that of the Standard Man and 6% as much as five times that of the Standard Man.

3) As expected, the dose experience of the population studied showed a wide variation. The average dose was, however, only approximately 70% greater than that predicted for the Standard Man, where a long-lived isotope was involved. For a short half-life activity (12 hr) the average was equal to the Standard-Man value.

4) Whether the route of entry of activity is through food (at meal time) or through water (between meals) does seem to grossly affect the dose received.

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

Irradiation Under Anoxia (Frank V. Comas)

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

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

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

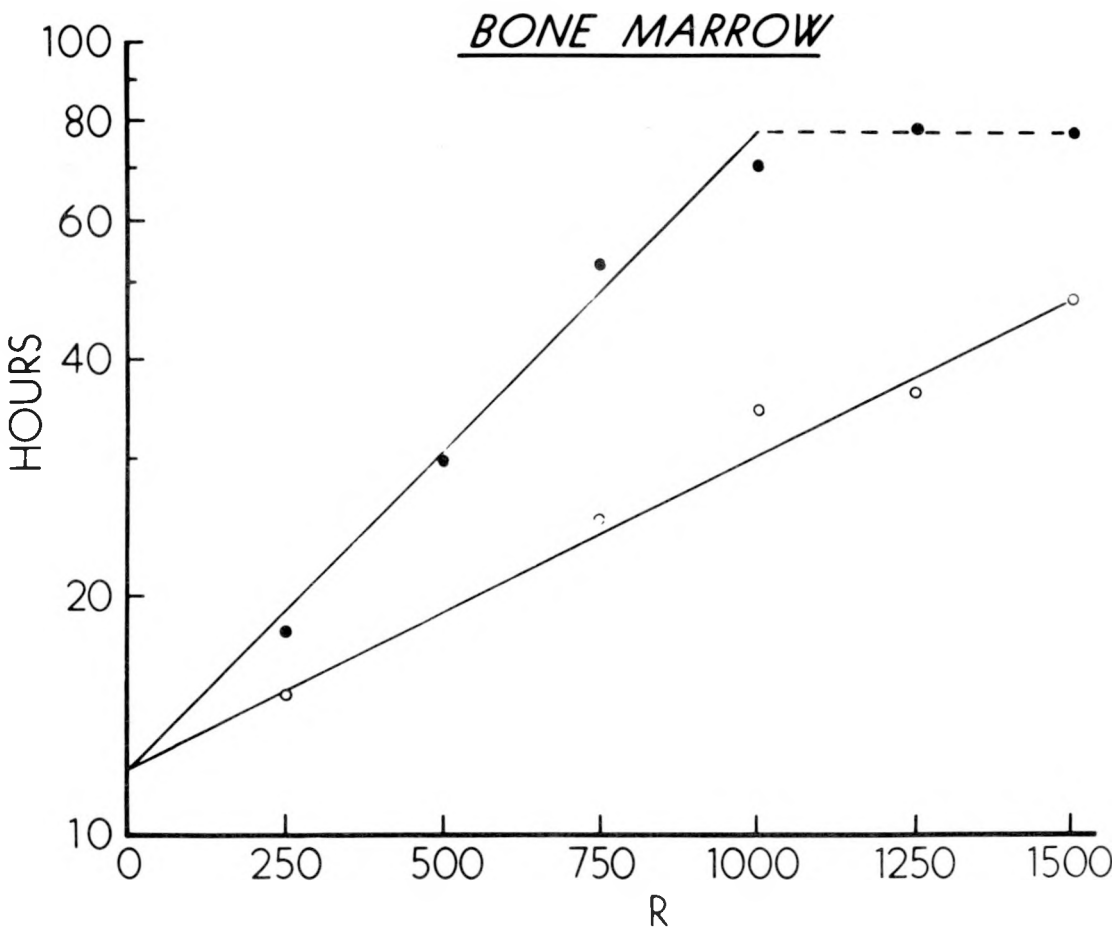

Fig. 1. Plot of duration of DNA synthesis depression for oxygenated (closed circles) and anoxic (open circles) bone marrow, versus radiation dose. The ratio of the slopes of the two lines is 2.03.

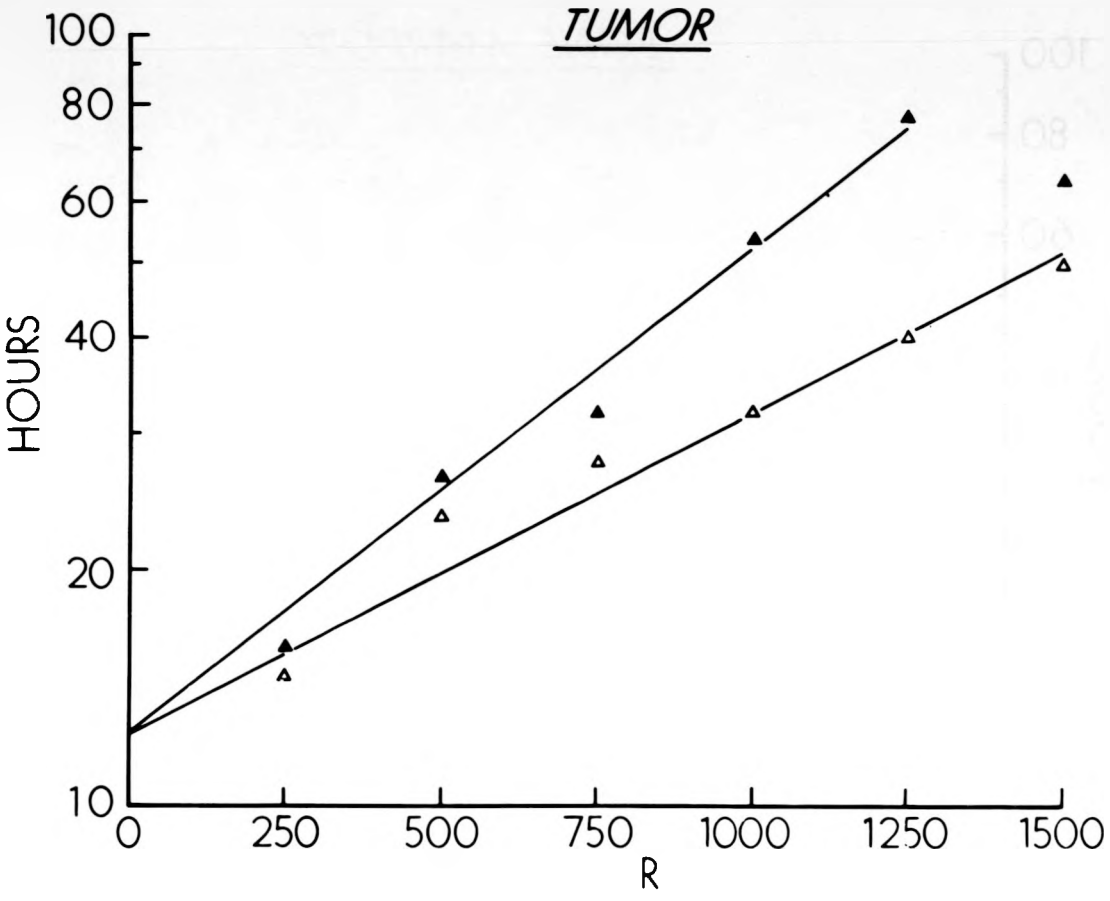

Fig. 2. Plot of duration of DNA synthesis depression for oxygenated (closed triangles) and anoxic (open triangles) tumor, versus radiation dose. The ratio of the slopes of the two lines is 1.58.

LIPID METABOLISM AND RADIATION

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

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

Bone Marrow Lipids and their Metabolism

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

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

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

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

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

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

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

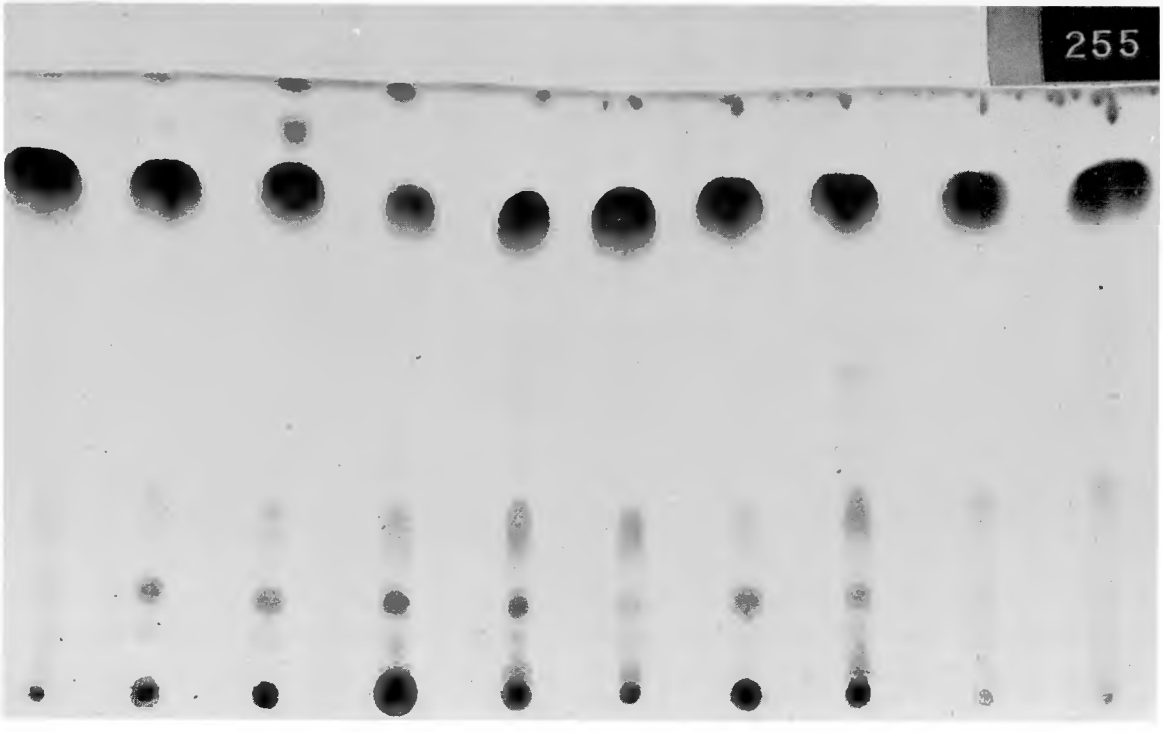

Fig. 1. Thin-layer chromatogram of bone-marrow lipids from different species. Left to right: chimpanzee femur, monkey femur, human rib, guinea pig femur, rat femur, rabbit femur, dog rib, pig rib, sheep femur, steer femur. Thin-layer chromatography separation of total marrow lipids in a 90:10:1 (hexane: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

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

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

* % of dry tissue

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

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

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

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

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

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

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

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

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

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

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

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

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2. V. D. Courtenay. Studies on the protective effect of allogenic marrow grafts in the rat following whole-body irradiation at different dose-rates. Brit. J. Radiol. 36: 440-447 (1963).

3. F. Snyder, E. A. Cress, and N. Stephens. USAEC Report ORINS-42, 1962, p. 12.

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

Orally administered glyceryl ethers

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

Organic synthesis of batyl alcohol

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

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

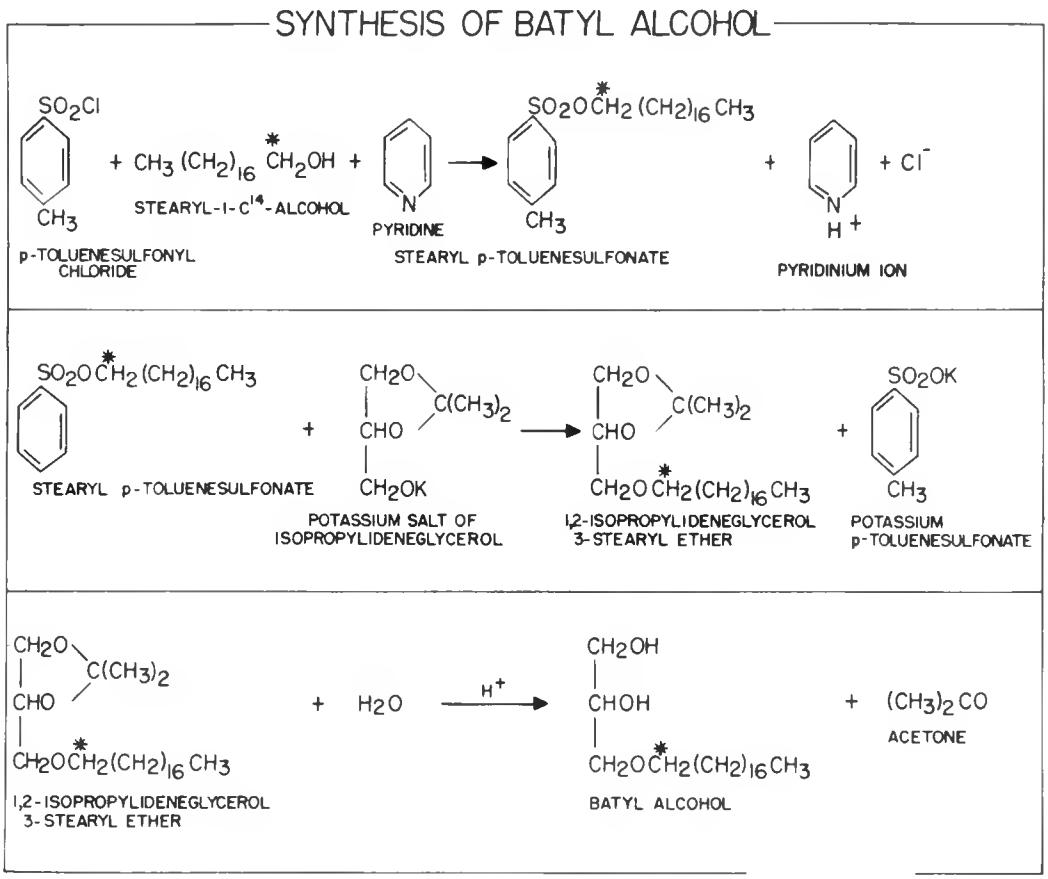

Fig. 1. Reaction sequence for the synthesis of batyl alcohol.

