ACRH-2

ARGONNE CANCER RESEARCH HOSPITAL
950 EAST FIFTY-NINTH STREET • CHICAGO 37 • ILLINOIS

Semiannual Report to
THE ATOMIC ENERGY COMMISSION

SEPTEMBER 1954

LEON O. JACOBSON, M. D.
Editor

OPERATED BY THE UNIVERSITY OF CHICAGO
UNDER
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DISCLAIMER

I

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ARCH-2

A CLINICAL INVESTIGATION OF THE CHRONIC EFFECTS OF RADIUM SALTS ADMINISTERED THERAPEUTICALLY (1915-1931)*†

By

R. J. Hasterlik, W. B. Looney‡, A. M. Brues**, and E. Skirmont**

INTRODUCTION

Within two decades of the isolation and identification of radium by the Curies, the therapeutic administration of this element, its isotopes, and other closely related radioactive elements had become an established medical procedure. As early as 1914, an extensive literature had developed relating to the therapeutic efficacy of radon administered by inhalation, orally, and parenterally; of soluble radium salts, orally and parenterally; of thoron by inhalation; and thorium X, orally and parenterally. Mesothorium, radiothorium, and polonium had also been used. (1-9) The diseases for which these radioactive materials had been exhibited were legion and included such clinical diversities as syphilis, hypertension, gout, infectious polyarthritis, "muscular rheumatism," leukemia, pernicious and other anemias, epilepsy, and multiple sclerosis. (10-36)

The parenteral and oral use of radium and its congeners was well established in the United States and Europe by 1913. Rowntree and Baetjer(9) summarized the treatment of 1038 persons injected with radium in various clinics and reported clinical improvement in 837. Early enthusiasm was based on an apparent high degree of therapeutic efficacy, the absence of clinically observable acute toxic manifestations, and the erroneous belief that the radioactivity was totally eliminated from the body after a period of months. The appearance of toxic effects at a very late period (10 to 20 years after exhibition of the medication) was not considered. Parenteral dosages, administered weekly, varied from 5 to 100 μg and were continued to a total dose as great as 1000 μg. On occasion, 1000 μg of radium were administered as a single dose. The scientific literature that appeared between 1913 and 1916 contains case reports of more than 70 individuals treated intravenously with between 50 and 1000 μg of radium. (4, 13, 15, 31, 32, 37, 38)

Until the late 1920's, the administration of radium, orally and parenterally, and radon and its daughters, orally, parenterally, and by inhalation flourished. One physi-

* An extension of this text, which includes a series of individuals who were patients in the Elgin State Hospital, Elgin, Illinois (see ANL-4666), has been submitted by W.B. Looney, R.J. Hasterlik, A.M. Brues, and E. Skirmont for publication in the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine.

† A large part of this investigation was conducted at the Argonne National Laboratory, Lemont, Illinois.

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cian[39] stated in 1921 that, since 1913, he had given over 7000 injections of radium element in doses ranging from 10 to 100 μg.[40-43] Over the years, he had treated "several thousand" patients with intravenous injections of radium.[44] Data published by other clinicians record the administration of radium to at least a hundred patients, as late as 1926-1927. It is also known that the administration and taking of soluble radium salts has continued sporadically to the present time in the United States. There must, therefore, exist in the United States a residuum of perhaps hundreds or thousands of individuals who have radium salts deposited in their bodies.

With the publication in 1924, of the first paper by Blum[45], soon followed by that of Hoffman[46], and the classic papers of Martland and his co-workers [47-52], evidence became available of the long-term retention in the body of radium, mesothorium, and radiothorium with the attendant delayed toxic effects manifested as bone necrosis, neoplasia, and hematopoietic dyscrasias. However, the therapeutic use of radium salts administered orally and parenterally continued until 1932, at which time radium for internal administration was removed from listing in the New and Nonofficial Remedies of the American Medical Association.

Data derived from experiments done on small laboratory animals with the α particle-emitting, long-lived elements are not readily extrapolated to man because of marked differences in life span, in geometry of the biologic systems studied, in metabolism, and in the rates of calcium deposition and turnover. Fundamental in the calculations involved in the determination of permissible body burdens of radioelements deposited in bone have been the studies done on the radium dial painter cases by Martland and his associates. Inherent, however, in an unbiased analysis of these data is the requirement that the groups studied not be unduly weighted with individuals seen by physicians first because of the appearance of clinical symptoms. It seemed desirable to the authors to extend the past and current investigations by Aub, Evans, Hempelmann, and Martland[53], by an analysis of data derived from as many patients as were available from the residuum that had received radium therapeutically and that had been selected, if possible, for this reason alone, rather than on the basis of their seeking medical care because of the presence of symptoms. Close attention has been directed to an evaluation and detection roentgenographically of a "minimal" bone lesion that would objectively indicate bone damage not necessarily associated with clinical symptoms. To this end, the authors undertook to find as many individuals as possible in the Chicago area who had been given radium therapeutically, as well as those included in the original elgin State Hospital series.*

It has been possible to find and study 20 individuals in the Chicago area who were given radium as a therapeutic procedure from 20 to 30 years ago (Table 1). In addition, 6 "dial painters" from an Illinois watch dial factory have been studied.

* See Bibliographical item 54.

Table 1

ESTIMATED BODY BURDEN OF 20 INDIVIDUALS ADMINISTERED RADIUM THERAPEUTICALLY IN THE CHICAGO AREA 20 to 30 YEARS AGO

Patient code	Sex	Dose administered (μg)	Age at examination	Approximate age in years at time of administration	Route of administration	Estimated body burden in μg of radium			Radon/radium ratio
						ɣ ray	Rn	Total	
119	M	unknown	30	6-8	orally	.01	.02	.03	.66
115	F	unknown	72	59	intramuscularly	--	--	.06	--
109	F	unknown	65	33	orally	.03	.04	.07	.67
120	M	unknown	32	8-10	orally	--	.097	--	--
117	M	unknown	44	16	intramuscularly	.11	.4	.5	.80
101(1)	F	250	73	47	180 μg orally 70 μg intramuscularly	.1	.41	.5	.80
116	F	160	53	31	intravenously	.21	.5	.7	.71
105	F	unknown	74	unknown	unknown	.17	.69	.86	.80
113	M	unknown	58	30	intramuscularly	.4	.6	1.0	.60
112	F	unknown	48	20	orally	.7	.6	1.3	.46
102	M	unknown	81	unknown	unknown	.5	1.1	1.6	.69
114	F	unknown	57	unknown	unknown	.61	1.0	1.6	.62
111	F	300-400	67	33-36	intramuscularly	2.0	2.2	4.2	.52
110	M	700-800	82	51-58	intramuscularly	1.6	3.8	5.4	.42
106	M	circa 200	54	25	intramuscularly	2.3	3.3	5.6	.59
103	F	unknown	63	unknown	unknown	2.27	4.30	6.57	.65
K.B.	F	unknown	66	46	orally	--	--	2-10*	--
104(2)	M	circa 330	78 †	51-56	intravenously	5.0	5.7	10.7	.54
108	F	unknown	69	37-42	unknown	4.4	6.9	11.3	.61
107	M	more than 250	70	43	intramuscularly	4.4	9.5	13.9	.68

(1) (Patient III) (2) (Patient VII) – as described in Barker, H.H. and H. Schlundt, 1930. The detection, estimation and elimination of radium in living persons given radium chloride internally. II. Am. J. Roentgenol. Radium Therapy, 24:4.

* Estimated in 1947 from count of ɣrays by Geiger-Müller counter.

† Age at death.

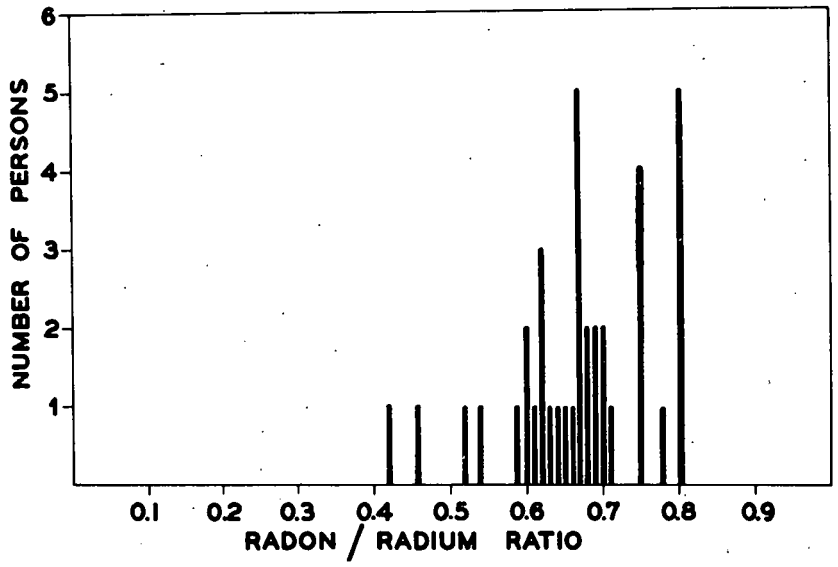

Figure 1 Distribution of radon: radium ratios in 37 individuals. This figure includes data from the Elgin series of patients.

In this study, we have fulfilled the following criteria: a period sufficient for the development of "late" effects has elapsed (20 to 30 years); the patients were selected (with but 6 exceptions) on the basis of having received radium and not because they were ill. Conclusive evidence has been secured by the authors from the papers furnished by the U. S. Radium Corporation that the luminescent paints used by the young women studied in the Martland-Aub series were composed of the phosphor and continu-

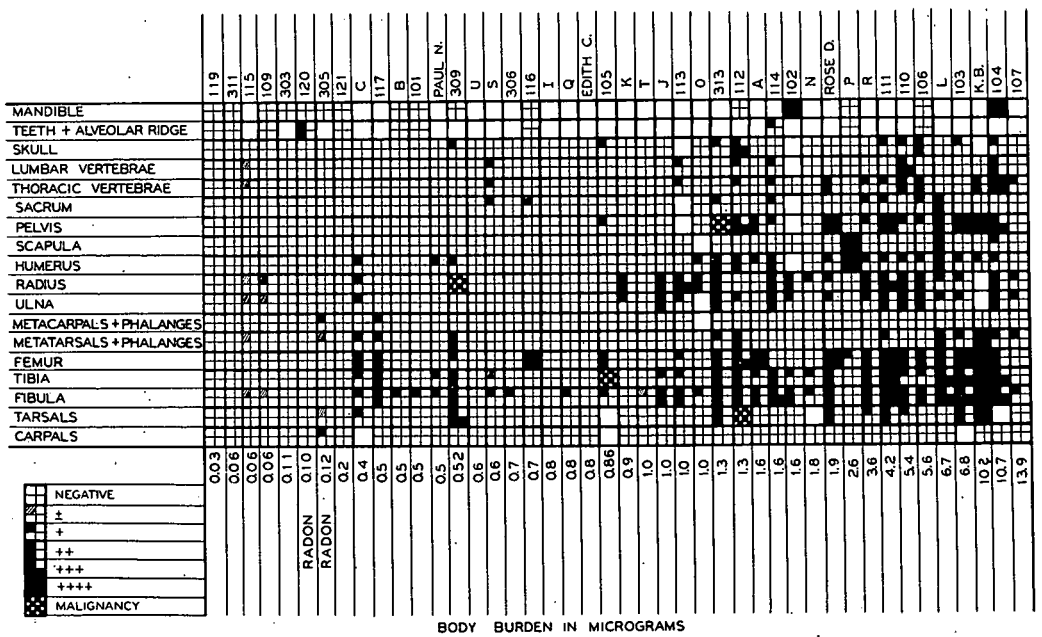

Figure 2. Relationship between body burden of radium and the frequency and severity of bone lesions. This figure includes data from Elgin patients.

ally varying proportions of radium and mesothorium, mesothorium and radiothorium, radium and radiothorium, or radium or radiothorium alone. It thus seems impossible at present to quantitate relative effects of these related radioelements in any one individual of the Orange, New Jersey, series.

Total retained radium and radon/radium ratios were determined for all patients (Figure 1).

CLINICAL STUDIES

Roentgenographic examination of the entire skeleton was done on all patients. In addition, as controls, roentgenograms were made of the extremities of 40 persons selected at random while undergoing a general physical examination at the Health Division of the Argonne National Laboratory. Each roentgenogram was examined, and the result of the study plotted on a master chart (Figure 2). Bone lesions were expressed as being questionably present, or of 1+, 2+, 3+, or 4+ severity.* All hematologic studies (Table 2) were carried out by one of the authors (E.S.); the general examinations were made by the physicians of the group.

ROENTGENOGRAPHIC FINDINGS

The major bone changes occurring in persons with radium deposited in the skeleton have been described in detail by Martland[48, 51, 52] and Aub et al.[53] In this evaluation of the late effects of radium, an attempt was made to recognize and describe the bone lesion, minimal in extent and appearing earliest, that could be associated with the presence of radium in the skeleton. It was soon recognized that since the pathogenesis of the bone changes would not be singular, so also, there could not be only one specific bone lesion.

The "major" roentgenographic changes seen in the Martland and Aub series of patients, mostly from the radium dial industry, have been well described by these observers. The following excerpts from the early article by Martland[51] give the essential features of bone damage in the "dial painter" cases.

> "As the jaw necrosis was the outstanding feature of the early cases, our attention was fixed mainly on this symptom and little attention was paid to changes in the other bones of the skeleton, although an extensive coxa vara was noted in one case. ... These bones were thought to contain a greater amount of radioactivity than did other bones of the skeleton. It was not until I began to see the later cases that I realized that this was not the correct interpretation of the lesion. In the later cases, localized pain had occurred in other bones and destructive lesions were demonstrated by the roentgenograms. These lesions were particularly noticeable in bones

* Those lesions classified as 1+ fall in Group I (vide infra), as 2+ fall in Group II, and as 3+ and 4+ in Group III.

Table 2

HEMATOLOGICAL STUDIES

Patient Code	Estimated total body radium (μg)	Date	Erythrocyte count (per cu mm)	Hemoglobin (photoelectric method) (g/100 cc)	Leucocyte count (per cu mm)	Polymorphonuclear count (%)	Stabs (%)	Metas (seen in 300 cells)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	Condition of red cells on differential	Mitotic lymphs (total seen)	Bilobed lymphs (total seen)	Immature lymphs (total seen)	Slightly immature lymphs	Atypical lymphs	Degenerating cells	Cells with vacuoles	Giant platelets	Atypical platelets	Sedimentation rate (mm in 1 hr.)	Hematocrit (%)
109	.07	1/31/51	467	13.7	5650	48	-	-	40	7	5	-	Normal	-	-	-	-	occ.		-	occ.	-	35.0	41
		4/27/51	424	12.6		49	-	-	47	2	1	1	Normal	-	-	1	-	-		-	-	-	-	-
117	.5	3/16/51	440	13.2	7700	53	2	-	34	4	6	1	Slight anisocytosis	2	-	1	3	-	-	1% eosinophil	few	few	22.0	44
116	.7	11/17/50	467	12.7	11200	72	-	-	26	1	1	-	Normal	-	-	-	-	-	-	2 lymphs	-	-	27.0	39
113	1.0	4/25/51	485	15.2	7700	51	3	1	44	1	1	-	Normal	3 lymphs & lg. azure gran.			2	-	-	-	-	-	12.0	48.5
102	1.6	4/4/51	504	14.9	10000	61	3	1 Basophilic	30	4	1	1	Normal	1	3	-	3	-	-	3 lymphs	few	few	13.0	52
114	1.6	4/27/51	426	13.8	6400	64	-	-	34	1	1	-	Normal	-	-	-	-	-	-	-	-	-	32.0	43
112	1.3	1/11/51	374	11.2	4900	59	2	-	36	1	2	-	Marked anisocytosis Mod. poikilocytosis Slight hypochromia	polys toxic			-	few		1 lymph with vacuoles	- -	- -	54.0 54.0	36 -
		5/1/51	414	11.5	7550	63	2	-	30	2	3	-	Slight anisocytosis and hypochromia	polys toxic			3	-	2 lymphs					
106	5.6	4/17/51	494	16.0	9750	65	1	-	31	1	2	-	1 Polychromatic red cell	-	-	-	-	occ.	-	1 poly 1 eosinophil	-	-	20.5	50.5
104	10.7	3/13/51	356	11.9	10600	67	7	1	23	1	1	1	Moderate anisocytosis Slight poikilocytosis and hypochromia	few toxic polys			4	-	-	few polys and eosinophilis occ. lymph	-	-	38.0	35
108	11.3	5/10/51	485	14.0	7000	68	-	-	27	4	1	-	Normal	-	-	1	2	-	2 lymphs	2 lymphs	-	-	32.0	44

exposed to trauma, like the scaphoid in the feet (tight shoe), and those carrying considerable weight, such as the spine and heads of the femurs. ... In other words, the lesions were quite evenly distributed over the entire skeleton, although in each individual bone the distribution, while diffuse, was distinctly patchy and concentrated in areas."

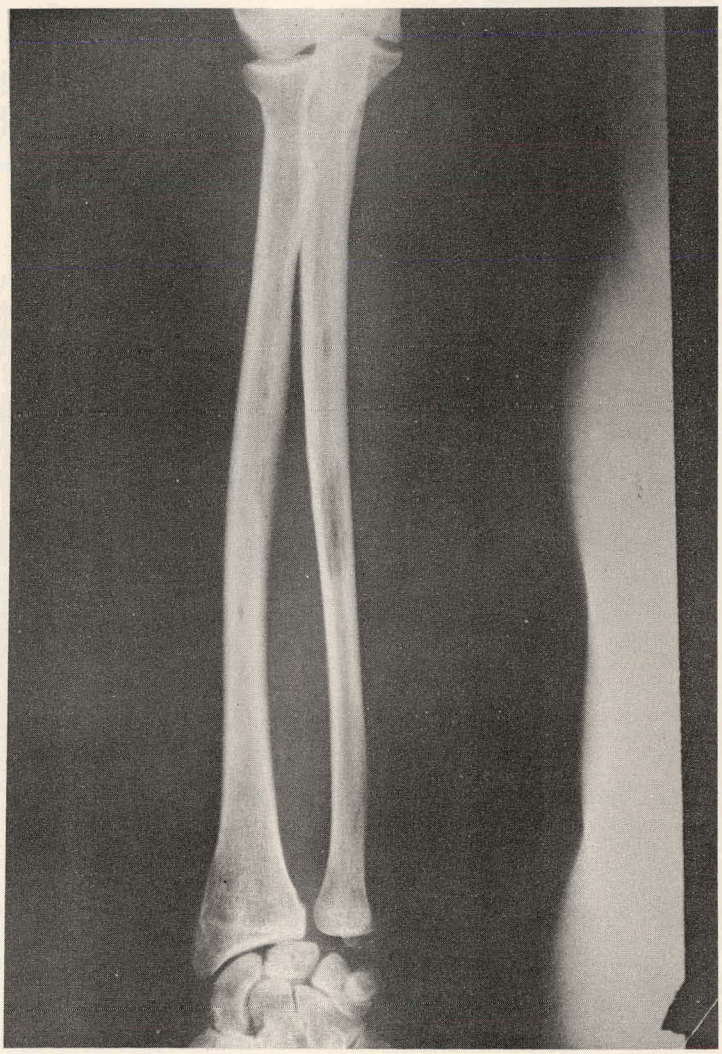

Figure 3. Small and large isolated areas of bone resorption in the radius and ulna.

Martland[48], as early as 1931, demonstrated the roentgenographic appearance of the skull showing areas of rarefaction, and Flinn[55], in 1934, considered these "punched out" areas in the skull to occur with sufficient frequency in "radium dial" patients to assume diagnostic value. Aub,[56] in 1938, described the roentgenographic changes in the heads of the humeri and femora as well as in the tarsal bones.

ROENTGENOGRAPHIC CLASSIFICATION OF PATHOLOGIC CHANGES

Group I. The significant minimal roentgenographic lesions observed by the authors have been multiple 1- to 2-mm x 5- to 20-mm areas of decreased density occurring with great frequency and located most prominently in the fibula, tibia, radius, ulna, humerus, and femur (Figures 3 and 4). Related lesions are the "punched out" areas

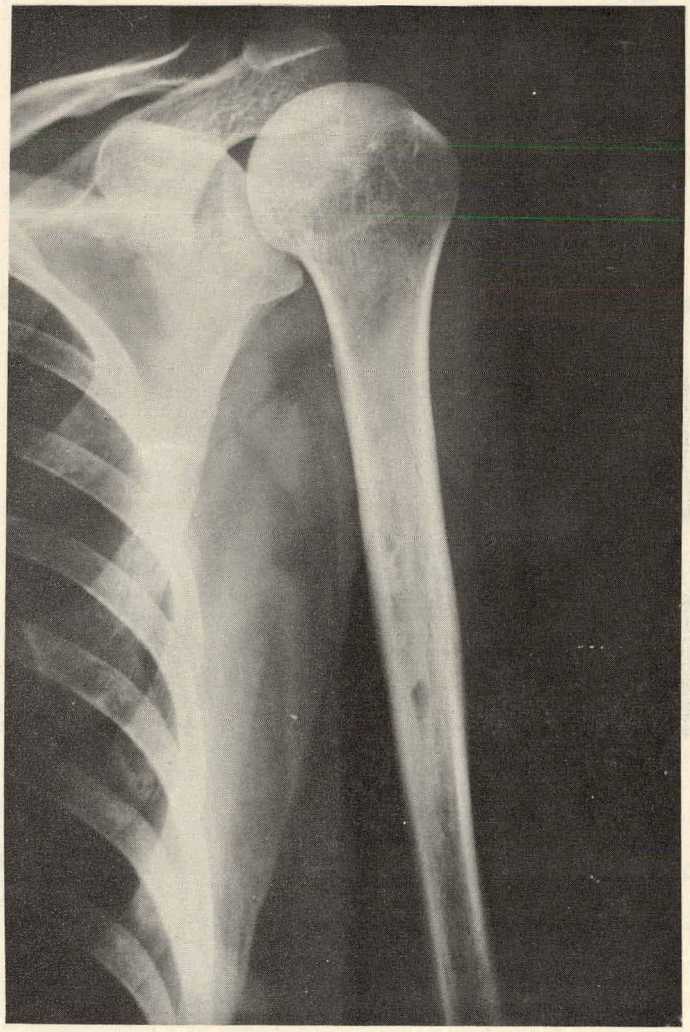

Figure 4. Areas of bone resorption in the humerus of a patient carrying a body burden of 6.7 μg of radium.

(Figure 5) of decreased density in the skull that closely resemble, but must be differentiated from, the lesions of multiple myeloma. The skull lesions vary in size from 2 to 10 mm in diameter and may be irregular or smooth in outline. In one patient (Patient 112), we observed a striking increase in the number and size of these skull lesions over a 3-year period.

When the small areas of decreased density that occur in the long bones become multiple, they lend a "streaked" appearance to the bone (Figure 6). Infrequently, these lesions may be solitary and as large as 3 to 4 cm in diameter. In these instances, the site of predilection is usually the tibia or femur. Occasionally, these larger lesions may be accompanied by diffuse "streaking" of the bone.

We have noted small areas of bone resorption in the humerus, radius, ulna, phalanges, femur, tibia, fibula, and tarsals in a patient carrying a body burden of 0.4 μg of radium. It is concluded that this lesion is not diagnostic of, but is most suggestive of the presence of significant quantities of radium in the skeleton, especially when noted in the cortex of the extremities of the long bones.

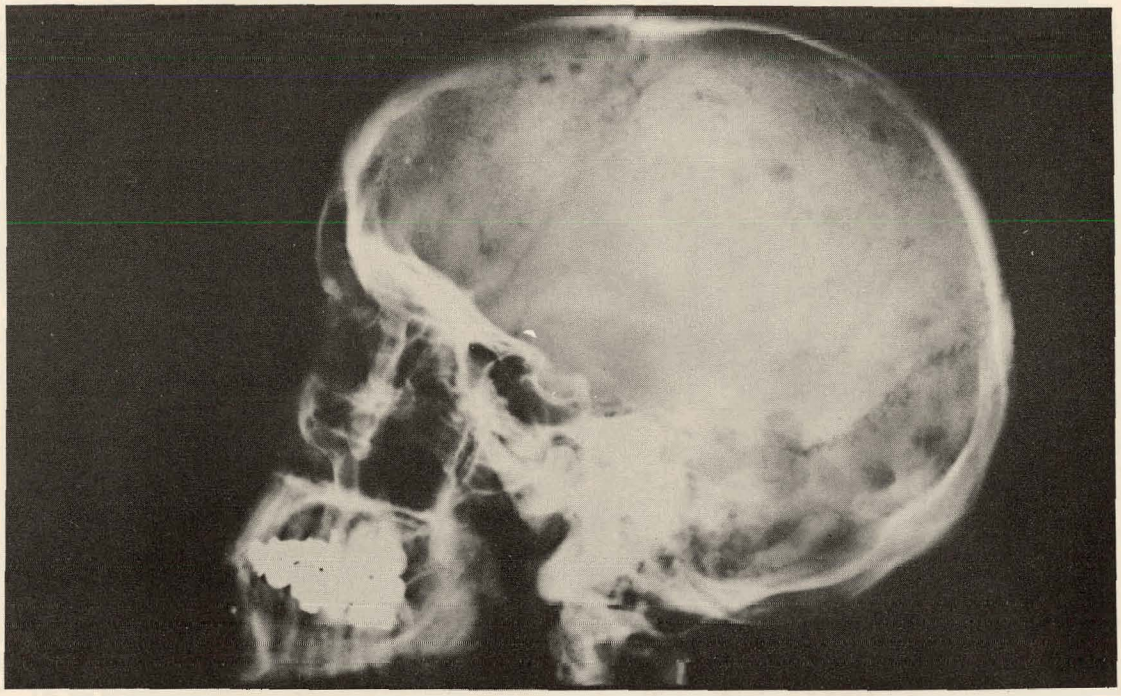

Figure 5. Multiple "punched out" areas of bone destruction in the skull in patient with a body burden of 1.3 μg of radium.

That these areas of decreased density do not represent roentgenographic artifacts but are true areas of bone resorption has been demonstrated by the histologic and autoradiographic studies of thin bone sections by Looney and Woodruff[57] (Figure 7). Accordingly, we believe this to be the minimal roentgenographically recognizable lesion. Its importance lies in the fact that it can be noted in patients who are asymptomatic, in the absence of other more marked lesions, and it occurs with a lesser body burden than any other lesion.

Group II. Areas of apparent increased density usually associated closely with areas of decreased density and showing varying degrees of change in trabecular pattern are characteristic of this group (Figure 8).

Certain bones have areas of apparent increased density of irregular and inconstant shape and size. The lesions may be as large as a centimeter in diameter, resembling the localized bone infarcts seen in "caisson disease," and are not specific for radium poisoning. They appear most frequently in the alae of the ilium and the heads of the femur and humerus.

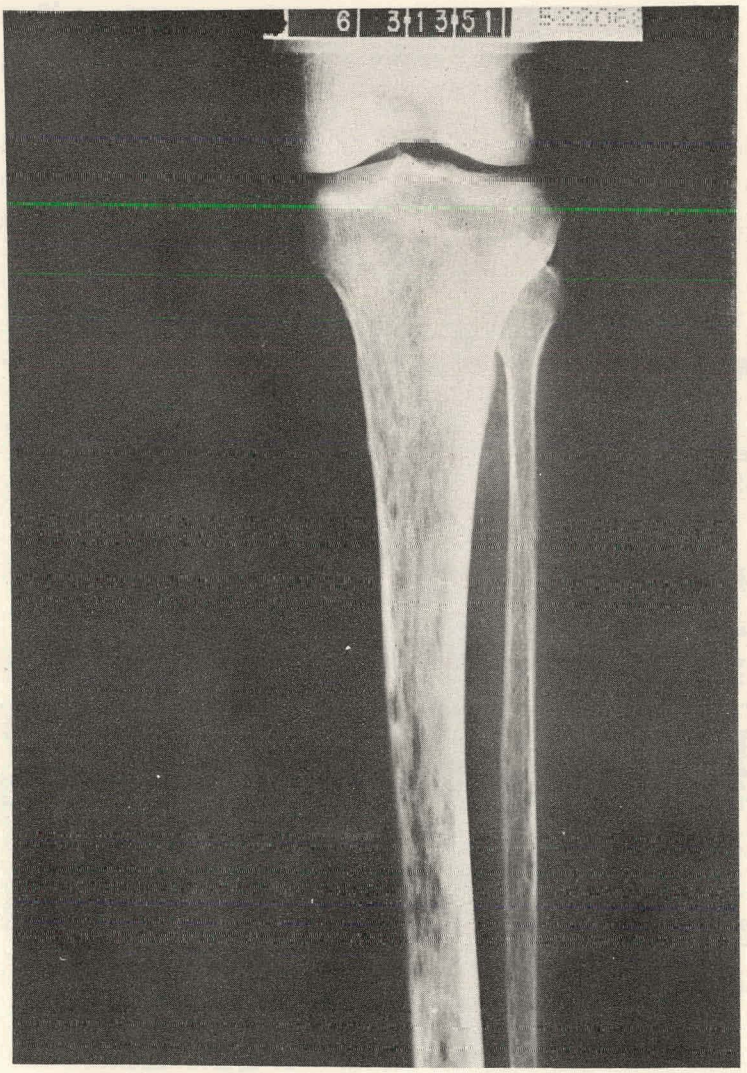

Figure 6. Multiple small areas of bone destruction and resorption lend a "streaked" appearance to the long bones.

A variant of this lesion is characterized by multiple areas of bone resorption contiguous with areas of apparently increased bone density and a broadening and coarsening of the trabeculae, which lend a "mottled" appearance to bone (Figure 9). This is seen in the ilium, the shafts of the femur and humerus and their heads (Figure 10), and in the glenoid process of the scapula where it may be so extensive that the bone appears "moth eaten."

The bodies of the vertebrae, in several instances, show a marked coarsening of the structure of the trabeculae of the spongiosa. In a few instances, small areas of apparent increased density have been noted in the vertebral bodies. It is believed that although these changes are not diagnostic of radium poisoning, and may be nonspecific reactions to several noxious processes, they do occur with increased frequency in patients retaining large burdens of radium (Figures 11, 12, and 13).

Figure 7. Photomicrograph of localized areas of bone destruction in tibial cortical bone from patient with total-body burden of 1.3 μg.

Group III. Certain "major" changes have also been observed in the bones of the patients in our series. From the roentgenographic standpoint they may be described as follows: areas of "aseptic necrosis," often with sequestration, occur in certain sites of predilection. These are the head of the femur (Figure 14), the mandible (Figure 15), tarsal scaphoid (Figure 16), and vertebral bodies (Figure 17). Occasionally one observes necrosis with sequestration in portions of the head of the radius (Figure 18)

and the superior aspect of the acetabulum (Figure 19). Fragmentation of the patella (Figure 20) is not infrequently seen. The dynamics of the skeleton draw attention to a correlation between these sites of vulnerability for bone necrosis and the areas that, during normal activity, regularly undergo greatest mechanical stress. In the pathogenesis of this type of bone lesion, the association of radium deposited in bone and

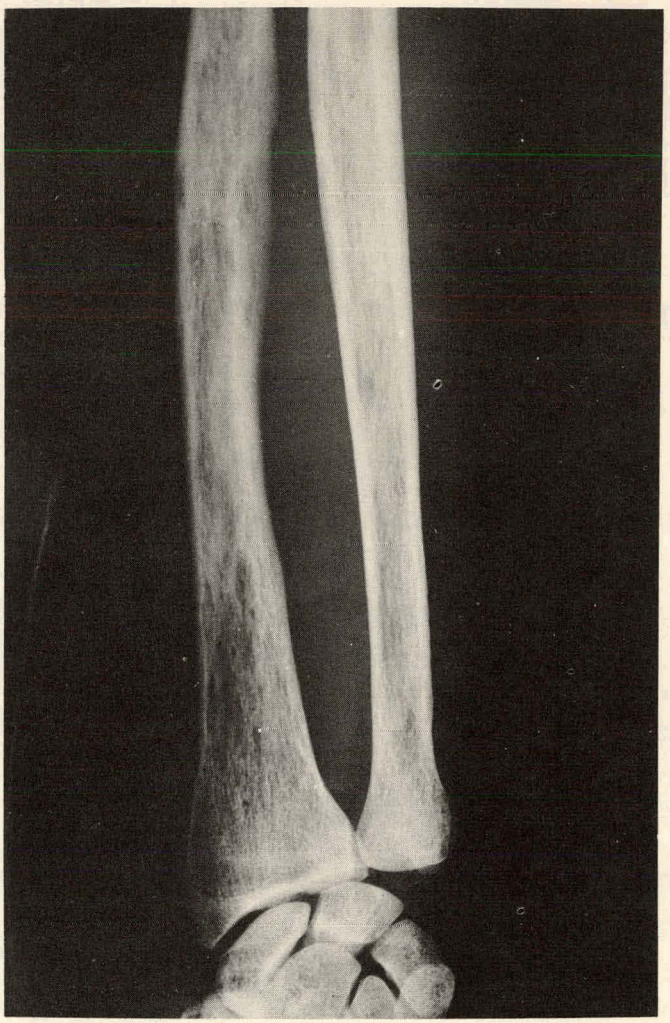

Figure 8. Extensive changes in the trabecular pattern in the radius and ulna.

repeated mechanical insult seems apparent. Taken by itself, the appearance of one of these areas of "aseptic necrosis" in an otherwise apparently "healthy" adult is <u>not diagnostic</u> of radium poisoning, but <u>is suggestive</u>.