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

Acknowledgements

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

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Radioassay of Thin-layer Chromatograms (Fred Snyder)

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

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

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

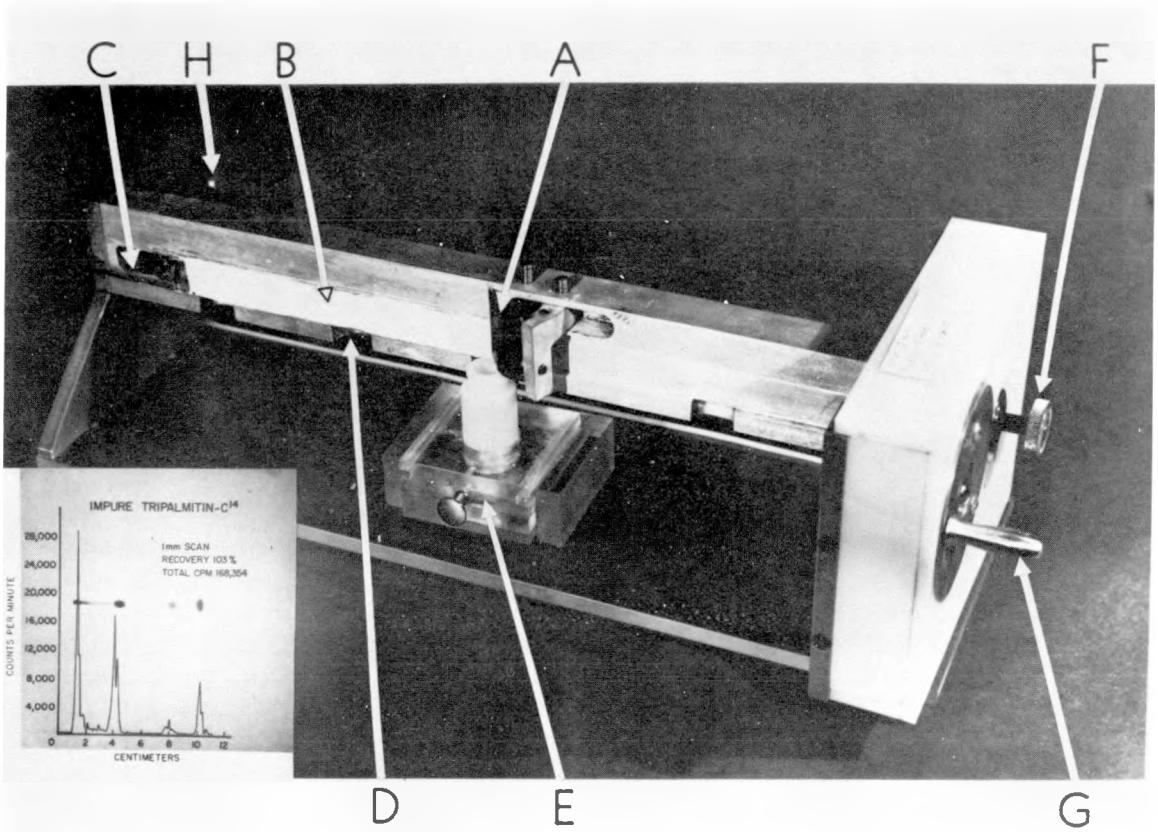

Fig. 1. Thin-layer chromatogram scraper.

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

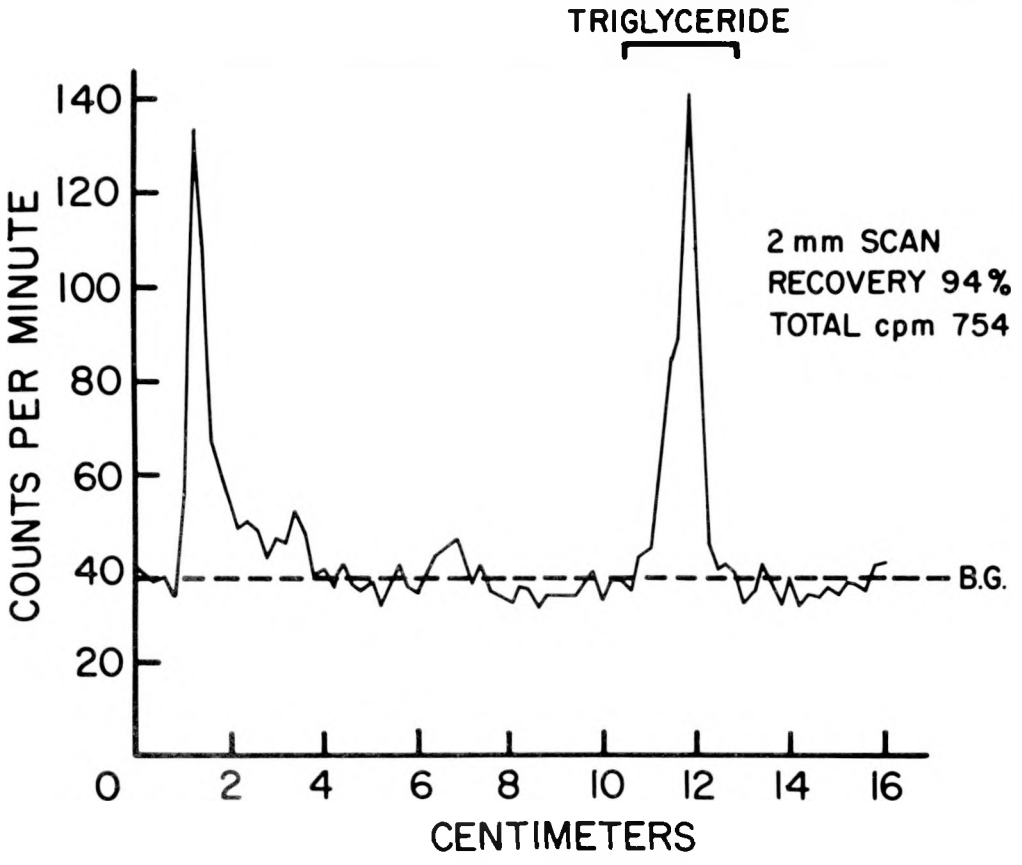

Fig. 2. Carbon-14 distribution in bone marrow lipids 6 hours after the oral administration of palmitic-1-C^{14} (in corn oil) to an irradiated rat. (4 days after 800-r total-body irradiation).

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

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

Acknowledgements

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

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AMINO ACID METABOLISM AND IRRADIATION

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

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

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

* Oak Ridge National Laboratory

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

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

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

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

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

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

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

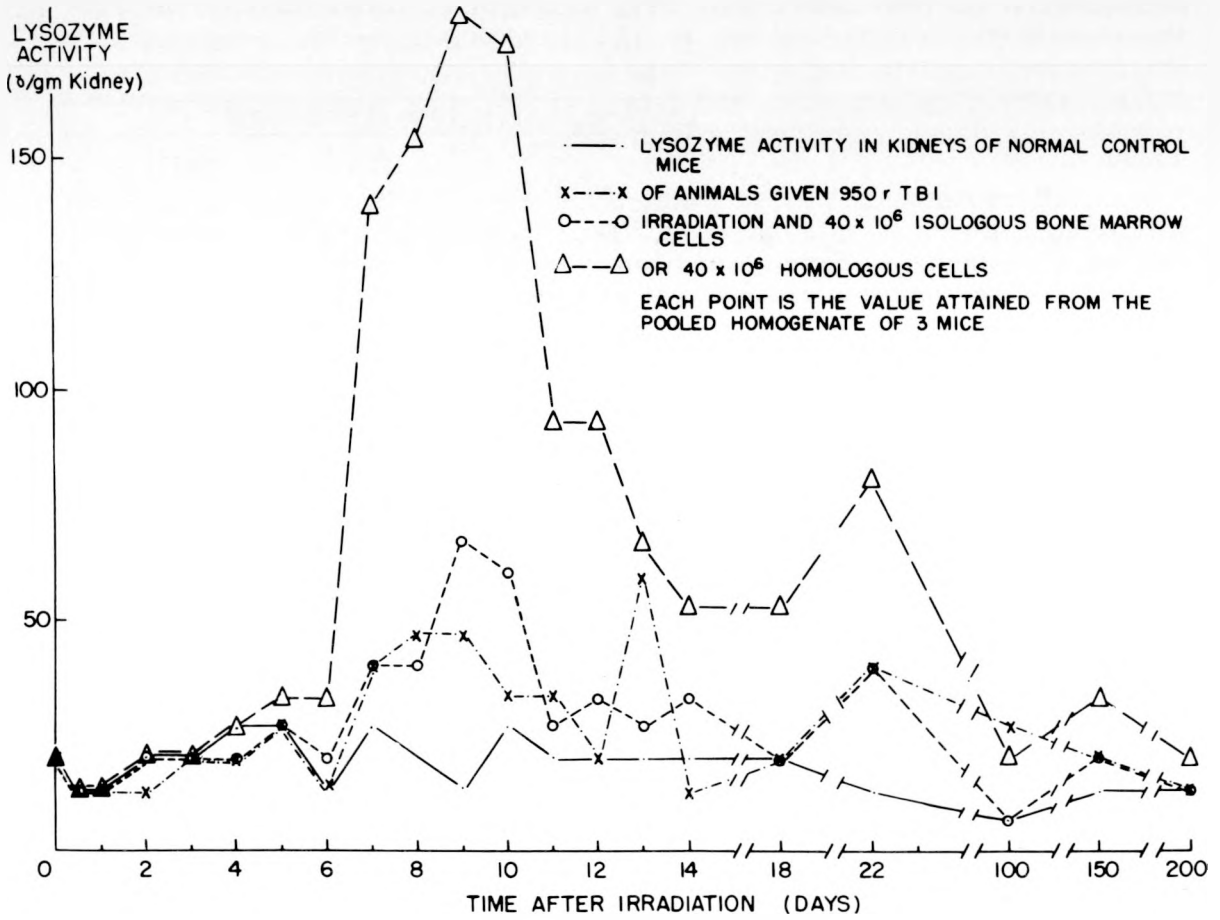

Fig. 1. Lysozyme activity in the kidneys of irradiation chimeras.

Table 1

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

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

* The number of mice is given in parenthesis.

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

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

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

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

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

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

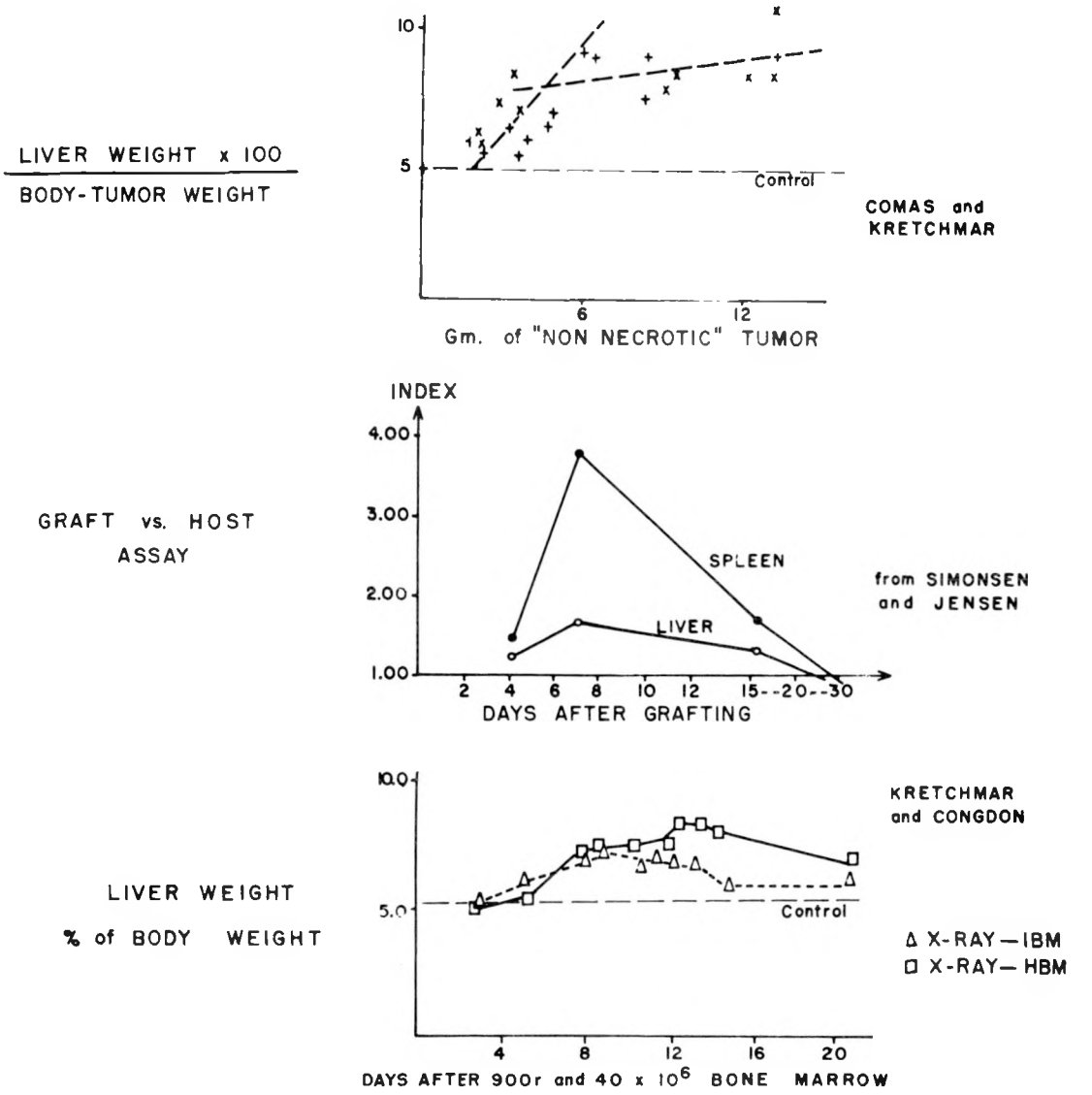

Fig. 1. Graphs showing the relation between liver weight and size of tumor or time after grafting or irradiation and injection of bone marrow cells. Upper graph shows liver weight divided by body weight minus tumor weight as a percentage; the middle graph shows liver and spleen weight as a calculated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

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

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

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

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

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

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

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

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

Table 1

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

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

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

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

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

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

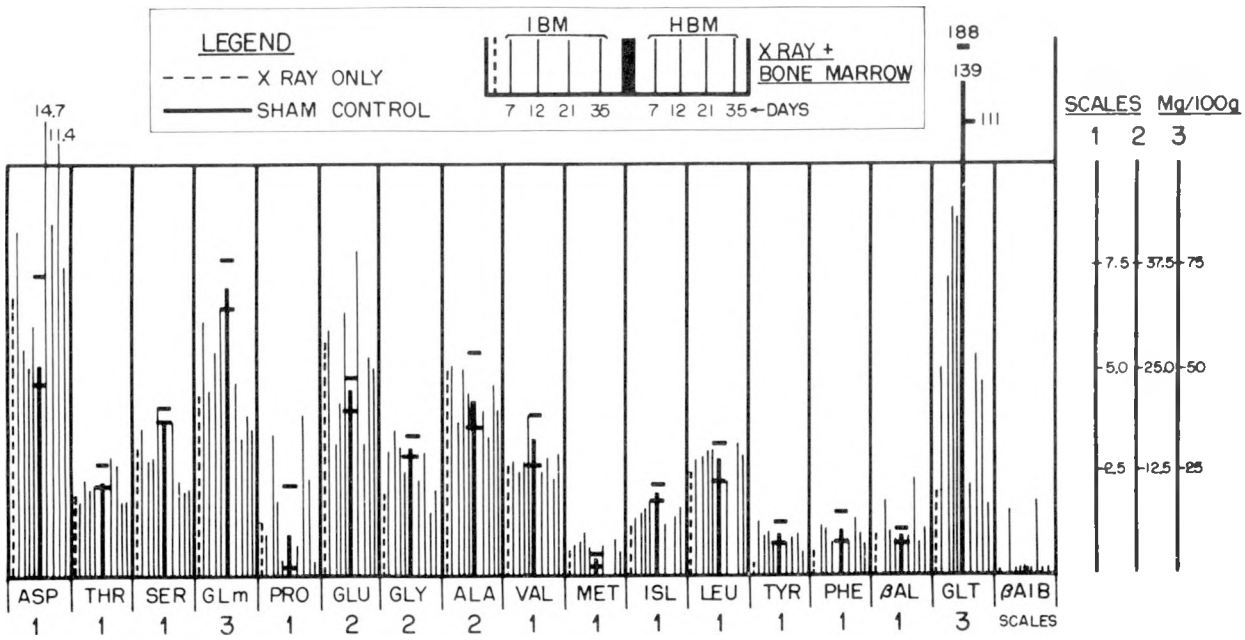

Fig. 1. Free amino acids of liver.

IMMUNOLOGY

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

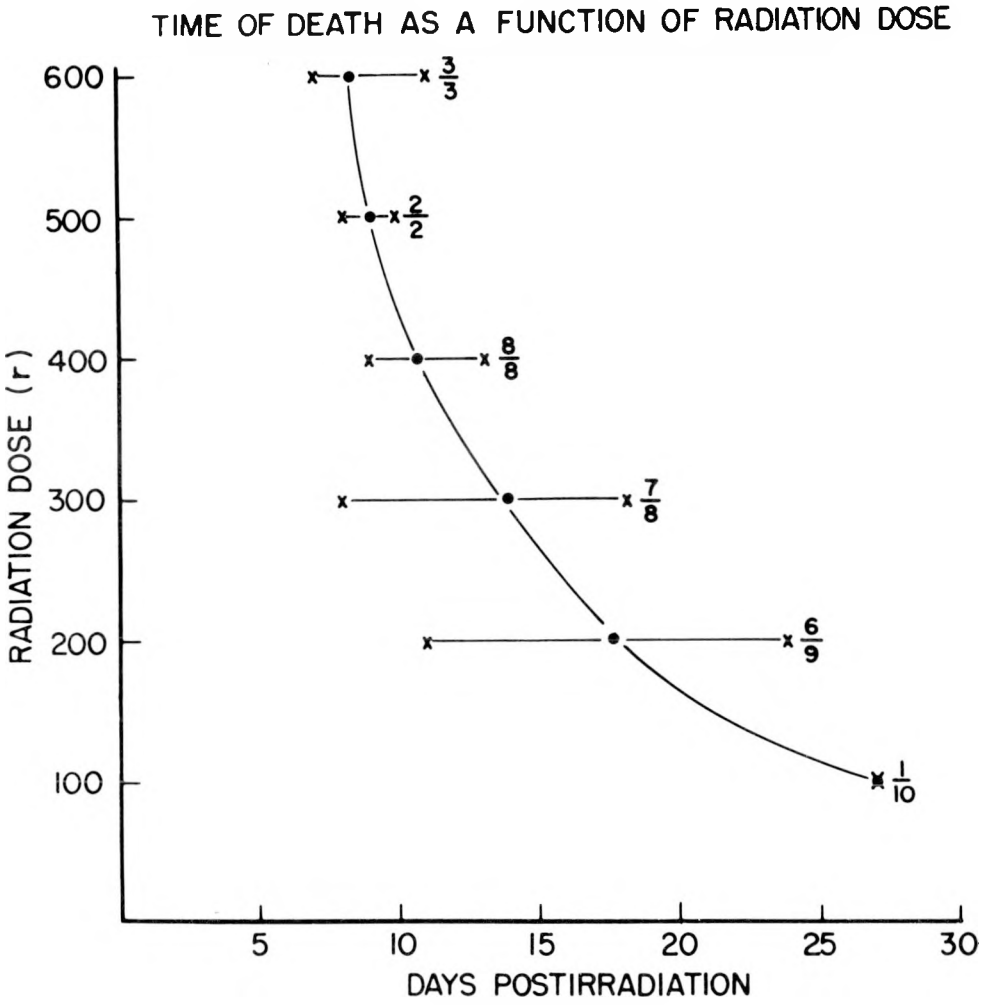

Fig. 1. Plot of mortality versus radiation dose indicating an LD_{50} of about 170 r with an LD_{100} of 400 r.

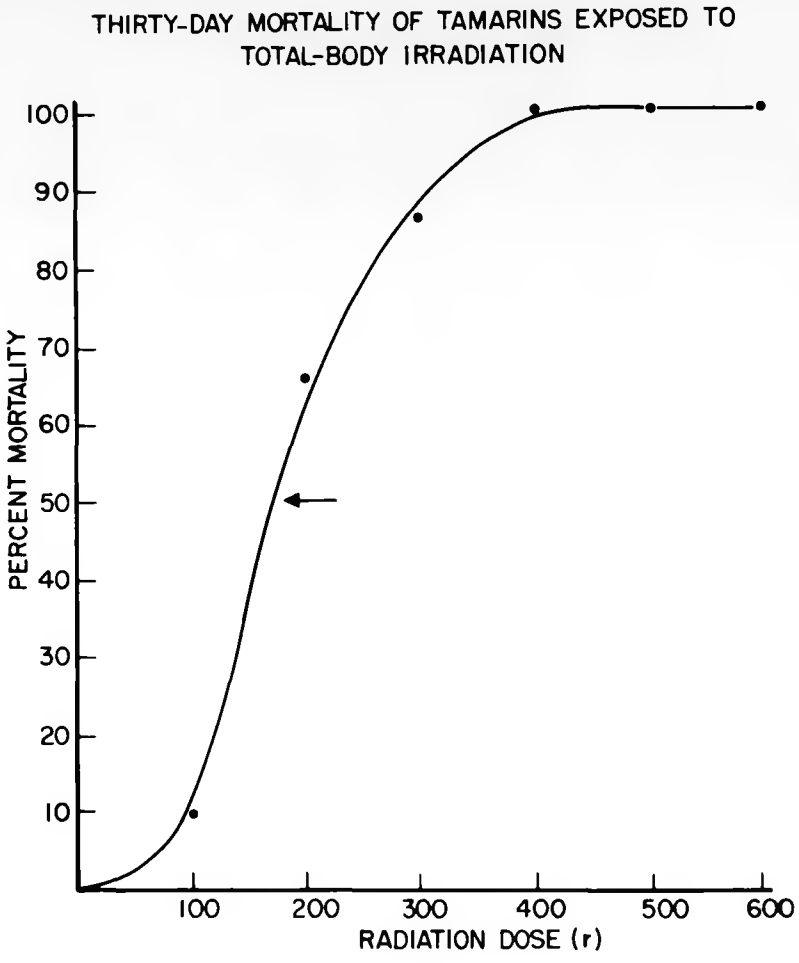

Fig. 2. The 30-day mortality among six groups (100 through 600 r) expressed as a function of time of death and radiation dose.