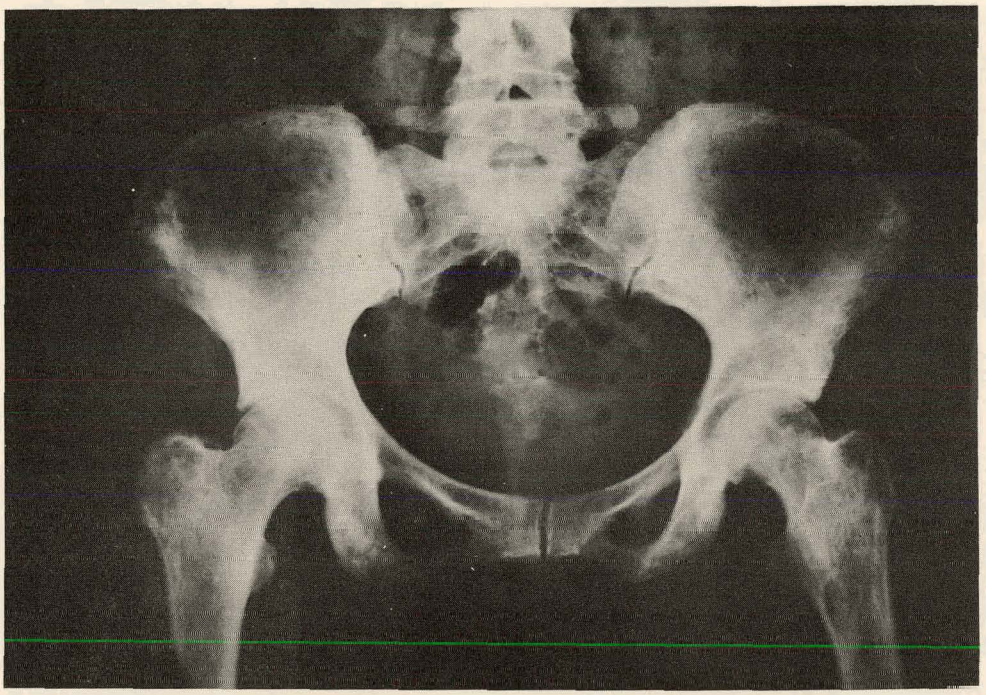

Figure 9. Scattered areas of apparent increased density in the alae of the ilia with coarsening of the normal lacy trabecular pattern in patient carrying body burden of 1.3 μg of radium.

HISTORIES

The clinical histories of those patients who received radium therapeutically and those of the 6 "dial painters" are illustrative of the progress of the disease over a long period; the appearance of the lesions after a long latent period; and of the lack of realization by many of these individuals that they had been given or carried a body burden of radium.

Patient 119 - White, male, age 30. Total-body burden 0.03 μg

This man is the brother of Patient 120 and received radium orally from the age of 6 to 8 years. He has been asymptomatic.

Complete physical examination and roentgenographic study of the skeleton revealed no body lesions. The teeth do not demonstrate lesions similar to those present in his brother.

Patient 311 - White, female, age unknown. Total-body burden 0.06 μg

The patient worked as a radium dial painter in Ottawa, Illinois, from 1941 to 1944, and for 6 months in 1946. The clinical history, physical examination, and skeletal roentgenographic survey were all negative.

Patient 115 - White, female, age 72. Total-body burden 0.06 μg

In 1939, the patient received intramuscular injections of radium chloride in the lower back for "arthritis of the lower spine." There was a negative history and no

other parts were involved. The radium was given every other day; later reduced to once weekly until a total of 10 to 15 injections had been given. The quantity of radium administered per injection is not known. Within 1 month, the patient noted marked relief from pain, and, within 6 months, the pain had subsided. She has remained asymptomatic. The history and physical examination gave essentially negative data.

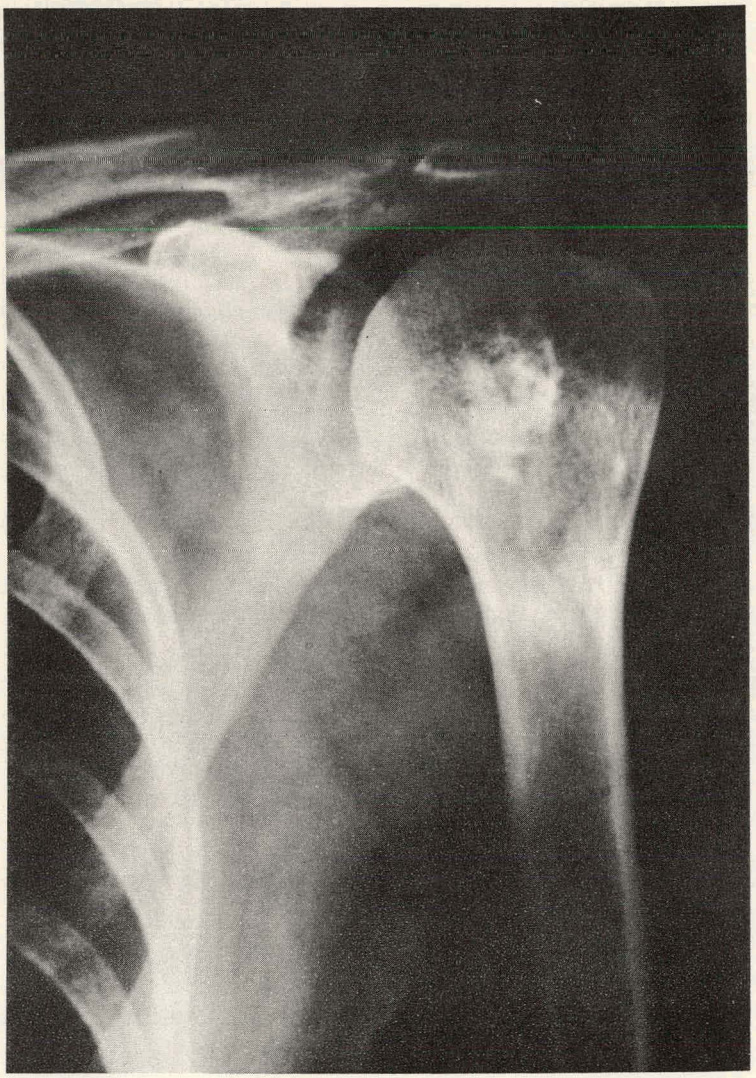

Figure 10. Localized area of apparent increased density in the head of the humerus of a patient carrying a body burden of 1.6 μg.

Patient 109 - White, female, age 62. Total-body burden 0.06 μg

In approximately 1930, the patient was prescribed radium water orally for a number of weeks for "sacro-iliac pain." The patient states that spontaneous "black and blue marks" have occurred intermittently for years, and that she has noted prolonged bleeding following skin lacerations. The teeth had to be removed with care because of

this bleeding tendency. Her mother and one other sister had similar difficulty with prolonged bleeding. The dental examination revealed an absence of all teeth. Skeletal roentgenographic examination was negative for bony lesions related to radium poisoning.

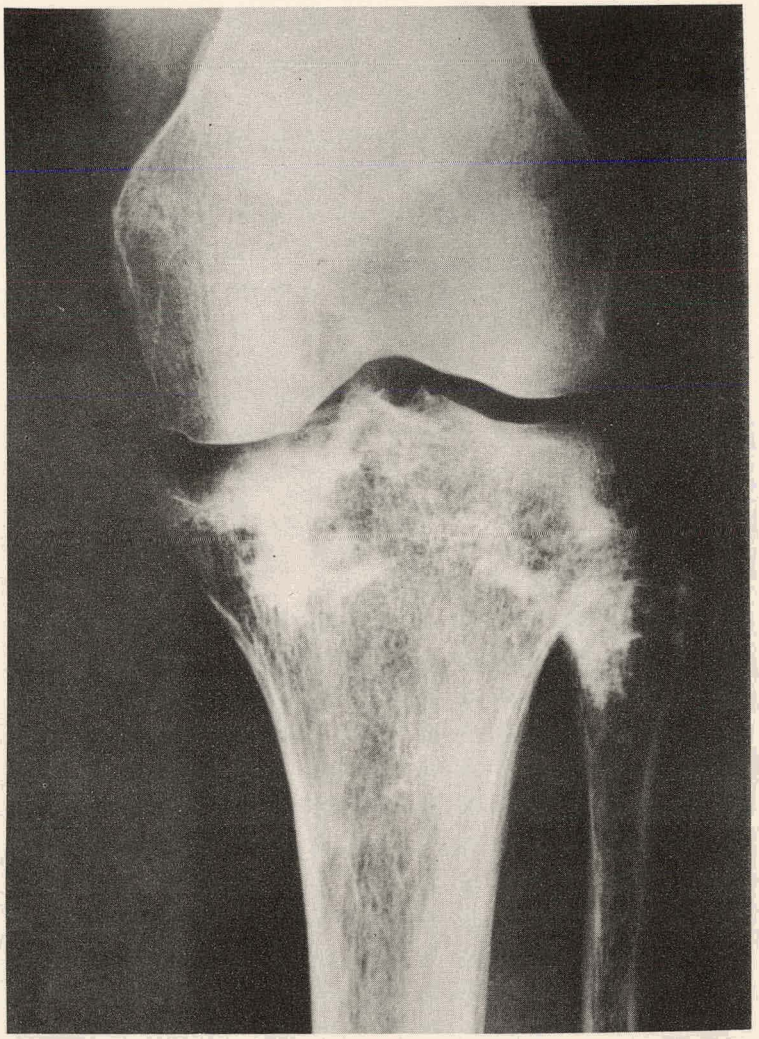

Figure 11. Areas of increased density in the proximal tibia and fibula in patient carrying a body burden of 4.2 μg of radium.

Patient 303 - White, female, age 50. Total-body burden 0.11 μg

The patient was employed as a radium dial painter in Ottawa, Illinois, for 6 months in 1922. In 1934, she noted the onset of an intermittent sharp pain in the hands that would last for 1 to 2 days and then subside for 2 to 3 months. Gradually, this type of pain also involved the shoulders, and by 1940, the hips. The patient also complained of difficulty in walking. During 1951, a sharp, aching pain developed in the long bones, which was increased on pressure over the bones.

The general physical examination and the complete skeletal roentgenographic survey were completely negative. The relationship of the pain in the bone to the presence of radium is difficult to establish in view of the negative roentgenographic findings.

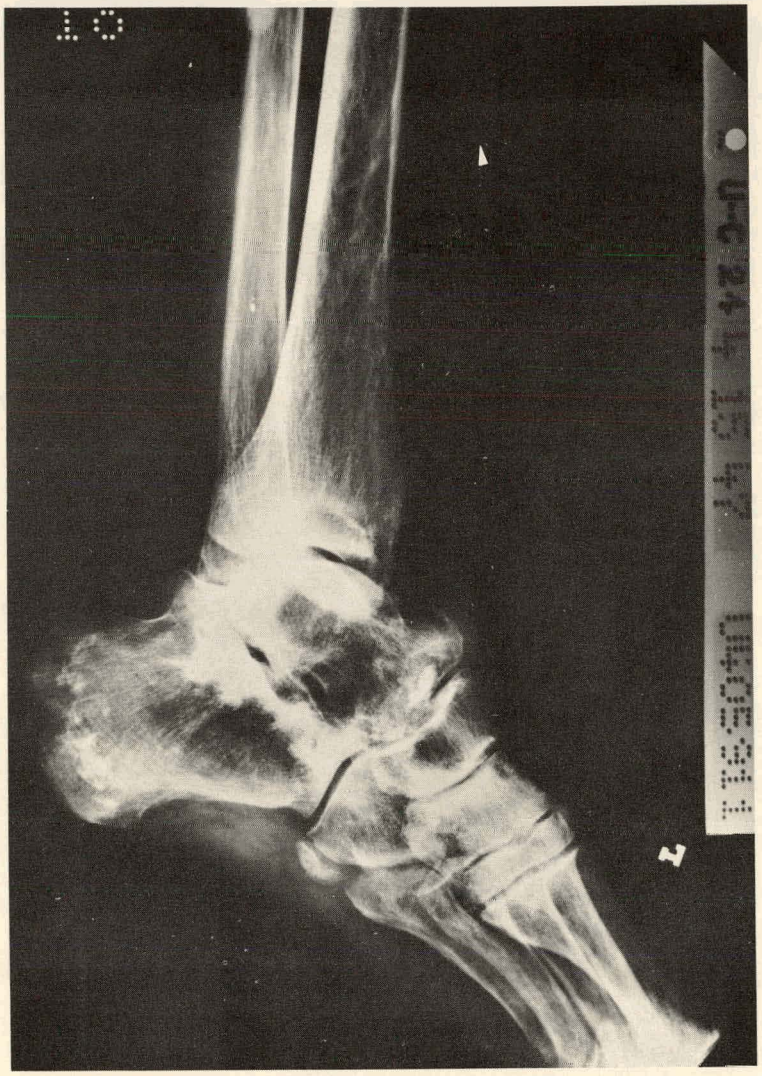

Figure 12. Areas of increased density in the tarsals and metatarsals of a patient carrying a body burden of approximately 10 μg of radium.

Patient 120 - White, male, age 32. Total-body burden 0.15 μg

The patient had been in excellent health all of his life until the age of 32 years at which time his oral surgeon noted a resorption of the teeth at the gum-line leading to the fragmentation and loss of the crown of a central incisor. Radiography of the teeth demonstrated a bizarre-appearing widening of the pulp cavity and destruction of the dentin arising within the teeth.

Dr. W. W. Wainwright of the School of Dentistry of the University of Illinois saw the patient in consultation and recognized these changes as similar to those that had been observed in radium dial paint poisoning cases as well as in some of the patients in the Elgin Hospital series. The patient was referred to our group for study.

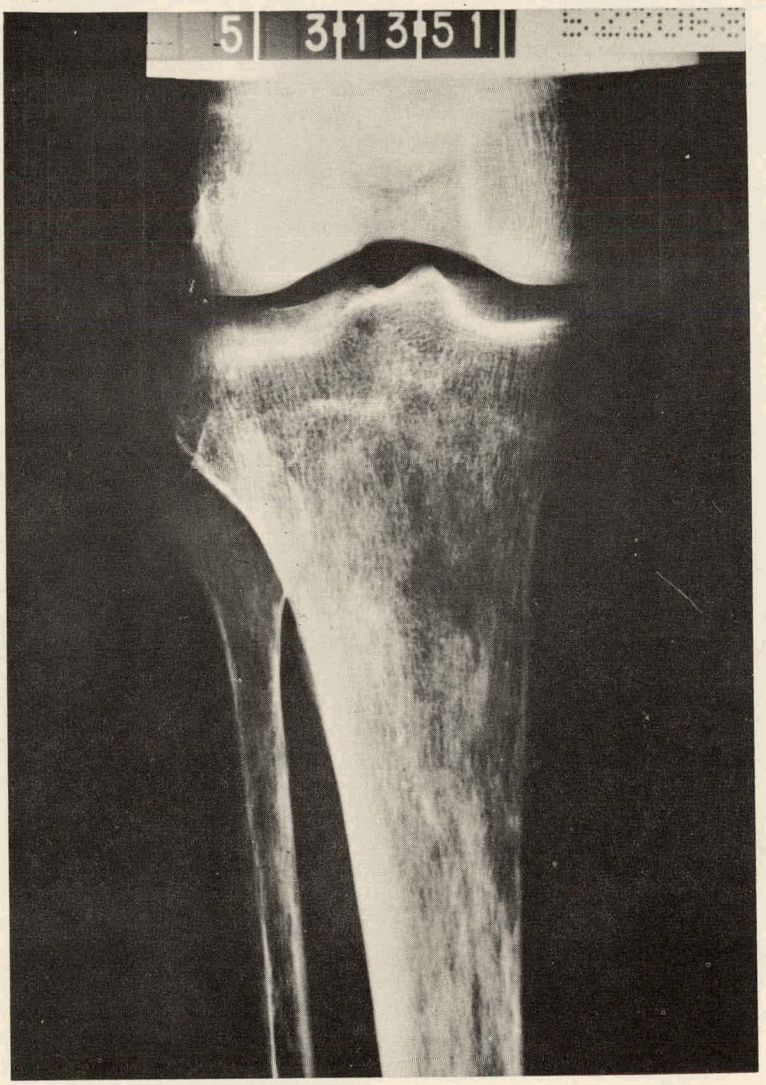

Figure 13. Note the loss of the normal trabecular pattern in the proximal end of the tibia in a patient carrying a total-body burden of 10.7 μg.