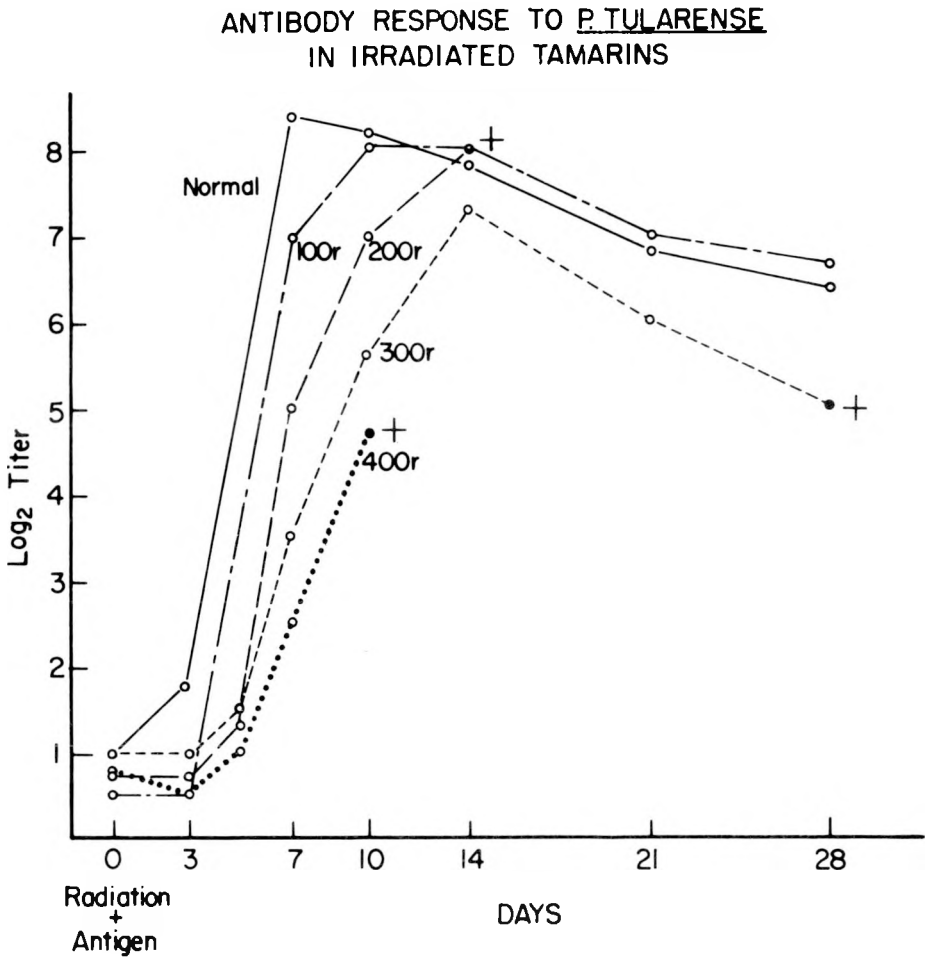

Fig. 3. Antibody formation by irradiated tamarins relative to the normal tamarin response curve.

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

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

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

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

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

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

Table 1

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

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

METALS METABOLISM AND MEDICAL RADIONUCLIDES

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

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

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

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

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

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

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

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

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

Table 1

Intralymphatic Localization of Radioactive Materials

(7-Day Dogs)

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

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

Table 2

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

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

* Radioisotope: Y^{90}; Ce^{144}

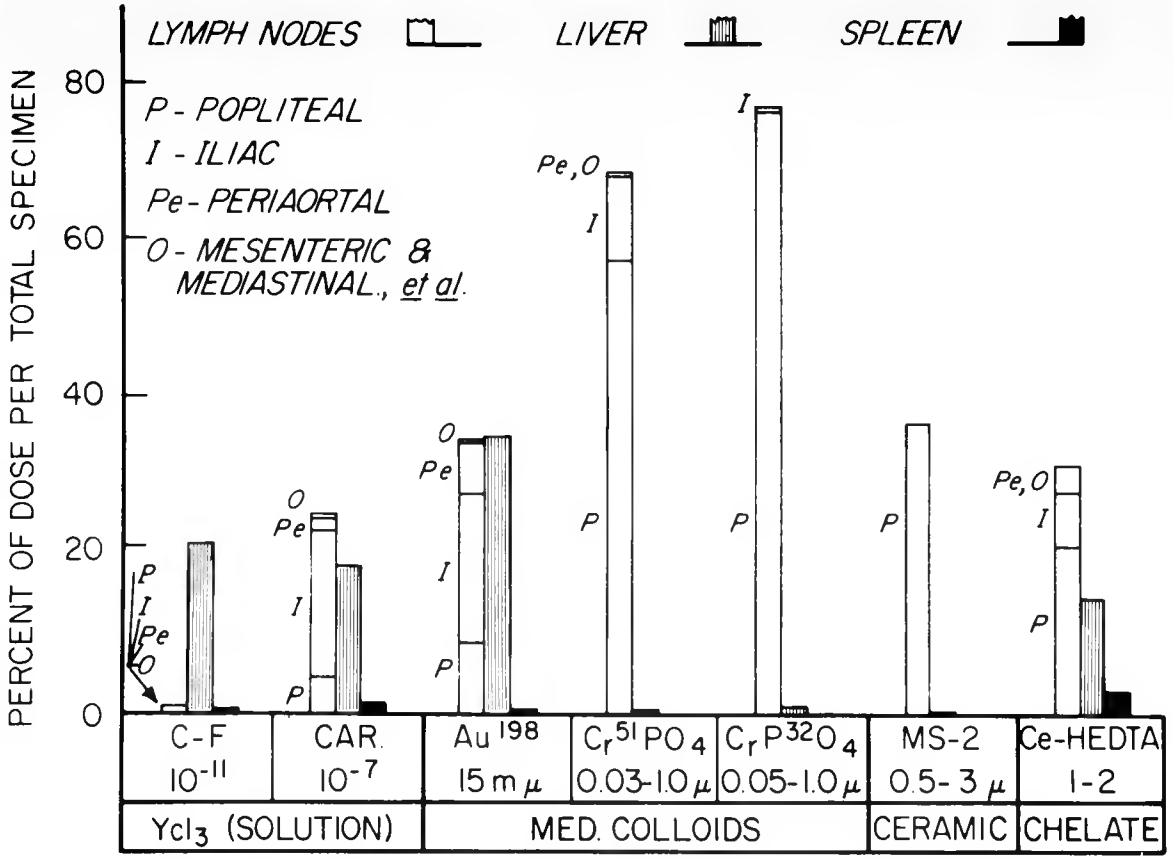

Fig. 1. Intralymphatic Localization in Dogs
(One Day)

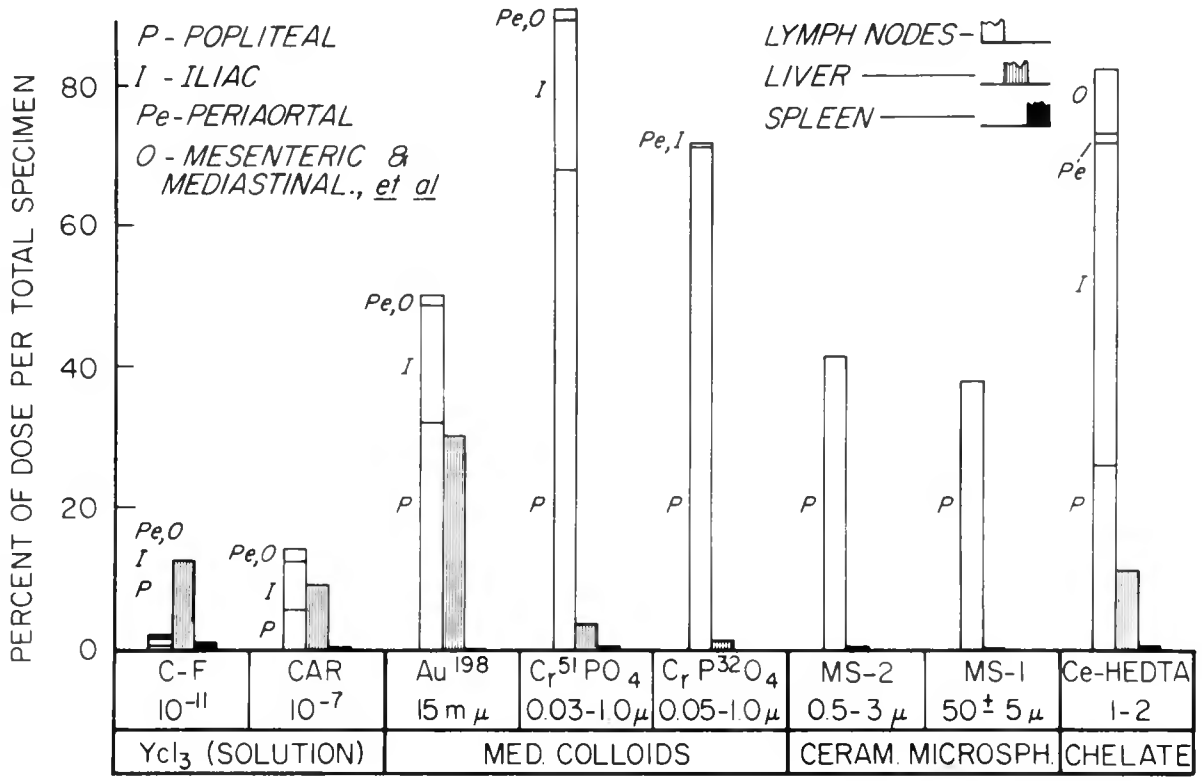

Fig. 2. Intralymphatic Localizations in Dogs
(Seven Days)

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

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

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

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

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

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

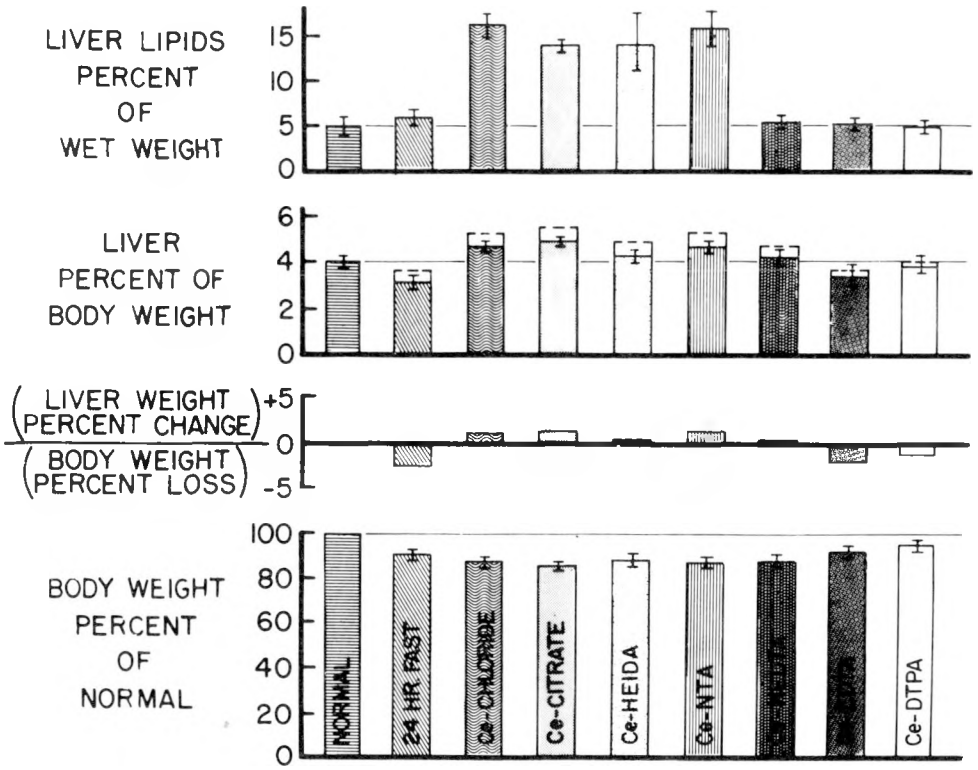

Fig. 1. Cerium Chelates - Intravenous.

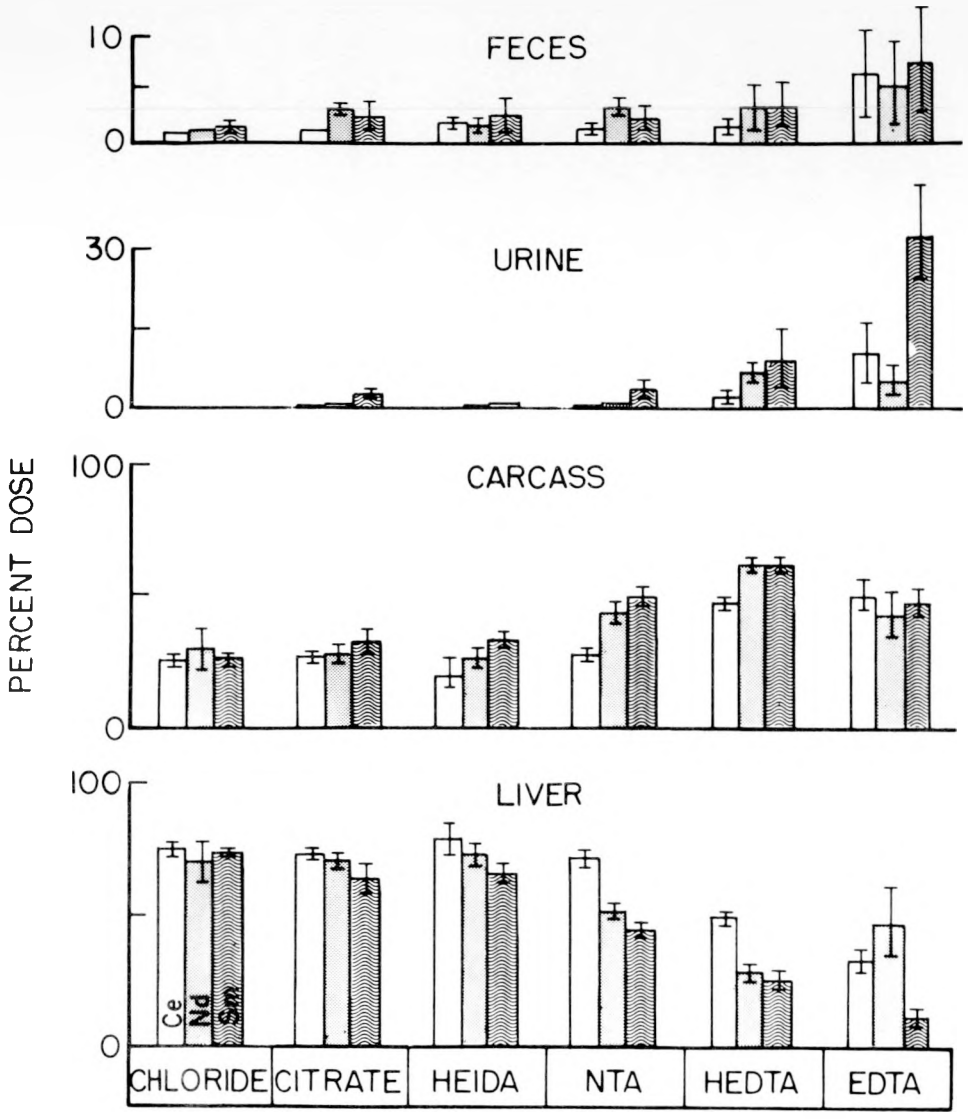

Fig. 2. Intravenous Lanthanon Chelates
Rats - 48 hrs.

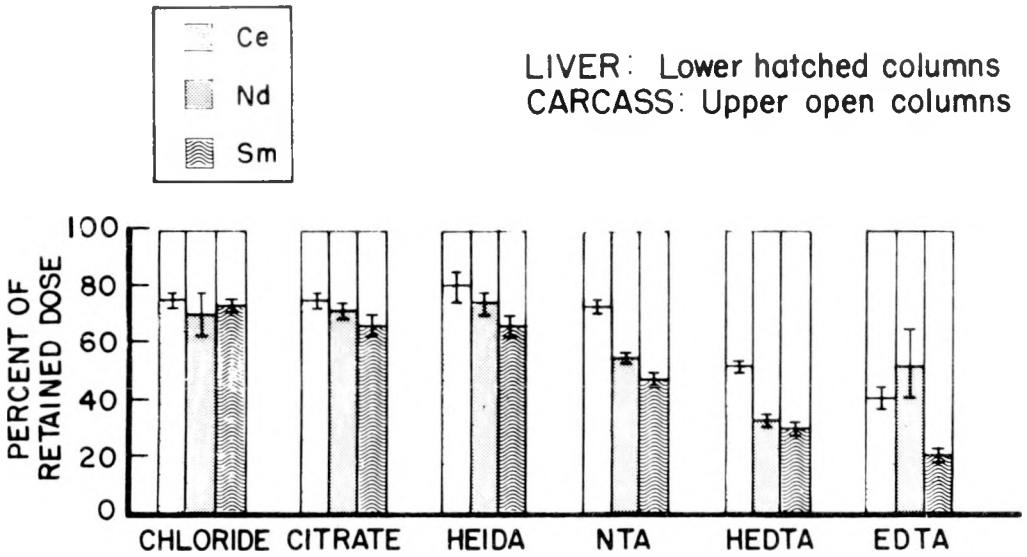

Fig. 3. Distribution of Retained Dose of Certain Lanthanon Chelates - Intravenous - Rats - 48 hrs.

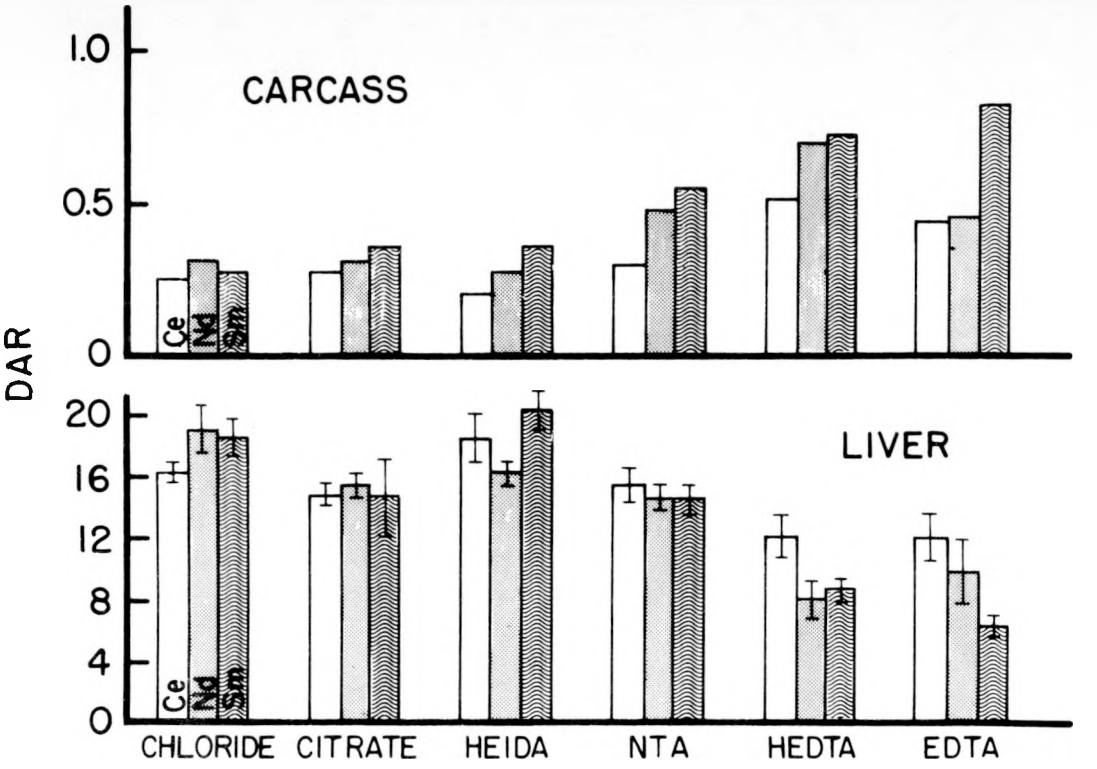

Fig. 4. Differential Absorption Ratio of Certain Lanthanon Chelates in the Liver and Carcass of Rats - Intravenous - 48 hrs.

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

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

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

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

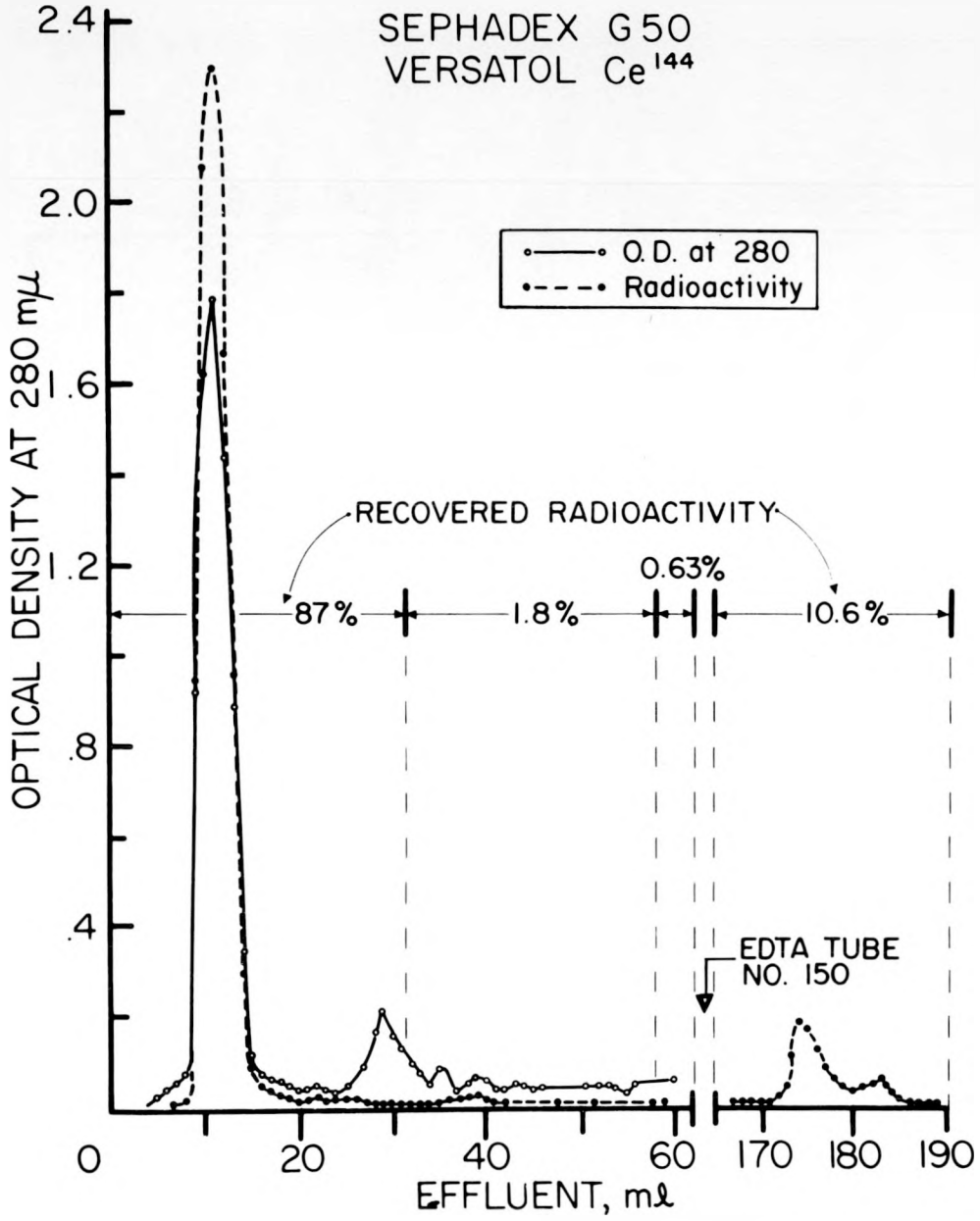

Fig. 1. Gel filtration of an incubated mixture of Versatol and cerium chloride (Ce^{144}) using Sephadex G-50 (See Experiment 2, Table 1 for column specifications).

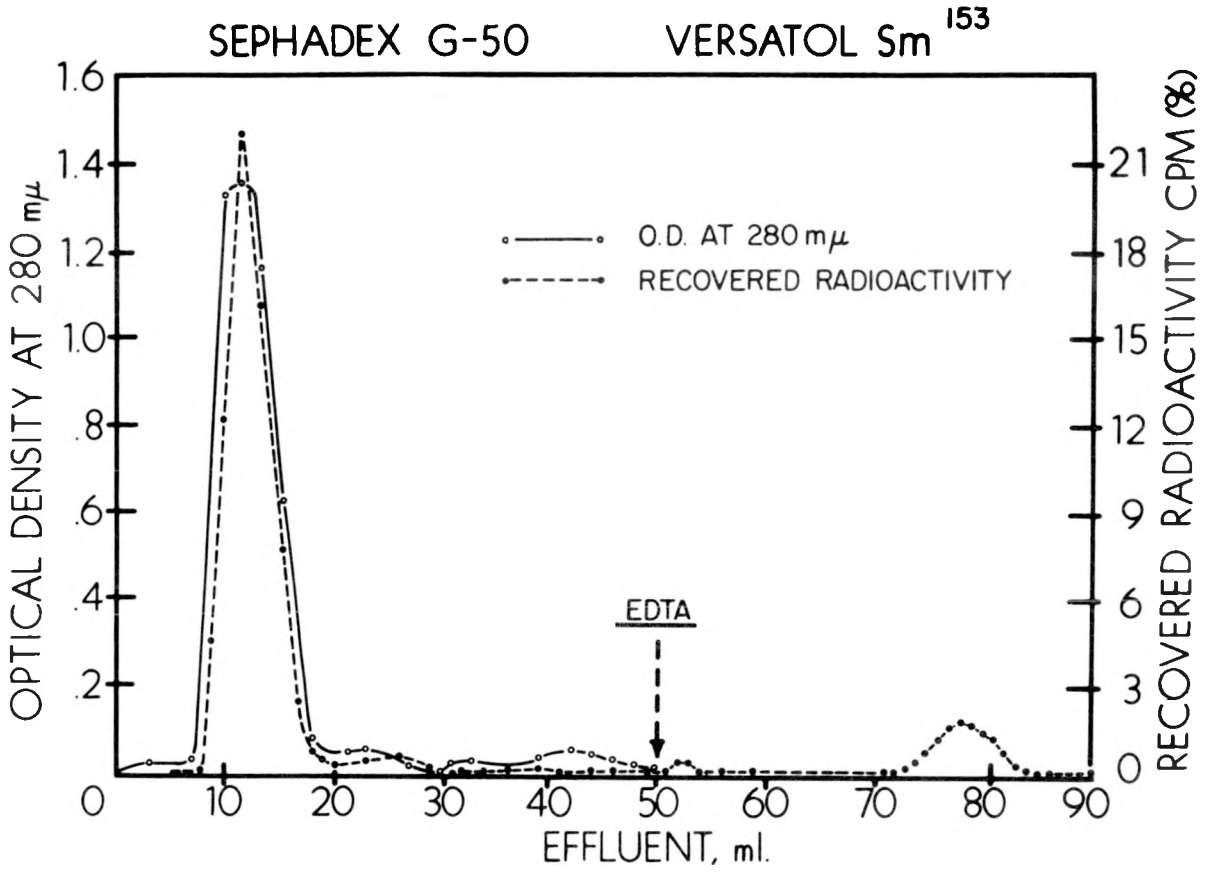

Fig. 2. Gel filtration of an incubated mixture of Versatol and samarium chloride (Sm^{153}) using Sephadex G-75 (See Expt. 4 in Table 1 for column specifications.)