On questioning, the patient at first did not recall ever having received radium, and because of his age it was difficult to conceive that he had been given radium therapeutically at the height of its popularity as a medicament. A perusal of the old files of Schlundt revealed, however, that the patient's father, a physician, had been a patient of Dr. ... (Patient 110) and had been given radium by him. The patient then remembered that for a period of about 2 years, when he was between the ages of 8 and 10,

his father had had his brother, mother, and himself each drink a glassful of "radium water" daily as a "tonic."

The general physical examination, hematologic studies, and radiographic examination of the entire skeleton were completely negative except for the destructive lesions in the teeth that are suggestive of radium poisoning.

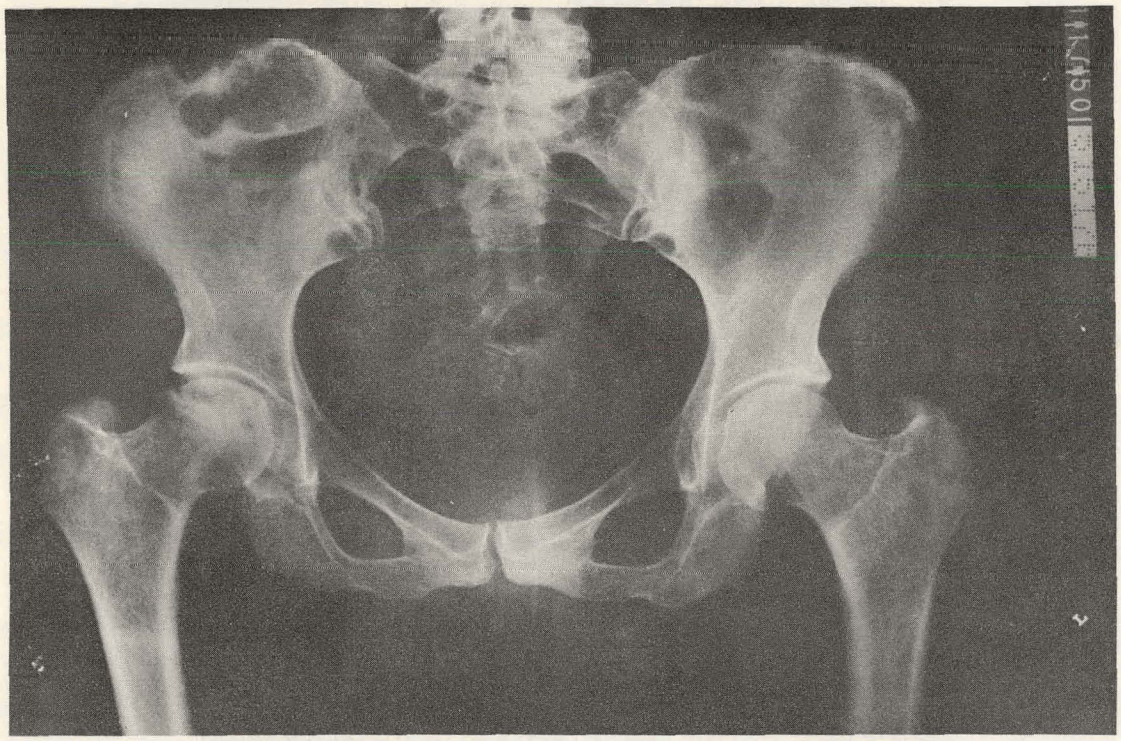

Figure 14. "Aseptic necrosis" with sequestration of the superior aspect of the head of the right femur in a patient carrying a total-body burden of 0.7 μg of radium. Radiography, which was done 3 years after this examination, demonstrated identical destruction of the head of the other femur.

Patient 305 - White, female, age 49. Total-body burden approximately 0.2 μg

The patient worked as a radium dial painter in Ottawa, Illinois, during 1924. In 1935, she noted the onset of pain in the hands, gradually involving the wrists, elbows, shoulders, and left knee. The pain was accompanied occasionally by redness, swelling, and stiffness of the joints. There has been little progression of the symptoms over the years.

A complete roentgenographic skeletal survey demonstrated joint changes, consistent with a diagnosis of rheumatoid arthritis. The changes in the hands suggested gouty arthritis. It is considered likely that the clinical condition is not related to the deposition of radium in this patient.

Patient 121 - White, male, age approximately 72. Total-body burden 0.2 μg

This individual, who has been asymptomatic, has been a radium chemist for the last 40 years. While visiting the Argonne National Laboratory, he kindly consented to

a determination of his total-body burden and a complete skeletal X-ray study. The skeletal survey revealed no bony abnormalities. The total-body burden of Patient 121 had been determined by the Aub group and is listed in their series as patient Dr. L. S. L.[53]

Figure 15. Necrosis of the mandible in patient carrying body burden of 1.6 μg of radium.

Patient 117 - White, male, age 43 (physician). Total-body burden 0.5 μg

At the age of 17 years, the patient had poliomyelitis, and as therapy for this received an unknown amount of radium parenterally in several injections from his physician (Patient 110). He was left with residual atrophy of the lower extremities but has had no symptoms referable to radium poisoning.

Roentgenography of the skeleton revealed minimal to moderately marked areas of bone resorption in the cortices of the femur, tibia, fibula (Figure 21), metatarsals, metacarpals, and the phalanges.

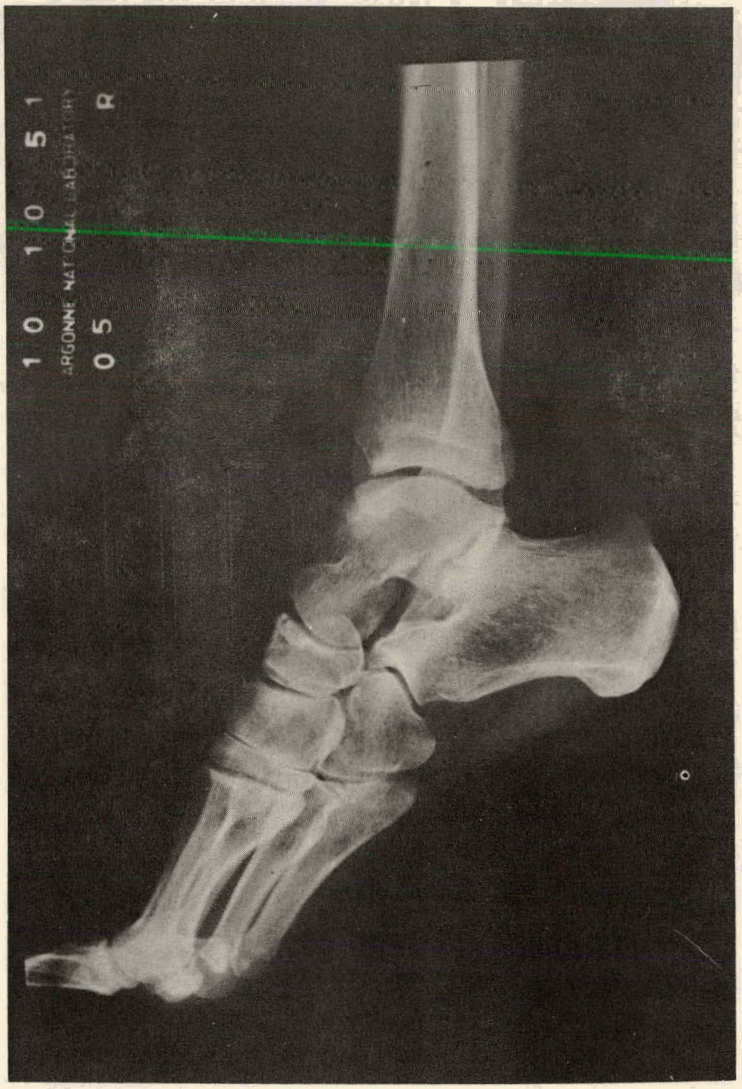

Figure 16. Localized area of bone necrosis in the tarsal scaphoid of a patient carrying a body burden of 1.3 μg. Also note small typical areas of bone resorption in the distal tibia and fibula.

Patient 101 - White, female, age 71. Total-body burden 0.5 μg

In June 1918, at the age of 37 years, the patient had her first episode of "arthritis." This involved the wrists, shoulders, neck, and knees and lasted about a year. She then had recurrent episodes of "arthritis," and in 1926 and 1927, a physician (Patient 110) gave her radium intramuscularly in an effort to alleviate the condition; apparently with some temporary relief of pain. Episodes of pain have continued

through the years, and there has occurred gradual limitation of motion of the joints of the hands, wrists, knees, and hips.

Physical examination revealed a blood pressure of 180/90. There was some weakness of the upper extremities. The joints of the hands were moderately deformed with some limitation of motion but there was an almost complete range of motion of the hip and knee joints; the ankle joint had a limitation of 20-30°.

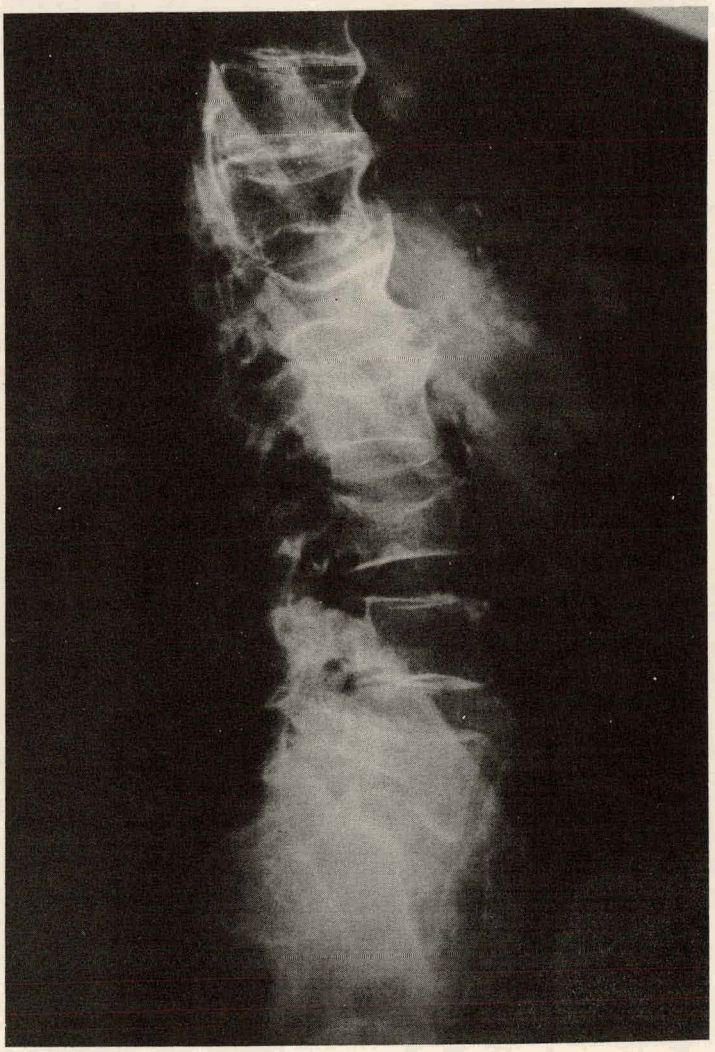

Figure 17. Destruction of vertebral bodies in patient carrying body burden of 10.7 μg of radium.

Roentgenography showed rather marked arthritic changes in most of the joints. Minimal areas of bone resorption were noted in the fibulae.

Patient 309 - White, female, age 46 years. Total-body burden 0.52 μg

When she was 16 years old, the patient worked as a dial painter for 1 year. At this time, it was a common practice to point the radium-containing brush with the lips; and this the patient did.

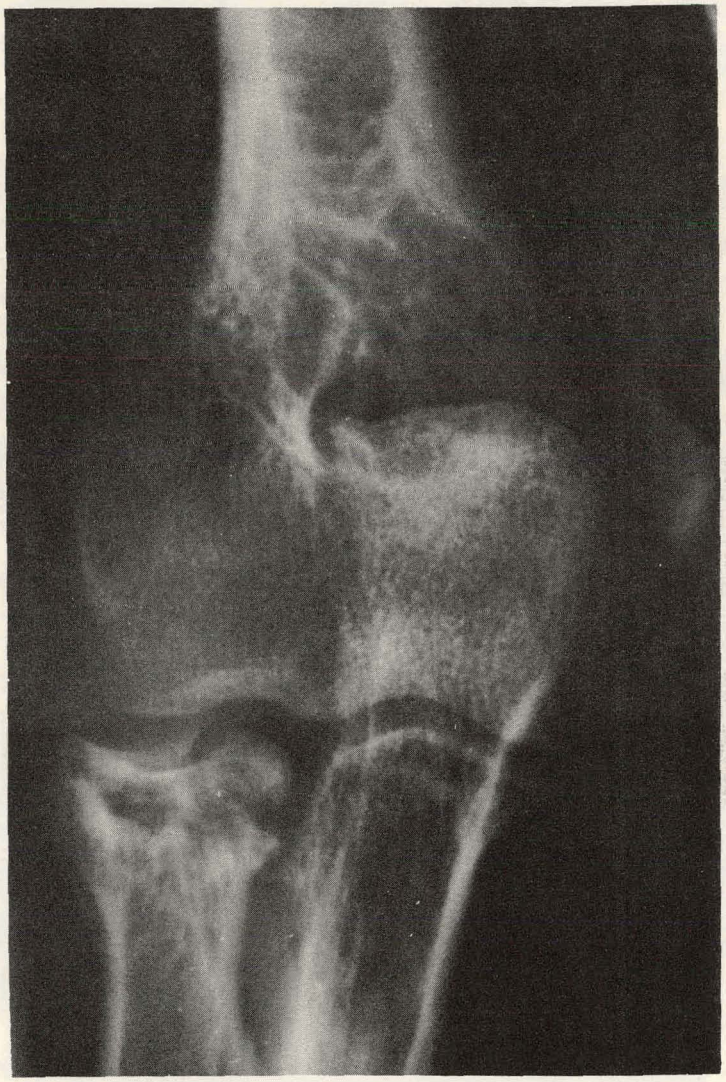

Figure 18. Necrosis and sequestration of the head of the radius in a patient carrying burden of 1.0 μg of radium.

In 1932, approximately 11 years after the last exposure to the ingestion of radium, she developed pain in the region of the left elbow that progressed. After 3 to 4 months, a golf-ball size mass was found to be present in the antecubital space. The arm was disarticulated at the shoulder at the Cook County Hospital, Chicago, Illinois, and a diagnosis of sarcoma was made on the basis of a microscopic examination of the tumor tissue. The original specimen was located in the Pathology

Museum of the Cook County Hospital in 1952 and, through the courtesy of Dr. Hans Popper, was made available to this group for study. The original histologic diagnosis of fibrosarcoma was confirmed. Radiography of the amputated arm revealed the characteristic typical areas of resorption in the radius, ulna, and humerus, as well as the osteolytic tumor of the head of the radius (Figure 22).

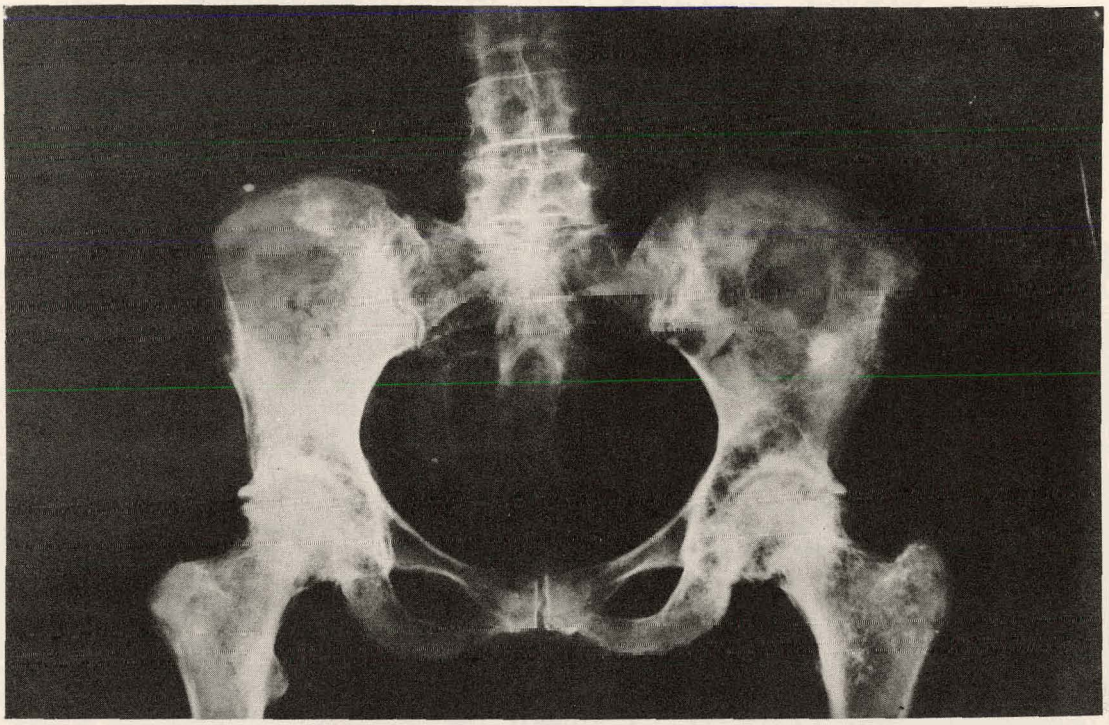

Figure 19. Multiple lesions in the pelvis of a patient carrying body burden of 4.2 μg. Note the destruction of the heads of both femora and the acetabula, the loss of joint space, change in trabecular pattern, and the localized areas of increased density in the pelvis and femora.