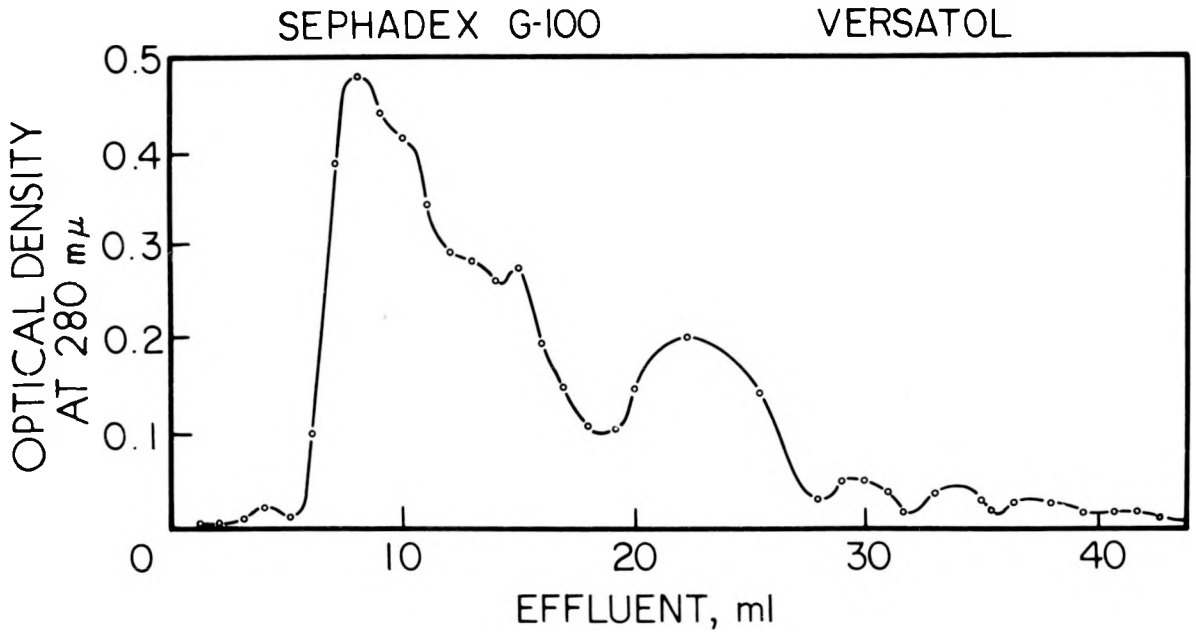

Fig. 3. Gel filtration of Versatol under conditions defined in Table 1, Experiment 5.

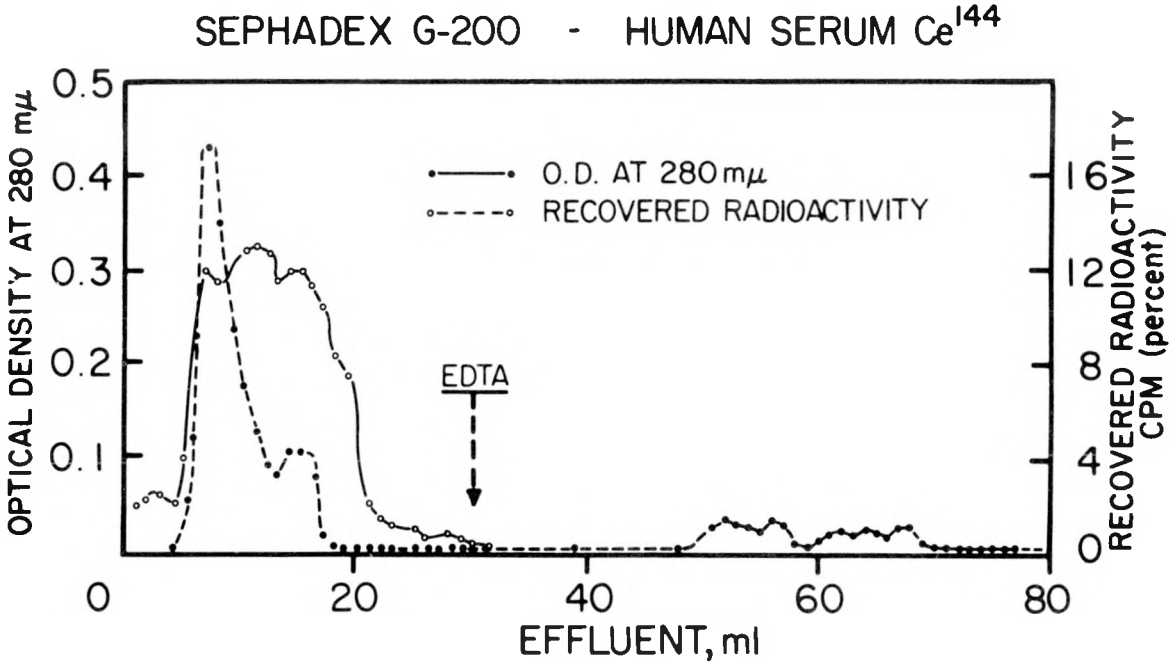

Fig. 4a. Gel filtration of an incubated mixture of human serum and cerium chloride (Ce^{144}) using Sephadex G-200. (See Experiment 6 in Table 1 for conditions.)

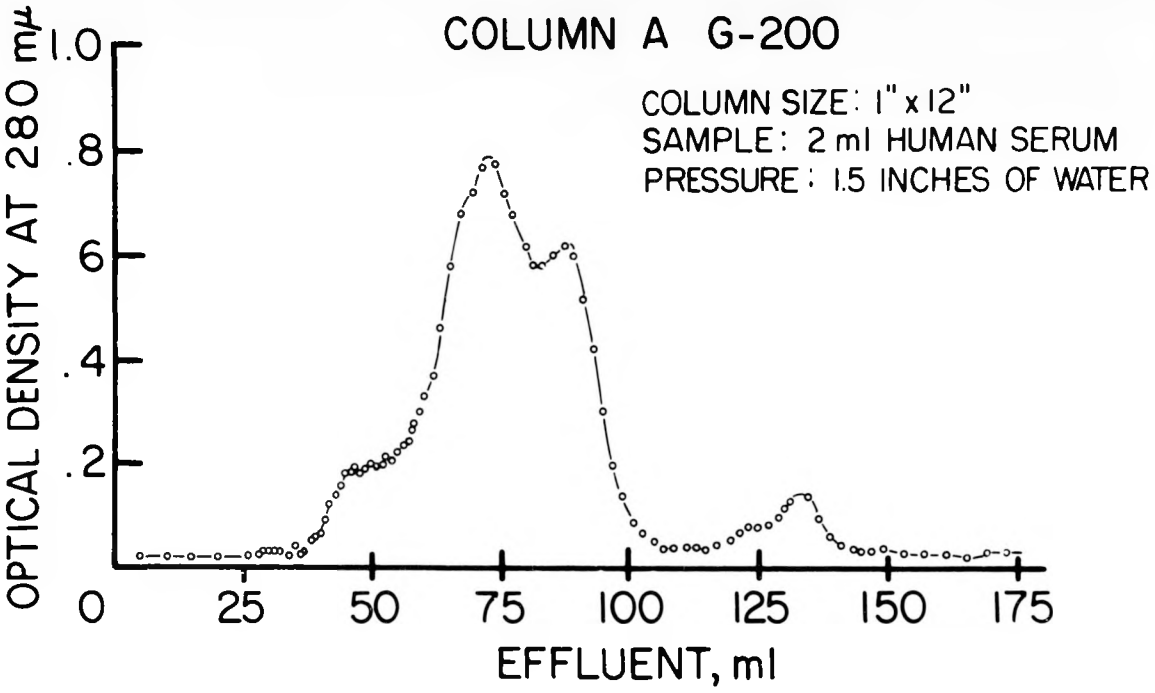

Fig. 4b. Compare with 4a: No radiotracer, different column dimensions, pressure, and flow rate. (See Table 1, Experiment 7)

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

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

Table 1. Flow Rates for Various Sephadex Grades

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

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

RADIOISOTOPES IN DIAGNOSIS AND THERAPY

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

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

Results of this experiment are shown in Table 1.

Table 1

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

* Corrected for long half-time component

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

* Visiting Internist assigned by the Public Health Service.

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

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

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

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

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

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

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

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

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

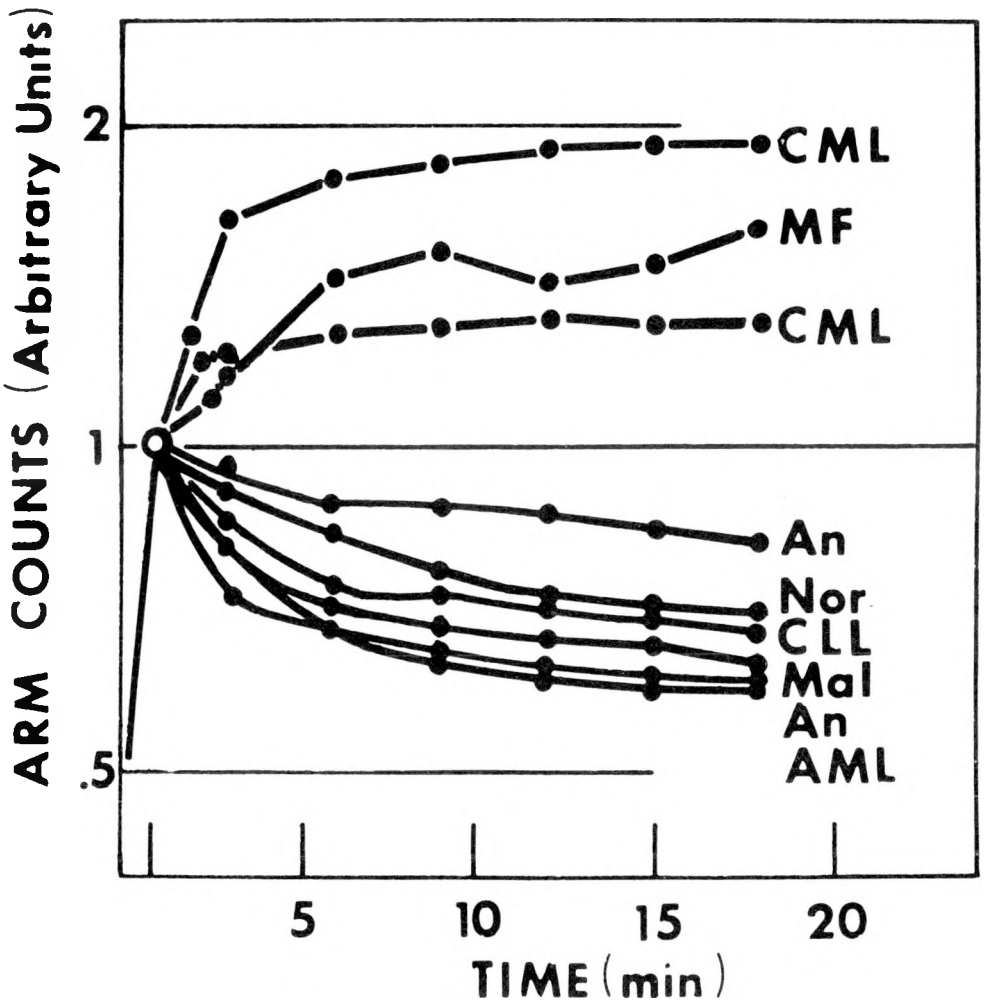

Fig. 1. Relative changes in arm radioactivity after intravenous 0.0005 millicuries cobalt-60 cyanocobalamin in chronic myelogenous leukemia (CML), myelofibrosis (MF), refractory anemia (An), chronic lymphatic leukemia (CLL), malabsorption syndrome (Mal), acute myelogenous leukemia (AML) and a normal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