Prior to the amputation, the patient had borne 3 normal children. Since the amputation of the arm, the patient has been in good health except for intermittent pain in the left ankle and the right knee.

Physical examination was noncontributory. Roentgenography revealed small areas of bone resorption in the skull, humerus, and femur. Moderately marked bone resorption and necrosis were present in the metatarsals, tibia, and fibula. One tarsal scaphoid demonstrated a typical moderately advanced bone necrosis.

Patient 306 - White, female, age 47. Total-body burden 0.72 μg

This patient worked as a dial painter in Ottawa, Illinois, from 1923 to 1934. For the first 2 to 3 years she tipped the brush with her lips, but this practice was discontinued, and a glass pencil was used thereafter. The patient has been in good health during the years since her employment.

The general physical examination was negative. Roentgenographic survey revealed the typical minimal lesions in the fibulae that are diagnostic of the presence of radium in the skeleton.

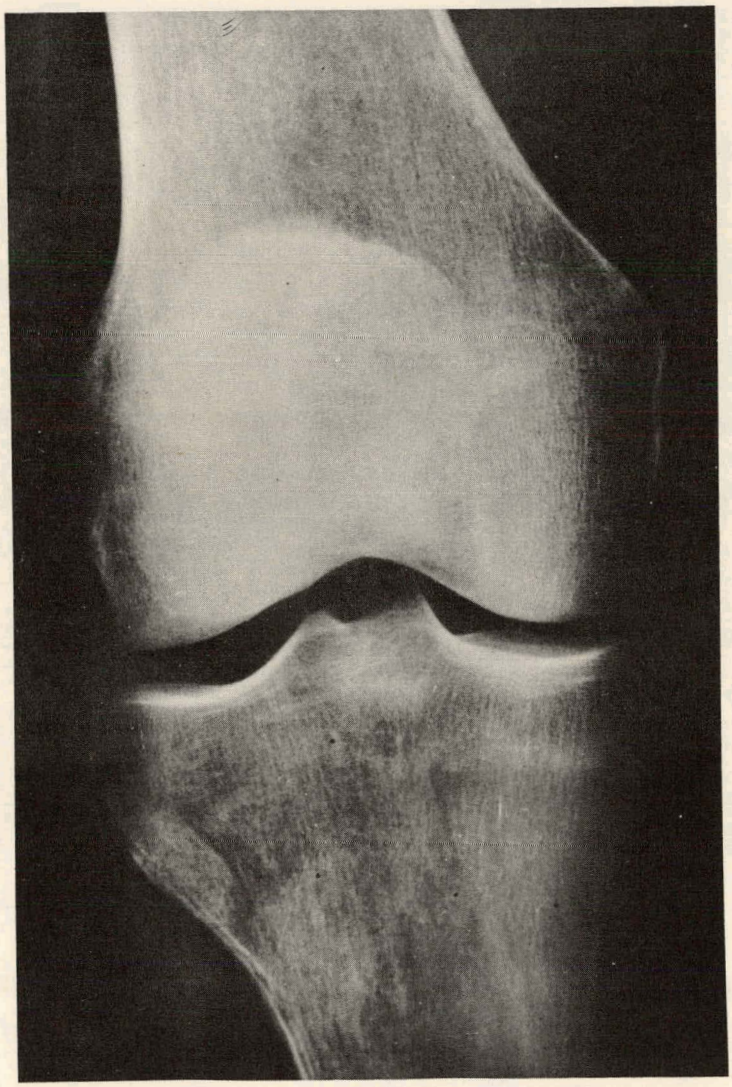

Figure 20. Fragmentation of the patella in a patient carrying a body burden of approximately 10 μg of radium.

Patient 116 - White, female, age 53. Total-body burden 0.7 μg

At the age of 28 years, the patient received 12 injections of radium chloride as therapy for a mental depression that followed the birth of her first child. Six years later, she was given 4 more injections for a recurrence of the depression after the birth of a second child. The physician who administered the medication informed us that each dose contained 10 μg of radium chloride.

In December, 1948, the patient first noted pain in the right groin, especially after much walking. During the ensuing few months, the pain spread throughout the entire right lower extremity and was associated with stiffness of the right hip and knee joints. In June, 1950, the patient consulted Dr. H. Sofield, who referred her to Dr. R. Potter for radiography. His study revealed an area of aseptic necrosis in

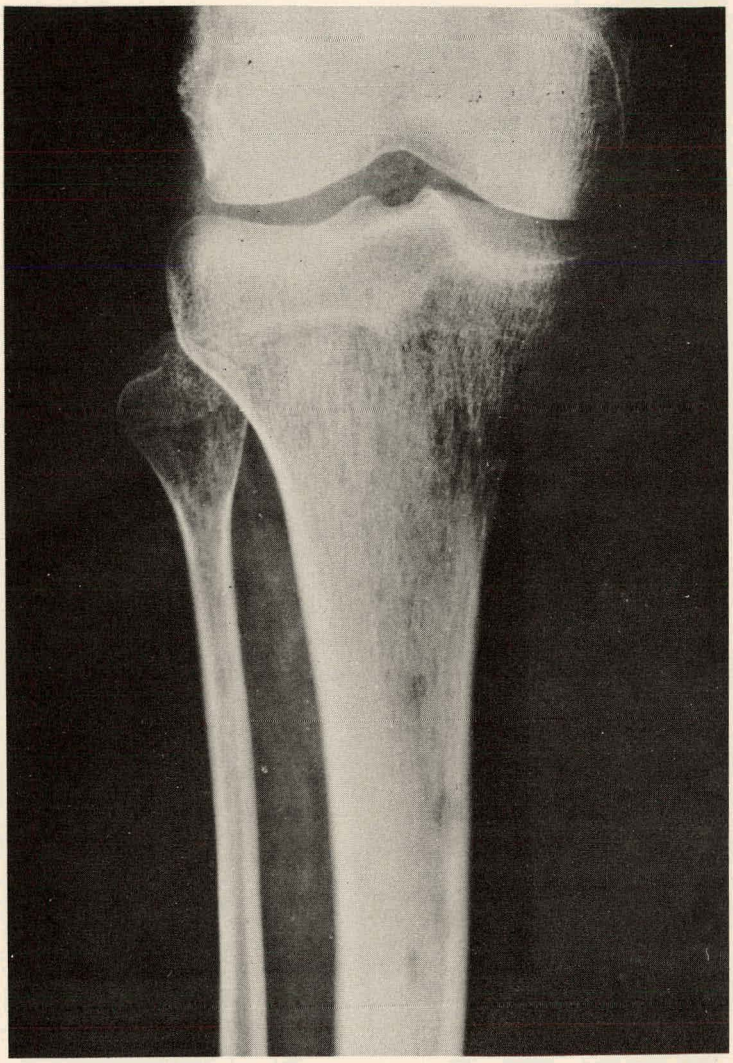

Figure 21. Patient 117. Body burden 0.5 μg. Areas of bone resorption in the tibia and fibula in an asymptomatic person who received radium injections at 17 years of age.

the head of the right femur that was suggestive of the bone changes appearing secondary to the presence of radium deposited in the skeleton. Radiography in 1954 demonstrated the development of an identical area of necrosis in the head of the other femur. (See Figure 14.)

Patient 105 - White, female, age 74. Total-body burden 0.86 μg

This patient did not know she had received radium. This fact was discovered when the radiographs of her husband, known to have been given radium, were reviewed by Dr. J. Clark of the Presbyterian Hospital, Chicago, Illinois, who had seen her films on a previous occasion. The similarity of the skeletal changes in the two

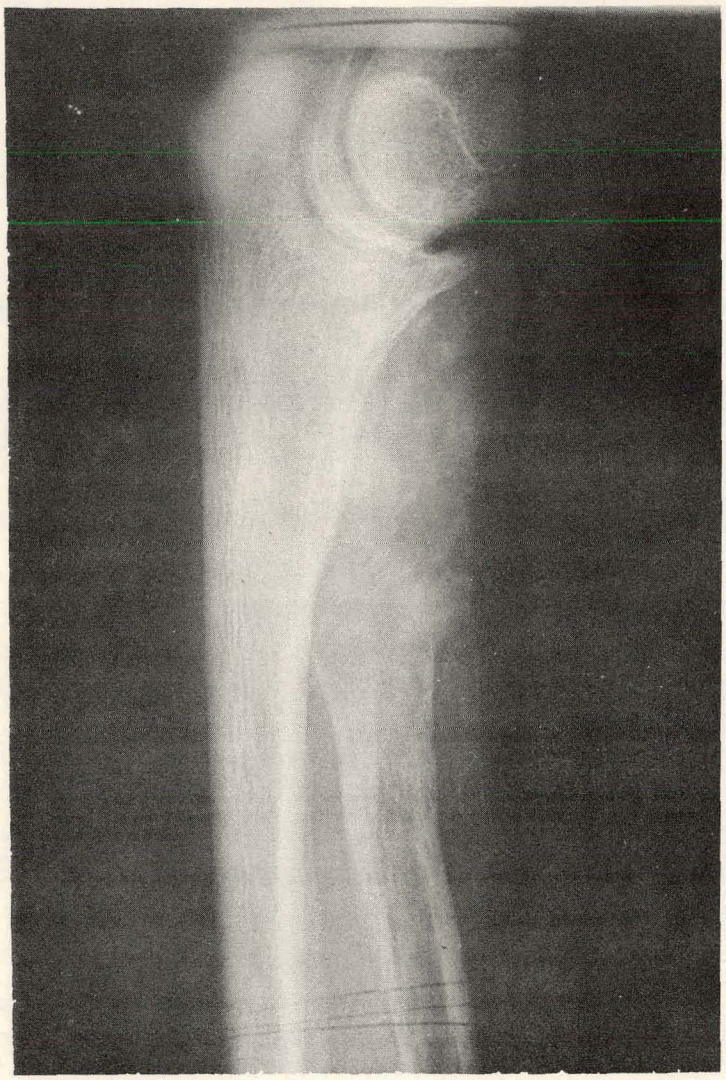

Figure 22. Patient 309. Body burden 0.52 μg. Fibrosarcoma of the head of the radius. Radiography done on anatomical specimen of arm amputated in 1932.

patients led to the conclusion that the etiological factors might be identical. The patient was referred to this group for study, and the presence of a total-body burden of 0.8 μg was demonstrated.

In 1947, the patient struck the lower part of her right leg and, because of the continued pain, swelling, and lameness, sought medical aid. A radiograph of the tibia was made (Figure 23). The bony changes were interpreted to be the result of osteomyelitis, and a course of 10 treatments of external X irradiation was given. The improvement of the condition following X-ray therapy suggest that the diagnosis in actuality could be Ewing's tumor or multiple myeloma.

Figure 23. Patient 105. Body burden 0.86 μg. "Osteoid-osteoma" of the tibia that later metastasized extensively to the patient's lungs. Note other typical changes in the patella, tibia, and fibula.

In 1948, a biopsy of the tibia was done, and a diagnosis of osteoid-osteoma was made. At a later date, the lesion was curetted and was considered to be a giant-cell tumor. There was little discomfort following the curettement; however, the

patient was unable subsequently to bear weight on the leg. In 1949, a pathological fracture was sustained, and a high amputation of the leg was performed because of the extensive bone involvement and absence of bony union.

The patient was examined by the authors on February 19, 1952. She was a small, thin, alert female who showed marked evidence of chronic illness and weight loss. No pain or discomfort in any of the joints had been experienced since 1949. She had noted pain in the mid-portion of her back to the right of the mid-line since January, 1952.

Radiographic examination revealed multiple area of increased density throughout both lung fields. These were suggestive of metastatic sarcomatosis. Osteolytic lesions were present in the ninth rib on the right side.

Shortly thereafter the patient suffered a cerebral accident either as a result of vascular disease of metastasis of tumor to the brain. The patient failed gradually and expired on October 8, 1952, from generalized sarcomatosis. Unfortunately, an autopsy was not performed.

Patient 113 - White, male, age 57. Total-body burden 1 μg

During 1925 through 1936, the patient received an unknown number of injections of radium from his physician (Patient 110) for the treatment of psoriasis. Five years ago, he developed pain in the right elbow, especially intense while playing golf. Roentgenography of the elbow in January, 1950, revealed "aseptic necrosis" of the head of the right radius.

In April, 1951, the total-body burden of radium was determined to be 1.0 μg. Roentgenography revealed changes in the trabecular pattern of the thoracic and lumbar vertebrae, minimal to moderately marked areas of bone resorption ("streaking") in the ulna, femur, tibia, and fibula in addition to the previously described changes in the head of the right radius. The psoriasis is still present.

Patient 313 - White, female, age 48. Total-body burden 1.3 μg

The patient had been employed as a dial painter in Ottawa, Illinois, since 1924, with the exception of 1 year, until her illness in 1953. During the first 2 years of employment, the patient tipped the brush with her lips.

The history was uneventful until 1948, at which time she tripped on a curbing and fractured the shaft of the left femur. Four months later, the femur was refractured in another fall. One year after the initial fall, the patient returned to her employment. In October, 1951, the patient was seen routinely with other former employees of the Ottawa, Illinois, radium dial plant. Roentgenographic changes typical of radium necrosis and resorption were present in the skull, head of the left femur, radii, ulnae (Figure 24), left tibia, left fibula, and left metatarsals. No tumors were present, nor was there evidence of change in the pelvis (Figure 25).

During the ensuing years, no changes in the state of her health occurred until January, 1953, when a persistent, severe pain deep in the right buttock developed and

continued unabated. Pelvic examination by her physician in 1953, revealed a carcinoma of the cervix. This was treated by radium needle implantation and pelvic X irradiation. A few months later, a total hysterectomy was performed because of the presence of fibroid tumors in the uterus.

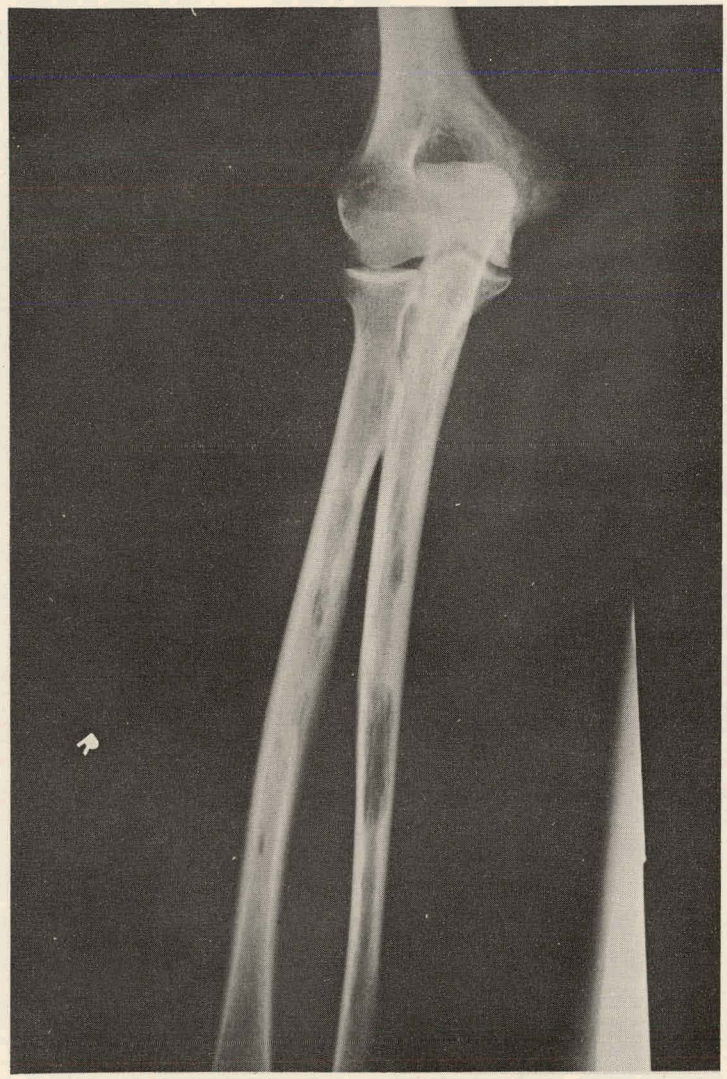

Figure 24. Patient 313. Body burden 1.3 μg. Extensive areas of bone resorption in the radius and ulna.