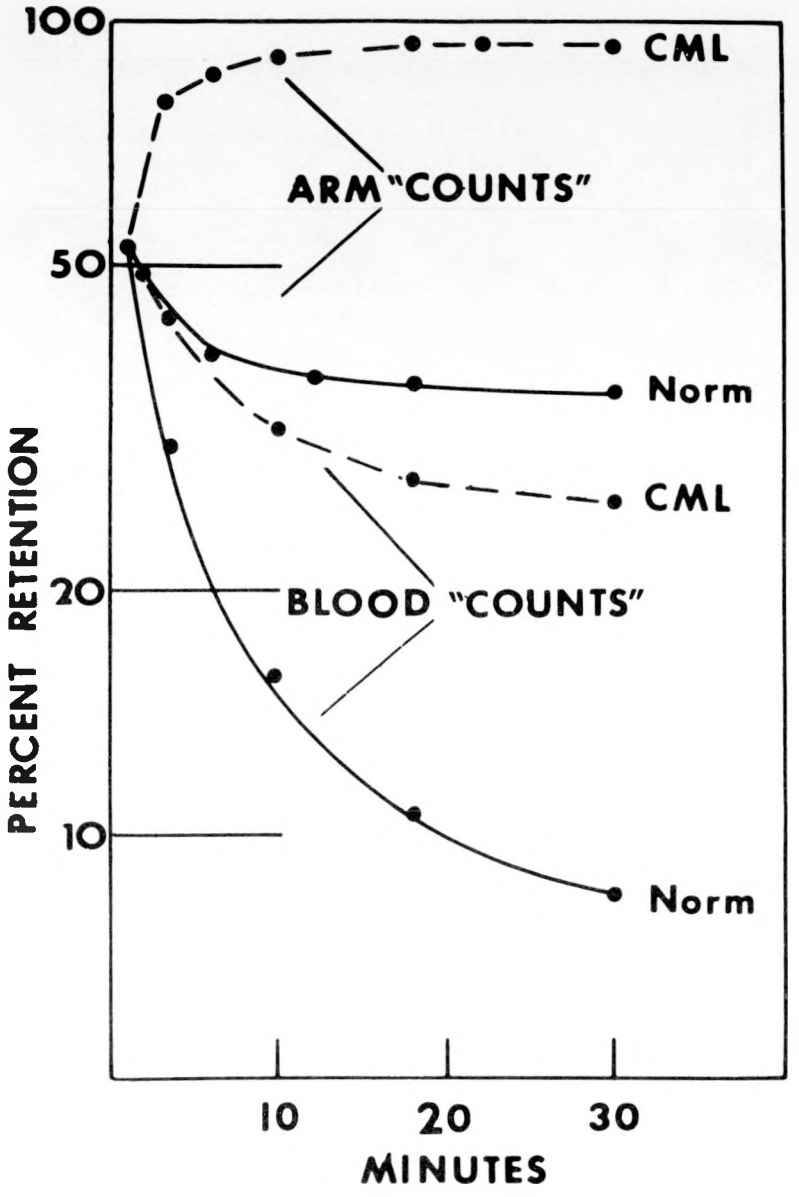

Fig. 2. Comparison of typical changes in arm and blood radioactivity in a normal person and a patient with CML after intravenous vitamin B_{12} labeled with radioactive cobalt, expressed as percentage of retention of dose to correct for differences in doses, blood volumes, and counter efficiencies.

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

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

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

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

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

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

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

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

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

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

Each value is the average of five determinations.

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

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

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

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

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

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

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

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

* IAEA Fellows, 1962, 1963 from Bankok, Thailand

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

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

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

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

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

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

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

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

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

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

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

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

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

References

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

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

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

MEDICAL INSTRUMENTS DEVELOPMENT

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

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

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

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

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

High-Level, Whole-Body Counter (Cyclops)

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

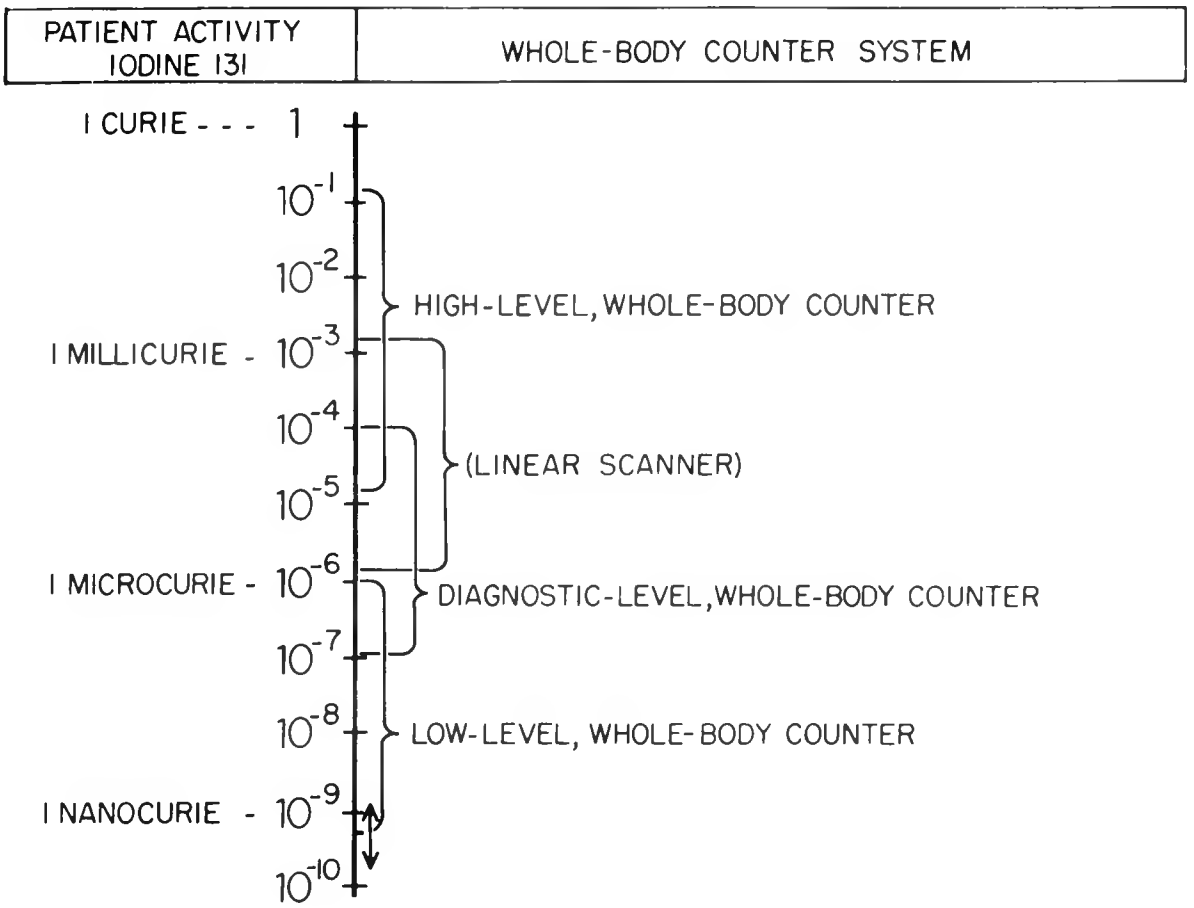

Fig. 1. Sensitivity ranges for the ORINS Whole-Body Counters.

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

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

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

Diagnostic-Level, Whole-Body Counter

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

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

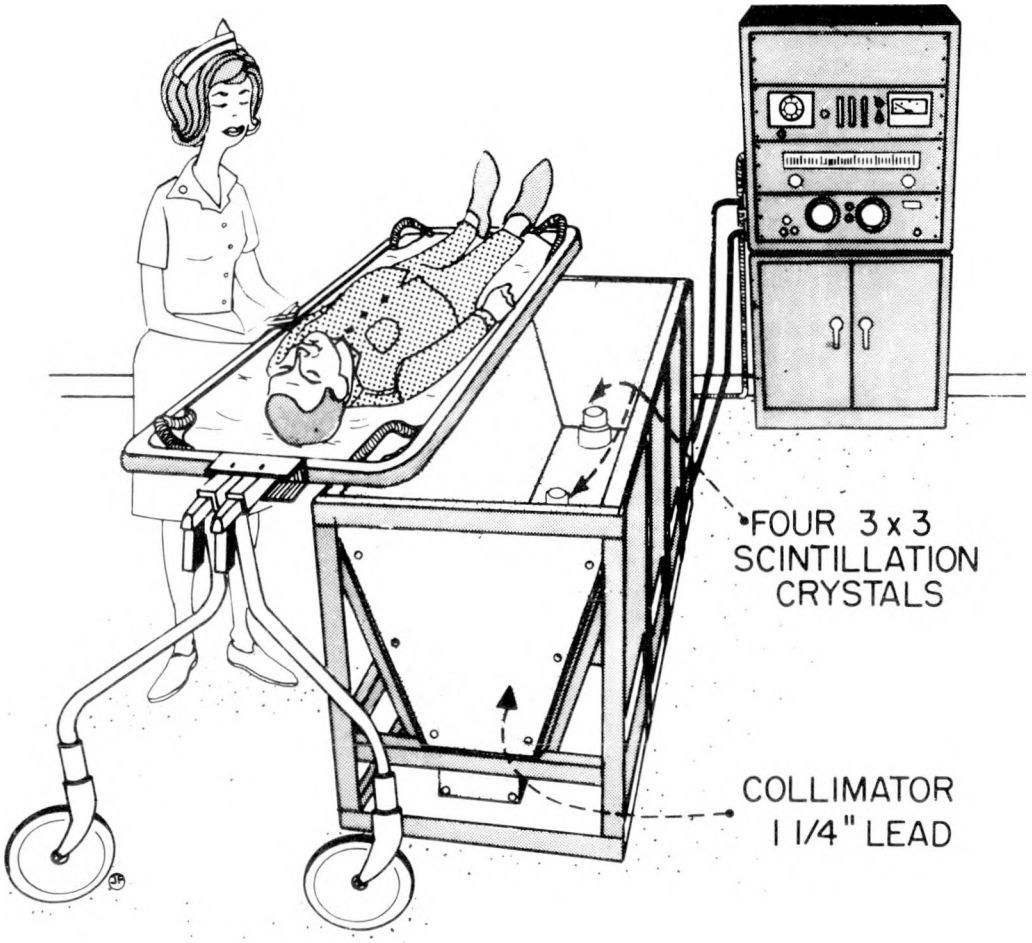

Fig. 2. The ORINS diagnostic-level whole-body counter.

Low-Level, Whole-Body Counting Facility

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

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

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

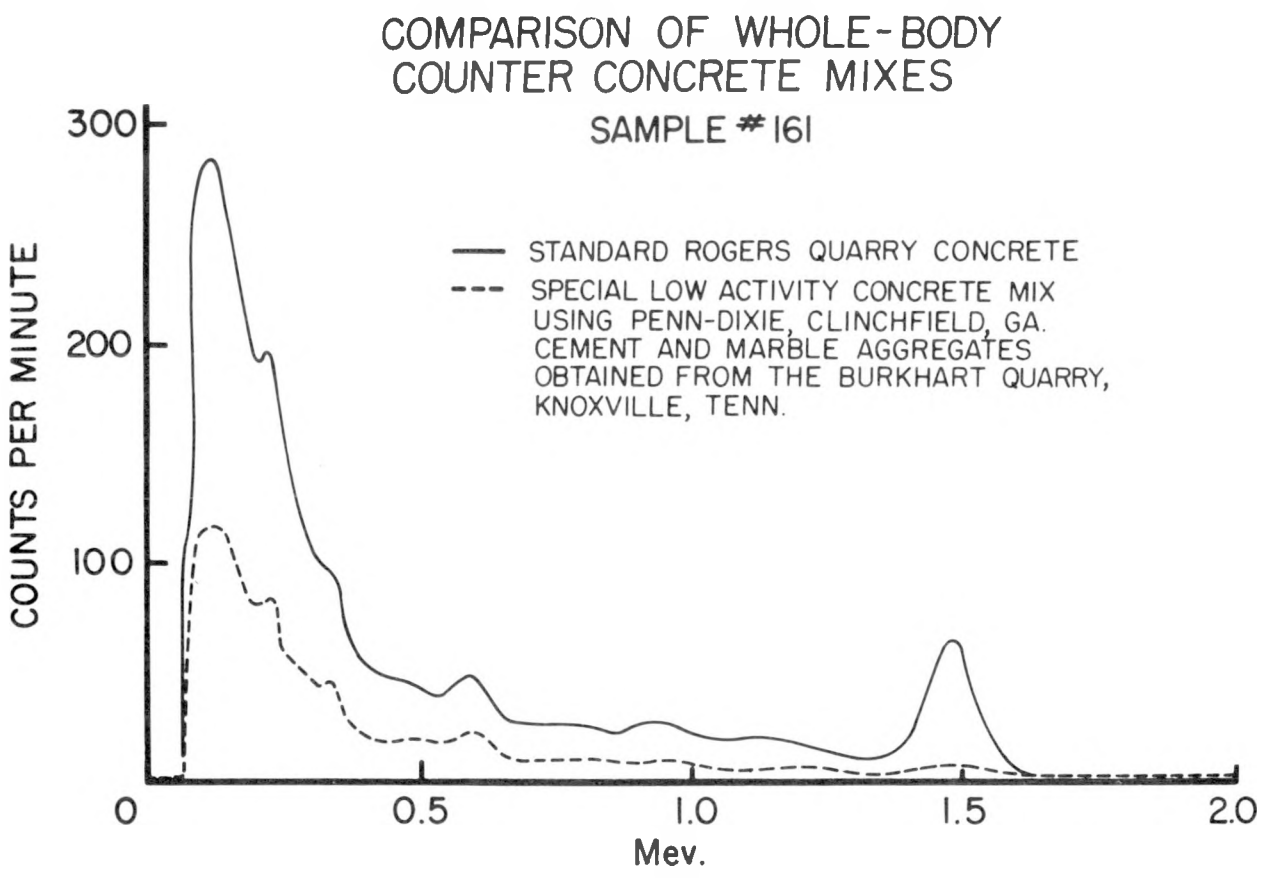

Fig. 3. Comparison of spectra of the locally available concrete mix and the special low-activity mix used in building the ORINS low-level whole-body counting facility.