Radiography of the pelvis in September, 1953, revealed an expansile osteolytic lesion in the right ischial tuberosity that also involved the pubis (Figure 26). This was presumed to be a metastasis from the cervical carcinoma, and the patient was given approximately 2000 rep of X radiation directed to the lesion.

The patient was then referred, in October, 1953, to this group for further study. It seemed apparent that the lesion did not resemble a metastasis, but rather an

osteogenic lesion. Dr. C. H. Hatcher of the University of Chicago Clinics performed a biopsy, which revealed a fibrosarcoma (Figure 27). Because a local resection was impossible, a right hemipelvectomy was done.

The patient re-entered the Argonne Cancer Research Hospital in May, 1954, with a local recurrence of tumor at the amputation site and expired on July 22, 1954.

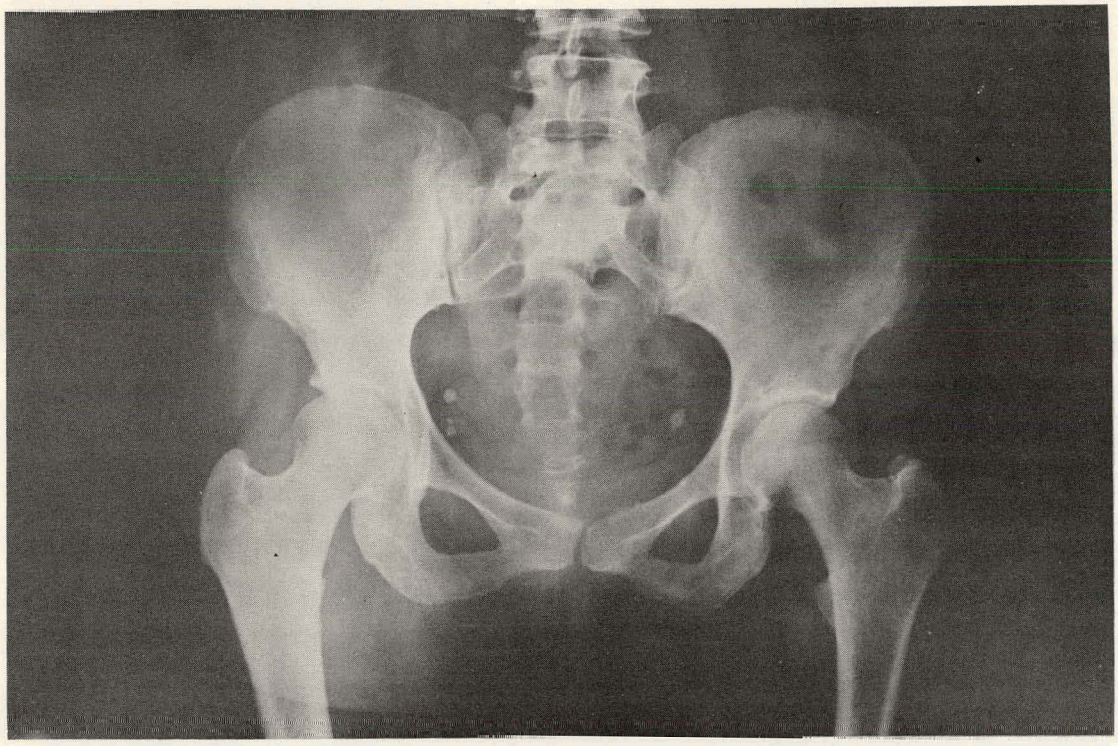

Figure 25. Patient 313. Body burden 1.3 μg. Note absence of typical changes from radium deposition in radiograph of pelvis made in 1951.

Patient 112 - White, female, age 48. Total-body burden 1.3 μg

At the age of 21, the patient was hospitalized with a vague illness diagnosed as "subacute bacterial endocarditis" and "polyarthritis" and at this time was confined to bed for 2 years. During the next few years, intermittent stiffness of the joints of the hands, shoulder, and lower back was present. For this, the patient's physician prescribed a solution of a soluble salt of radium of unknown concentration to be taken orally. The dosage is unknown. All that is known is that the patient took 15 to 30 drops of the solution once or twice daily for several weeks.

In July, 1947, about 19 years after the administration of radium, the patient was suddenly affected with a crushing type of pain of great severity in the left foot. She was admitted to Billings Hospital of the University of Chicago Clinics on February 3, 1948, where radiography of the entire skeleton was done and interpreted by Dr. P. C. Hodges. The examination revealed a large, irregular cavity in the body of the tarsal scaphoid of the left foot of undetermined nature (Figure 28). There was an unidenti-

fied negative shadow in the left occipito-parietal region of the skull. Minimal grade aseptic necrosis of the head of the left humerus and both glenoids was noted. An anthropoid-type of spongiosa in the lumbar vertebrae, pelvis, and upper ends of the femur, was observed. Its significance was not known at that time.

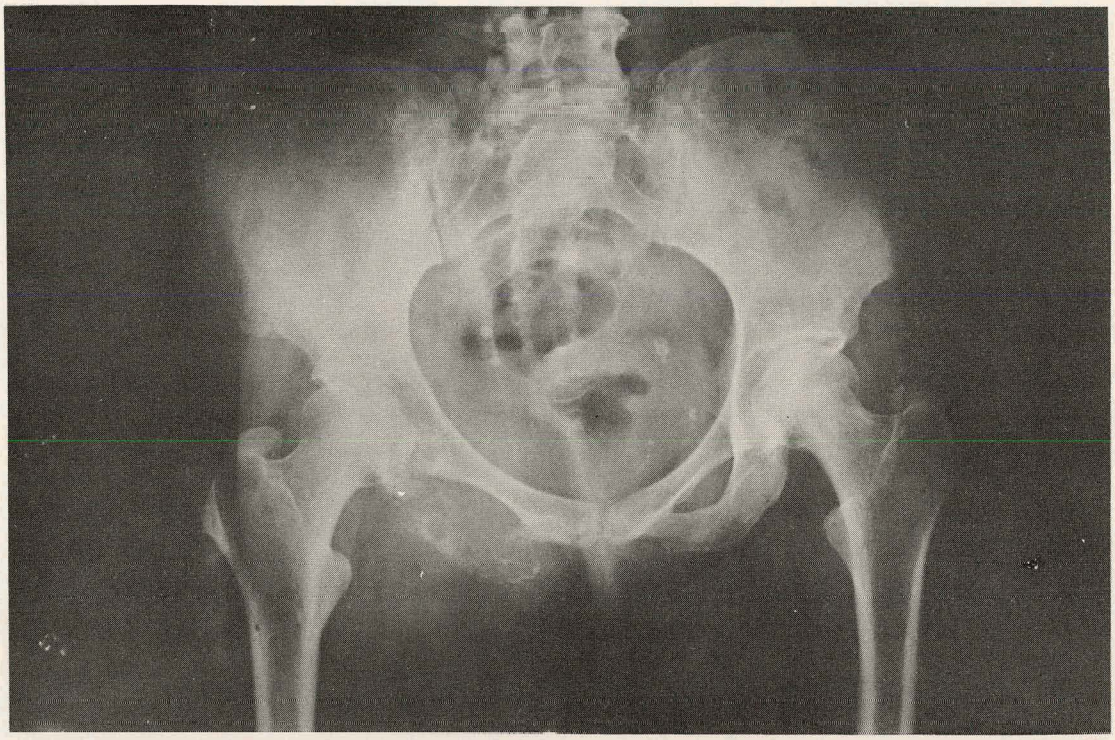

Figure 26. Patient 313. Body burden 1.3 μg. Note the fibrosarcoma of the right ischium and pubis present on radiographs made 2 years after previous radiograph, which shows no typical changes.

The patient was seen in consultation by one of the authors (A.M.B.), and determinations of breath samples in his laboratories at the Argonne National Laboratory revealed significant amounts of radon, diagnostic of radium deposits in the body. At his suggestion, she was placed on a low calcium, low phosphorus diet, and was given ammonium chloride and parathormone, until she was discharged on March 8, 1948. Although a measurable increase in radium excretion was observed as a result of this regimen, there was no roentgenographic evidence of bone healing. The peripheral blood picture at this time was not remarkable, but a study of the sternal marrow gave distinct evidence of over-activity. There was moderate erythroblastic activity without shift to the left, but it was the opinion of Dr. M. Block that more mitoses than usual were present. The blood picture was not that of a "radium aplasia."

Pain on walking increased after the patient was discharged from the hospital The onset of pain in other bones occurred in 1950 and later became generalized through the skeleton.

In January, 1951, determination of radium content of the body was estimated to be 1.3 μg by combined γ-ray and radon methods. Radiography of the skeleton demonstrated a progression of all lesions, especially in the left foot where destruction of the scaphoid was complete and partial destruction involved other tarsals (Figure 29). A biopsy of the scaphoid was done by Dr. F. Chandler, and a diagnosis of fibrosarcoma was made (Figure 30). On February 20, 1951, the left leg was amputated

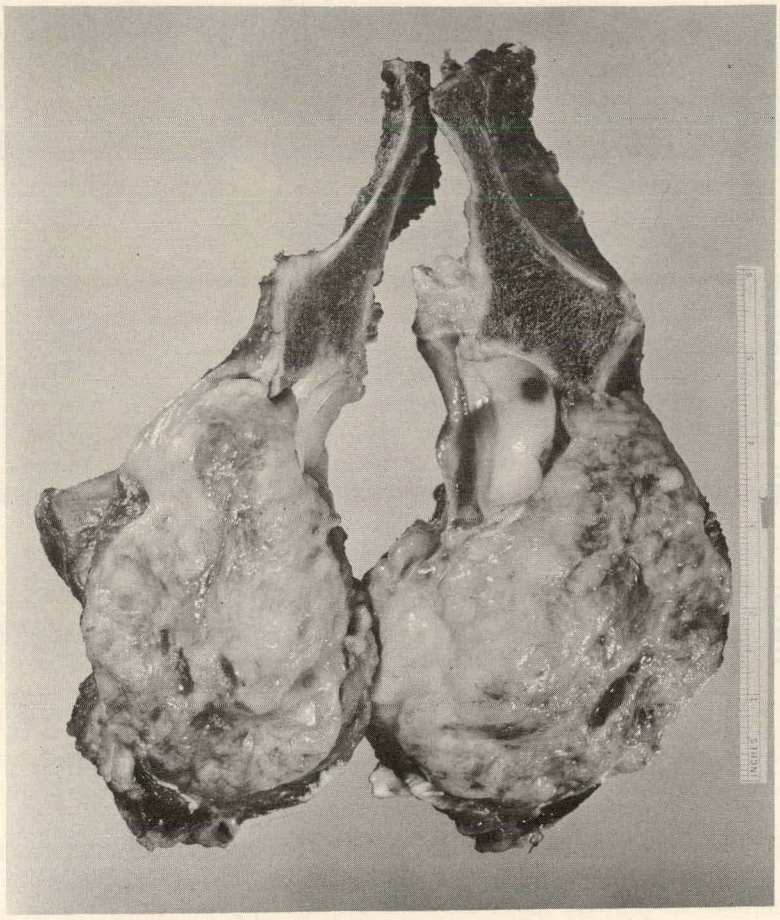

Figure 27. Patient 313. Body burden 1.3 μg. Surgical specimen following hemipelvectomy demonstrating fibrosarcoma of the ischium invading the acetabulum.

15 cm below the knee. The patient died of generalized sarcomatosis on August 15, 1951, 23 years after the administration of radium and 4 years after the onset of the symptoms. During the last 3 years of her life, the number of "punched-out" lesions in the skull approximately doubled (Figure 31).

Patient 114 - White, female, age 56. Total-body burden 1.6 μg

The patient, the wife of a physician, did not remember ever receiving radium. In the winter of 1950, her dentist noted a pinkish discoloration of the teeth and advised the patient that this phenomenon had been described and associated with radium

poisoning. She was referred by her husband for a determination that revealed a total-body burden of 1.6 μg.

Radiography demonstrated minimal trabecular changes in the thoracic and lumbar vertebrae; minimal changes of bone resorption and necrosis in the ilium and acetabulum; and similar moderately marked changes in the humerus, radius, ulna (Figure 32), tibia, and fibula. The patient is asymptomatic.

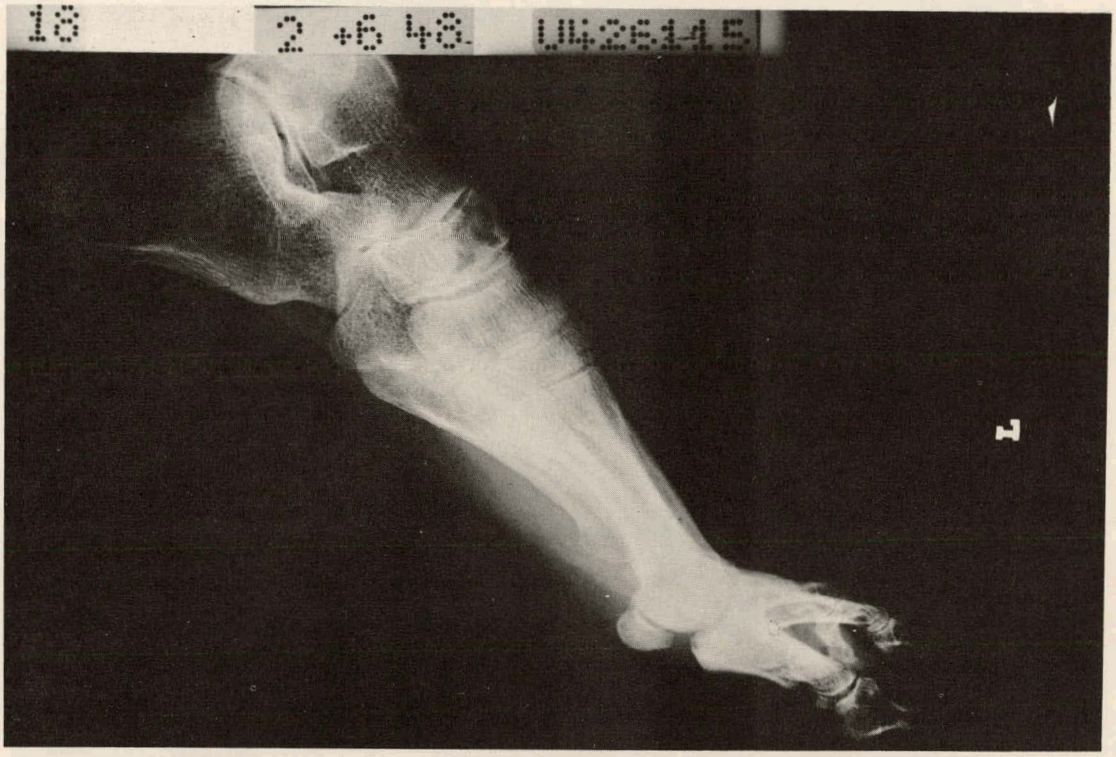

Figure 28. Patient 112. Total-body burden 1.3 μg. Radiograph revealing area of necrosis of tarsal scaphoid in 1948.

Patient 102 - White, male, age 81. Total-body burden 1.6 μg

In March, 1939, the patient noted a small spicule of bone protruding from the gum at the base of a left lower molar. This was accompanied by slight tenderness, redness, and swelling in the same region. He entered Billings Hospital on the service of Dr. D. B. Phemister. From that time until October, 1940, the patient had intermittent drainage from and sequestration of the left ramus of the mandible. On June 17, 1941, the defect of the mandible was repaired with a bone transplant from the right ilium. On October 5, 1941, the infected necrotic bone graft was removed.

The patient does not remember ever having received injections of radium or having taken radium water by mouth. However, since he was the husband of Patient K.B., who was a known "radium poisoning" case, Dr. Phemister referred the patient to the authors for study. (See Figure 15.)

Determination revealed the presence of 1.6 μg of radium in the body. A review of films made in 1941 and 1942 in the Department of Radiology of the University of Chicago Clinics demonstrated the mandibular defect, moderately severe areas of bone resorption ("streaking") in the fibula and radius, and minimal, similar lesions in the tibia and ulna.