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

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

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

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

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

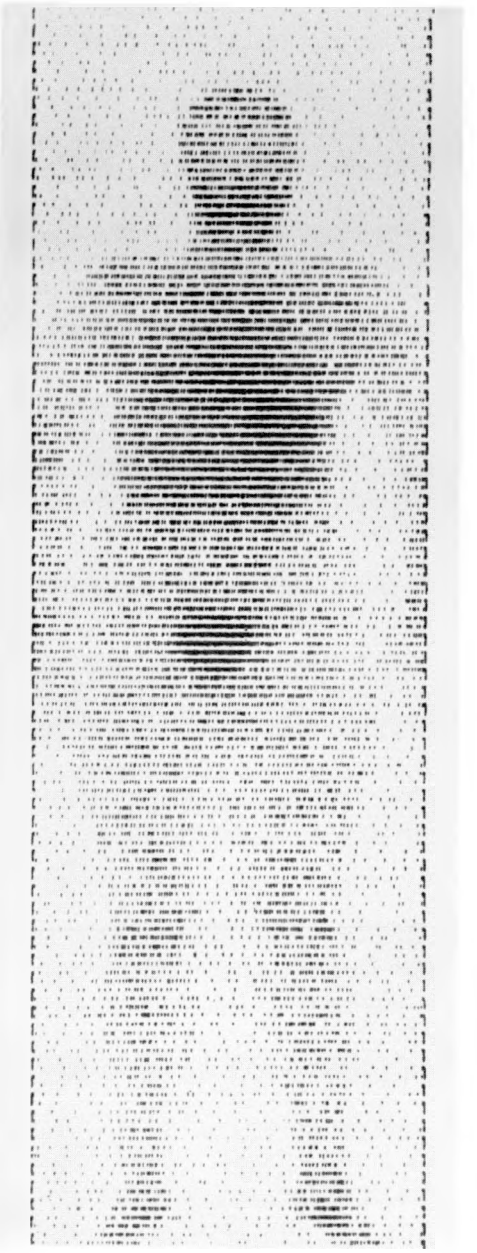

Fig. 1. Scan of a surgically athyroid patient made 5 hr after a 5-millicurie dose of I^{131} as NaI.

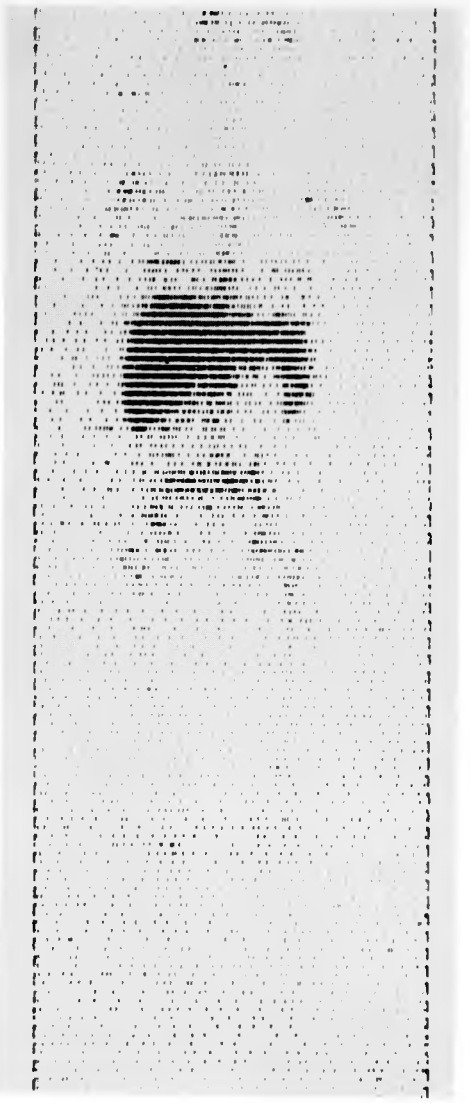

Fig. 2. Scan of a patient with acute granulocytic leukemia who had been given 2.5 millicuries of Au^{198}.

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

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

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

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

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

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

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

* IAEA Fellow from Istanbul, Turkey.

Fig. 1. ORINS arm counter.

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

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

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

Table 1

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

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

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

Table 2

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

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

SCANNING AND WHOLE-BODY COUNTING

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

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

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

Bone-Marrow Scans (till November 15, 1963)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

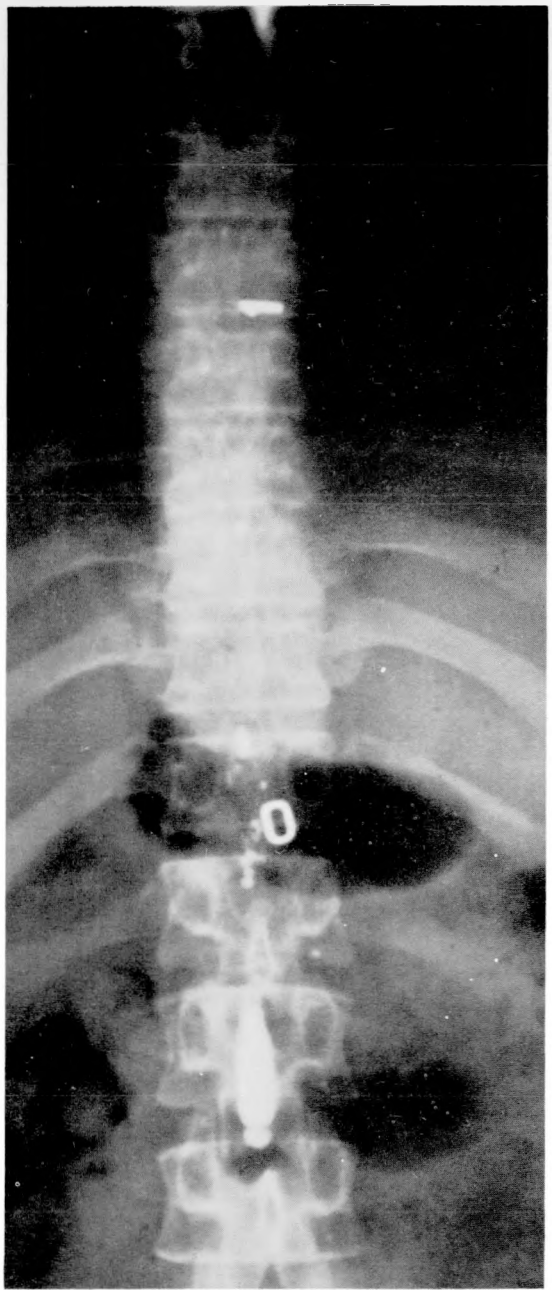

Fig. 2a. Pantopaque myelogram showing compression of the spinal cord at T 10 (metastatic tumor, liposarcoma).

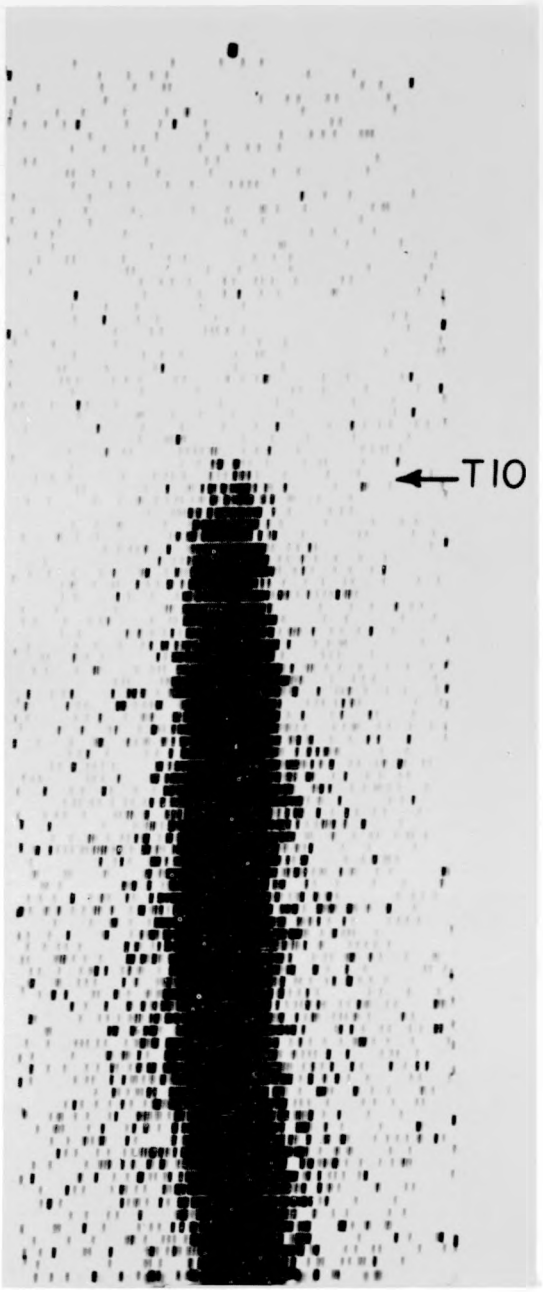

Fig. 2b. Scintigram showing block at T 10.

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

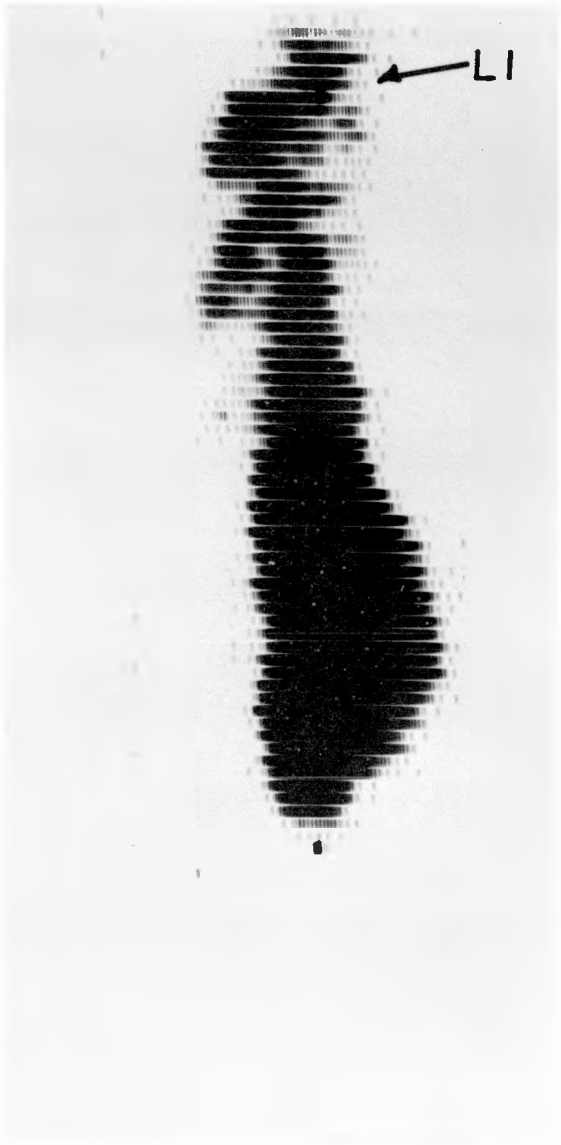

Fig. 3b. Rescan of Fig. 3a, an electronically obtained image made by using the ORNL rescan device to enhance the contrast of the primary scan.

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

* * *

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

* * *

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

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

The results to date are summarized in the following tables:

Table 1

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

Table 2

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

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

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

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

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

* Summer Research Participant, Sam Houston (Texas) Teachers College.

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

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

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

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Table 1

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

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

* Number of animals

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