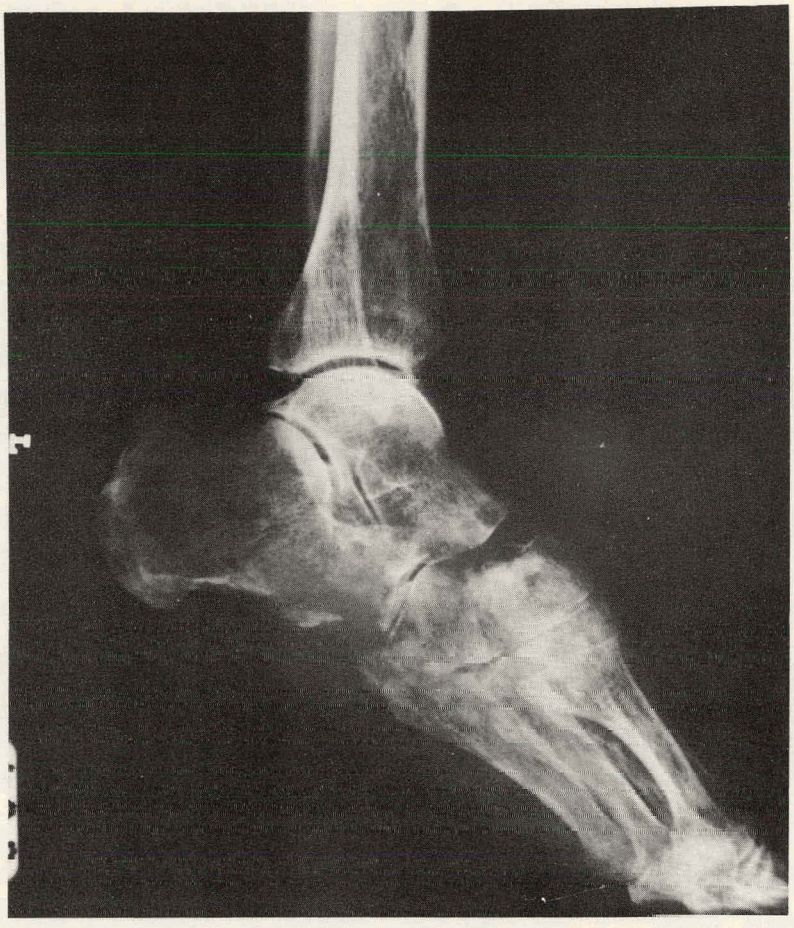

Figure 29. Patient 112. Widespread sarcomatosis of the same foot when reradiographed in 1951. There is complete destruction of the scaphoid and involvement of the other tarsals.

Patient 111 - White, female, age 66. Total-body burden 4.2 μg

This patient is the wife of Patient 110. In the interval of 1922 to 1923 she took some "emanation water." In 1929 and subsequently, she was given radium chloride by her husband for an intermittent tachycardia. The total dosage reached approximately 400 μg over a 2- to 3-year period.

Six years ago, she was told that she had "insufficient calcium in her back." At that time she had severe pain and wore a Taylor brace for 2 years. Four years ago, the patient caught her heel on a stairway, fell, and fractured her left femur and was on crutches for 14 weeks.

Determinations revealed a total-body burden of 4.2 μg. The patient denied symptoms referrable to "radium poisoning." Roentgenography revealed minimal lesions of bone resorption in the skull, thoracic vertebrae, humerus, and metatarsals, and moderate to markedly severe lesions in the ilium, acetabulum, radii, ulna, femur, tibia, fibula, and tarsals (Figures 33 and 34).

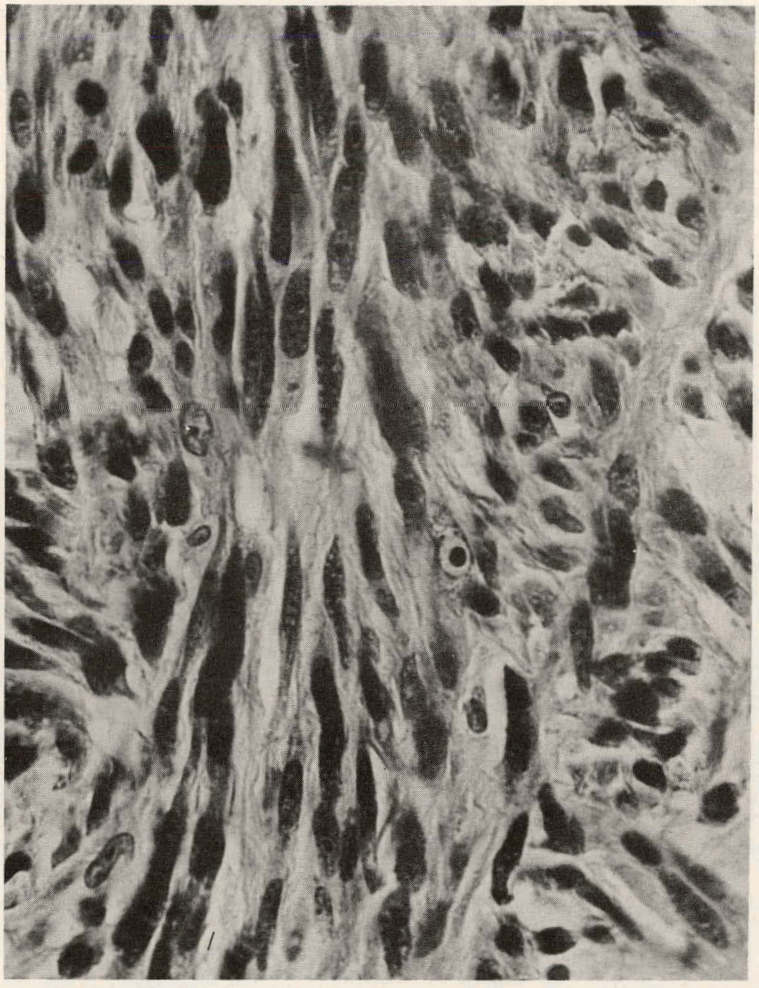

Figure 30. Fatient 112. Anaplastic fibrosarcoma arising in the tarsal scaphoid.

Patient 110 - White, male, age 81 (physician). Total-body burden 5.4 μg

This patient, one of the physicians who was most active in the administration of radium in the Chicago area, first took radium water orally for 6 to 8 weeks in 1922. Until 1929, he took radium intramuscularly in an intermittent manner, and estimates that over the years he has taken 700 to 800 μg of radium.

Determinations revealed a total-body content of 5.4 μg. Roentgenography demonstrated minimal to moderately severe lesions attributable to radium in the skull, thoracic and lumbar vertebrae, ilium, acetabulum, humerus, radius, ulna,

femur, tibia, fibula, tarsals, metatarsals, and the phalanges of the feet. The patient remains steadfast in his belief that the clinical administration of radium is an innocuous procedure and not attended by any late sequelae.

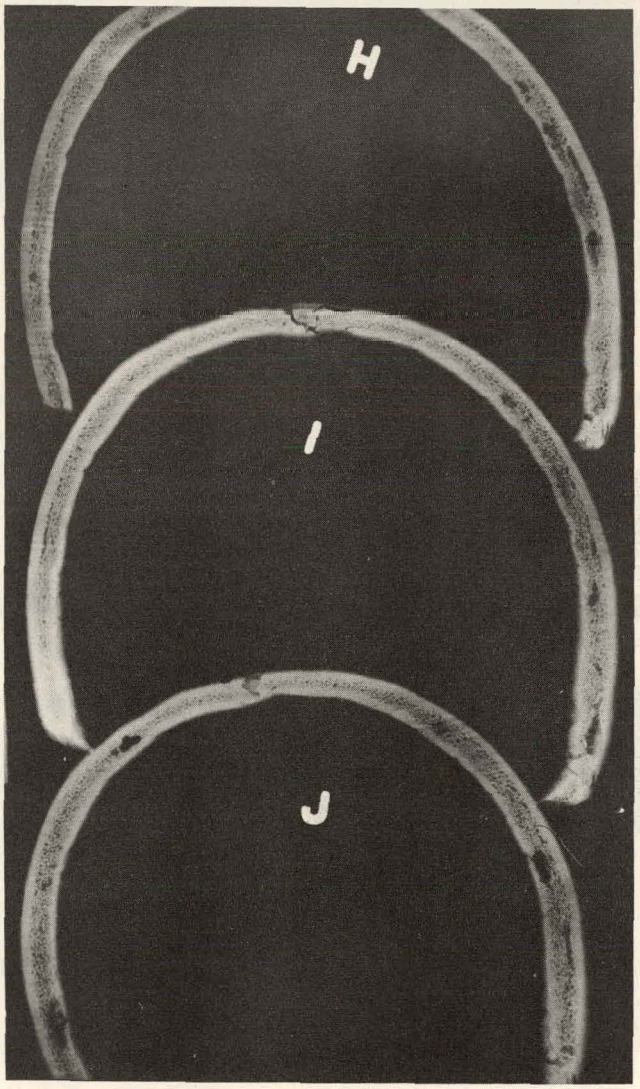

Figure 31. Patient 112. Postmortem coronal sections of the skull demonstrating areas of bone destruction visible on the radiographs as "punched out" areas.

Patient 106 - White, male, age 53. Total-body burden 5.6 μg

This patient, the son of Patient 104, received "about 20 injections" of radium intramuscularly 26 years ago at the age of 27 years for acne rosacea. These were administered and prescribed by the same physician (Patient 110) who had treated his father.

In 1948, he noted a bone sequestrum about 3/8" in diameter protruding from the left ramus of the mandible. This occurred 6 additional times, and all teeth have been extracted since the onset of the mandibular necrosis.

Physical examination revealed a hypertension of 230/130. The total-body burden of radium was 5.6 μg. Roentgenography demonstrated minimal lesions in the ilium, acetabulum, humerus, and tibia, and moderately severe to severe lesions in the mandible, skull, thoracic vertebrae, radius, ulna, femur, and fibula.

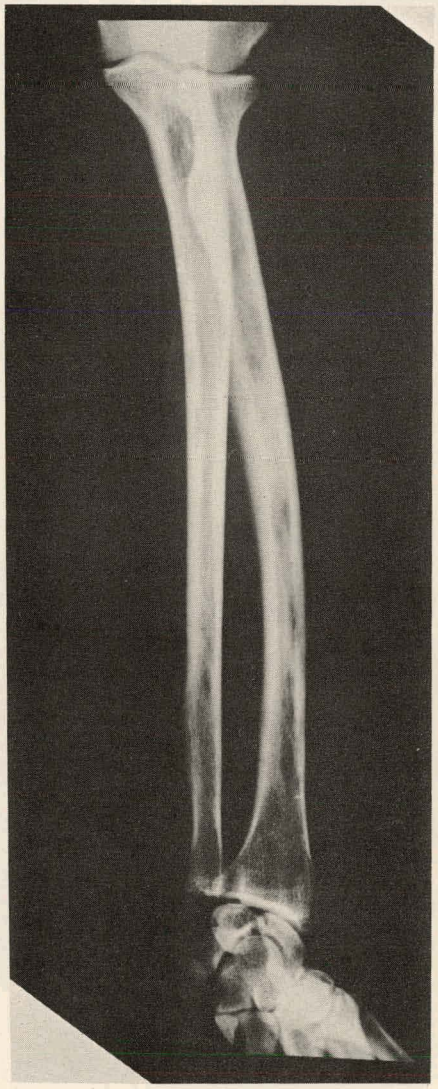

Figure 32. Patient 114. Body burden 1.6 μg. Multiple typical areas of bone resorption in the shafts of the radius and ulna in an asymptomatic person who was unaware that she had received radium.

Patient 103 - White, female, age 63. Total-body burden 6.8 μg

In July, 1951, the patient developed severe pain in her left hip, which rapidly increased in severity and limited her ability to walk. During the previous 28 years, the patient suffered from intermittent episodes of joint pain, malaise, and fever, which, on various occasions, were thought to be due to brucellosis.

She consulted a physician in Tuscon, Arizona, in 1947, because of pain in the joints and fever. Roentgenograms of the long bones and skull were made in the course of his study. Multiple areas of bone resorption were seen in these bones, and it was the impression of her physician that the patient was suffering from multiple myeloma, although further diagnostic procedures were not carried out.

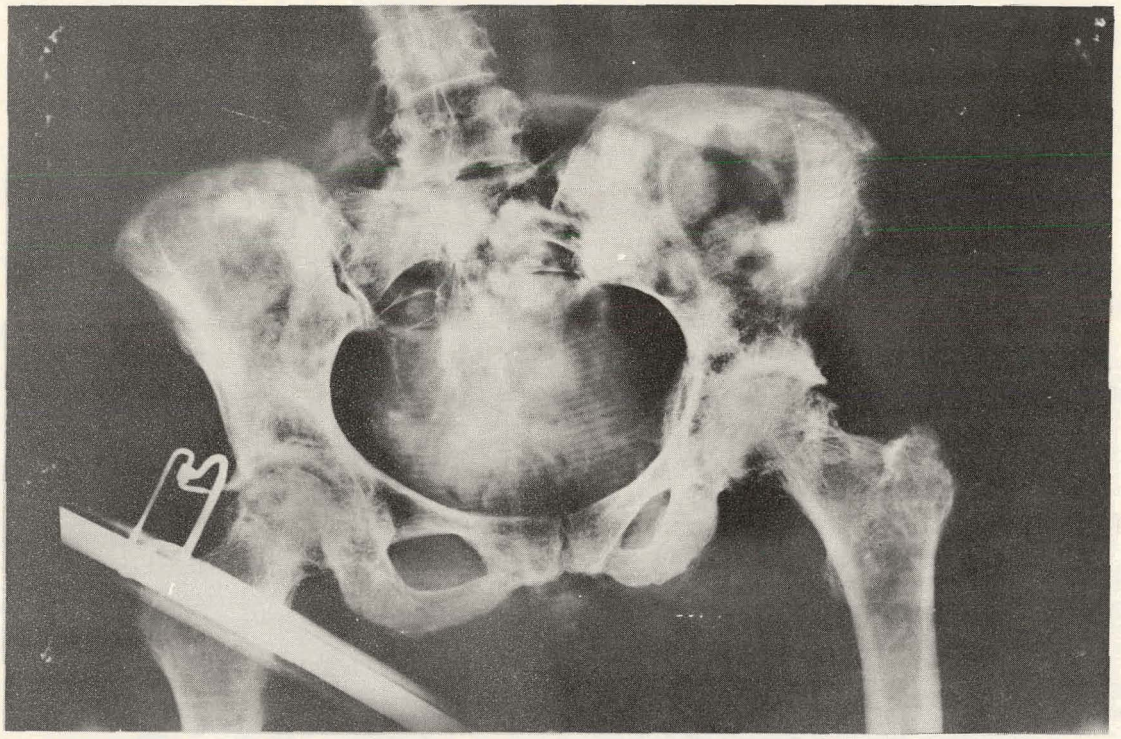

Figure 33. Patient 111. Body burden 4.2 μg. Loss of normal trabecular markings and areas of increased density in the heads of the femora, the pelvis, sacrum, and lumbar vertebrae.

The patient was seen in Chicago in June, 1952, by Dr. T. Coogan, who had also been the physician of Patient 112. He recognized the bone lesions as being typical of those seen in other patients in our series and referred Patient 103 to us.

The patient, formerly the wife of a physician, was completely unaware of having received radium, either orally or parenterally. However, in 1925-1926, she was hospitalized in Chicago because of her complaints of muscle and joint pain. During this hospitalization, she received intravenous injections of a medication, and it is known that the parenteral use of radium by certain members of the hospital's staff was not uncommon at that time.

The total-body burden of radium, in July, 1952, was estimated to be 6.8 μg. Complete skeletal radiography revealed severe destructive lesions, typical of radium poisoning, in the heads of the femur (Figure 35), distal ends of both tibiae and fibulae, and lesser typical lesions in the other bones of the skeleton.

Arthroplasty for the relief of pain in the left hip was carried out by Dr. Hatcher of the University of Chicago Clinics in 1953, and the bone fragments were analyzed for radium content (Table 3 - Norris et al.).* During 1953, extensive metabolic studies were done in the Argonne Cancer Research Hospital, and the excretion rates that were found have been summarized (Tables 6 and 7, Norris et al.).*

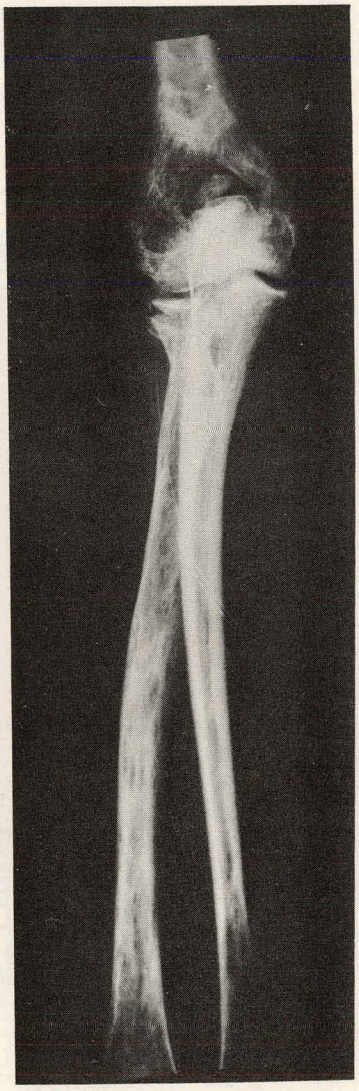

Figure 34. Patient 111. Body burden 4.2 μg. Necrosis with sequestration of the head of the radius and multiple areas of bone resorption in the shafts of the radius and ulna.

* Norris, W. P., T. W. Speckman, and P. F. Gustafson. Studies of the metabolism of radium in man. Submitted for publication in the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine.

Patient K.B. - White, female, age 66. Total-body burden 2 to 10 μg

The patient received 12 injections of radium chloride (probably 120 μg) intravenously from her physician in 1922 as a "tonic." The estimated body burden of radium based on urinary excretion rates in 1947 was 2.5 μg and between 2 and 10 μg by external γ-ray determination. In 1944, the patient developed "arthritis," which gradually involved the right hip joint and both knees, and by 1946, was bedridden most of the time as a result of the joint changes. Roentgenographic examination at this time demonstrated changes in the long bones and pelvis that were characteristic of radium toxicity.

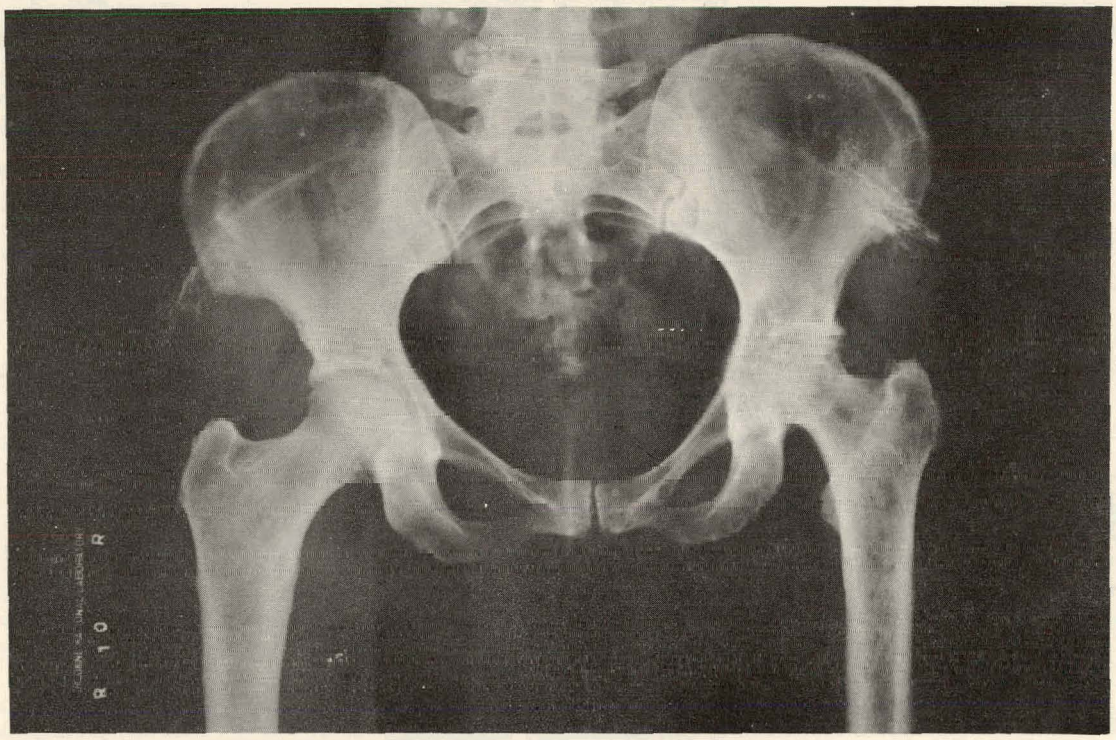

Figure 35. Patient 103. Body burden 5.6 μg. Destruction of the head of the left femur and early changes in the opposite femur in a patient who was unaware that she had received radium.

On April 13, 1947, she was admitted to the University of Chicago Clinics with the principal complaints of a defect in mental concentration and inability to speak correctly. There was a gross irregularity in the cardiac rhythm, and the blood pressure was 205/110 mm. Hg. The patient was digitalized and discharged as improved on May 19, 1947. The erythrocyte count was low, and the sternal marrow on April 18, 1947, was considered to be extremely atrophic. An incomplete skeletal roentgenographic survey during the hospitalization revealed marked aseptic necrosis of the head of the right femur (Figure 36), moderate necrosis of the left astragalus, collapse of the vetebral bidies T-8 and T-12, fragmentation of the left patella, "mottling" of the head of the femur, and "streaking" of the tibiae and fibulae.

Patient 104 - White, male, age 76. Total-body burden 10.7 μg

The patient, a physician and dentist, was treated intravenously about 18 years ago with radium chloride by another physician (Patient 110) for vague "arthritic" pains in both shoulders. Over a period of 9 months, the patient probably received about 700 to 800 μg of radium intravenously.

Approximately 2 years after the injections of radium, the patient began to have difficulties with his jaw, and, in the ensuing years, lost all of his teeth and the anterior half of the mandible. About 5 years ago, he began to experience "arthritic" pain in the right hip joint, which progressed in severity and resulted in limitation of motion.

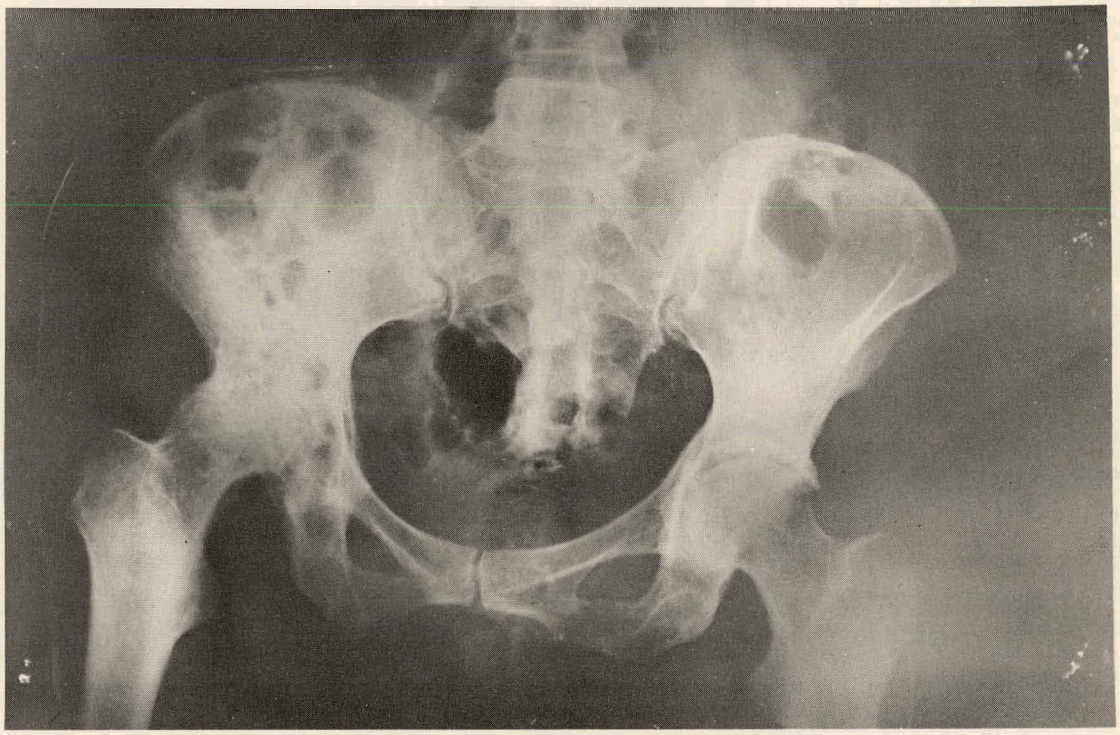

Figure 36. Patient K.B. Body burden approximately 2 to 10 μg. Destruction of the head of the femur.

Examination revealed a total-body burden of 10.7 μg. Roentgenography demonstrated slight to severe bony changes attributable to radium in the mandible, skull, thoracic and lumbar vertebrae, ilium and its acetabula, glenoid, humerus, radius, ulna, femur, tibia, fibula, metatarsal, and phalanges of the feet. The patient developed a rapidly progressive aplastic anemia and died in 1952.

Patient 107 - White, male, age 69. Total-body content 13.9 μg

The patient's physician (Patient 110) gave him "about 25 injections" of radium at weekly intervals 26 years ago. The total quantity that was injected is unknown. He was suffering from no particular illness at the time; the radium was administered by his physician to "pep him up."

During the past 10 years, "arthritis" has affected the feet, knees, and right shoulder. In May, 1950, the patient suffered a right hemiplegia from which he made a satisfactory recovery.

The total-body radium burden is 13.9 μg. Roentgenography revealed a paucity of lesions attributable to the presence of radium. Only minimal changes of bone resorption were noted in the thoracic vertebrae, radius, ulna, fibula, metatarsals, and the phalanges of thc feet.

DISCUSSION

In the United States, there are hundreds, perhaps a thousand individuals, who were given radium salts parenterally or orally 20 or more years ago. For the most part, these people are unaware that they carry radium deposits. Moreover, until recently, the medical profession has not realized that these patients exist. The long latent period between the time of administration of the radium and the appearance of symptoms has, in several instances, led to a delayed recognition of the correct etiology of the bony lesions.

Hematologically, the findings in these patients are neither striking, nor diagnostic of radium poisoning (Table 2). One patient (Patient 104), a 78-year-old male, died at the age of 78 years of a rapidly progressive aplastic anemia. The usual hematologic criteria of radiation damage to the erythropoietic system, such as abnormalities of the total white cell count and atypical appearance of the white blood cells, did not differentiate in this series between those individuals carrying a very small and a very large body burden of radium. Moderate anisocytosis and poikilocytosis of the red blood cells seemed to be constant findings in those individuals carrying more than 2 μg of radium.

An analysis of several thousand radiographs done on 38 individuals who received radium therapeutically and 6 individuals who had been employed as "radium" dial painters demonstrates a general correlation between the level of body burden of radium and the frequency and severity of osseous lesions.

A study, which includes measurements done by Miller and Rowland, that is reported by Norris et al.*, indicates that the radium solution prepared by the U. S. Radium Corporation for intravenous use was not contaminated at the time of preparation with more than 0.66 per cent of mesothorium. The authors believe that it can be assumed with confidence that the individuals in this series who received radium only by the intravenous or intramuscular routes were not contaminated with significant quantities

* Ibid

of mesothorium or radiothorium. It is possible that the oral preparations may have contained mesothorium since it is a fact that one of the proprietary preparations, "Radiothor," used in the 1920's, contained relatively large amounts of mesothorium, and was so advertised. It must also be assumed that the dial painters from Ottawa, Illinois, included in our roentgenographic analysis, may have ingested mesothorium and radiothorium in addition to the radium present in the luminous paints.

R. D. Evans* reports the presence of radiothorium that may have been present as a daughter of mesothorium or as the original contaminant along with radium in bone samples from Patient 112. This patient received the "radium" orally. Studies are now in progress by Dr. A. Stehney and his associates at Argonne National Laboratory on bone specimens from several of our patients in an attempt to obtain further data on the relative importance of the presence of mesothorium and radiothorium in the induction of neoplasia and osseous lesions.

A clinically recognizable and typical osseous lesion (in the dentin of the teeth) has been observed in Patient 120, who carries a body burden of approximately 0.15 μg of "radium." Since he received this radium as a solution ingested orally, the possibility of contamination with mesothorium and radiothorium has not been excluded. The early age (8 years) at which the patient received the radioemitter may also be a factor of importance in the production of this lesion at such an extremely low level of total-body radioactivity.

At body burdens of 0.4 μg and greater, clinically recognizable and unique bony lesions are, with but three exceptions, present (Figure 2). These lesions increase in number, extent of distribution throughout the skeleton, and in severity with increasing body burdens of radioactivity. A notable exception is Patient 107, who carries 13.9 μg of radium and demonstrates only minor bony changes in the radius, ulna, and fibula. This unique exception to the general pattern will be the subject of further studies in an attempt to elucidate the factors that have mitigated against damage to the bone.

Sarcomata were seen in 4 patients included in this study and in 2 additional Ottawa dial painters observed but not included in this report. Patient 309, a radium dial painter from the Ottawa area, developed a sarcoma of the head of the radius in 1932. Following amputation of the arm, the patient has survived and, when examined in 1952, carried a body burden of 0.52 μg of "radium." The bones of the arm, amputated in 1932, are available for assay. Patient 105 received radium salts parenterally and probably orally. A metastasizing osteogenic tumor arose in this patient whose body burden was 0.86 μg. The ingestion of a mesothorium-containing oral preparation cannot be excluded. Bone samples are not available for assay.

* Personal communication: "The specimen was a 5.9 g. (wet weight) piece of the femoral epiphysis... . The sample contained 2.3 ± 0.4 μμc radiothorium and a radiothorium to radium ratio of 0.0024 ± 0.0005."

Patient 313, a radium dial painter from the Ottawa area, developed a fibrosarcoma of the greater tuberosity of the right ischium in January, 1953, and expired on July 22, 1954. In the 1926 to 1928 era, she tipped the dial painting brush with her lips. It is probable that the paint, at this time contained radiothorium and/or mesothorium. Bone samples are available for assay.

Patient 112 who received radium salts orally developed a sarcoma of the tarsal scaphoid. The presence of mesothorium or radiothorium in this oral preparation has not been excluded, and analysis of bone samples will be carried out.

No patient who received radium salts exclusively by the parenteral route (and thus probably received only radium) has developed a bone neoplasm attributable to the presence of radium in the skeleton. However, significant non-neoplastic lesions are present in those individuals containing as little as 0.4 μg of what has been shown to be only radium salts, uncontaminated with more than 0.66 per cent mesothorium.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation for the valuable contributions of the following indivuals who assisted in this project:

Mr. L. D. Marinelli and Mr. J. E. Rose participated in originating the program and contributed throughout its course.

Drs. D. L. Steinberg and A. A. Lieberman, formerly of the Elgin State Hospital, aided in the original search for patients and made available diagnostic facilities for the study of the patients at the Elgin State Hospital.

Dr. W. P. Norris, Dr. A. F. Stehney, and members of their groups made the physical determinations on all patients.

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The authors are also indebted to the many physicians of Chicago who made available their patients for study.

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KILOCURIE REVOLVING COBALT-60 UNIT FOR RADIATION THERAPY*

By

L. H. Lanzl, D. D. Davison, and W. J. Raine

SUMMARY

A revolving cobalt-60 unit for radiation therapy has been designed, built, and placed in operation at the Argonne Cancer Research Hospital. The unit is designed to hold a source of 1750 curies, although it can accommodate a source of twice that strength. (At this writing, the unit contains a 650-curie source.) The source shield revolves either completely or in sectors about a recumbent patient. In addition, the shield itself can be angulated. The distance from the source to the center of revolution is fixed at 81.6 cm. The treatment cot is aligned by means of one rotational and three linear motions. Rectangular and square field shapes of arbitrary size up to 15 x 15 cm at the center of revolution are available.

Figure 1 is a photograph of the unit. The main shield, treatment cot and support, and one of the auxiliary control panels are visible. The patient-viewing window appears in the background. A schematic cross-sectional drawing of the main shield is shown in Figure 2. The shutter is in the open position; the closed position is indicated by dotted lines. To minimize the weight and size of the shield, uranium was chosen as the shielding and collimating material.

Figure 3 is a photograph of the shield (without collimator) within a steel cradle. The beam emerges through the hole at the top when the shutter is in the "open" position. The shutter gear train is partially visible, as well as one of its two support trunnions. Another photograph of the unit, taken during construction (Figure 4), shows the main shield with the variable collimator, the counterweight, side rollers, drive rollers, large rings, and a part of the drive motor.

Figure 5 shows, on the extreme left, the dual angular position controls with reversing switches for sector irradiation, main shield position indicator, revolution direction and jogging controls, and vertical motion controls for the treatment cot; in the center, the main shield with angulation controls and indicator. The treatment cot is visible to the right.

* Summary of a paper that has been accepted for publication in the American Journal of Roentgenology, Radium Therapy and Nuclear Medicine.

The control console (Figure 6) is located in a room adjacent to the treatment room. The right-hand panel of the console contains the shutter and revolution controls as well as an intercommunication unit. The left-hand panel contains the power key switch, scattered radiation meter, dose selector, and computer.

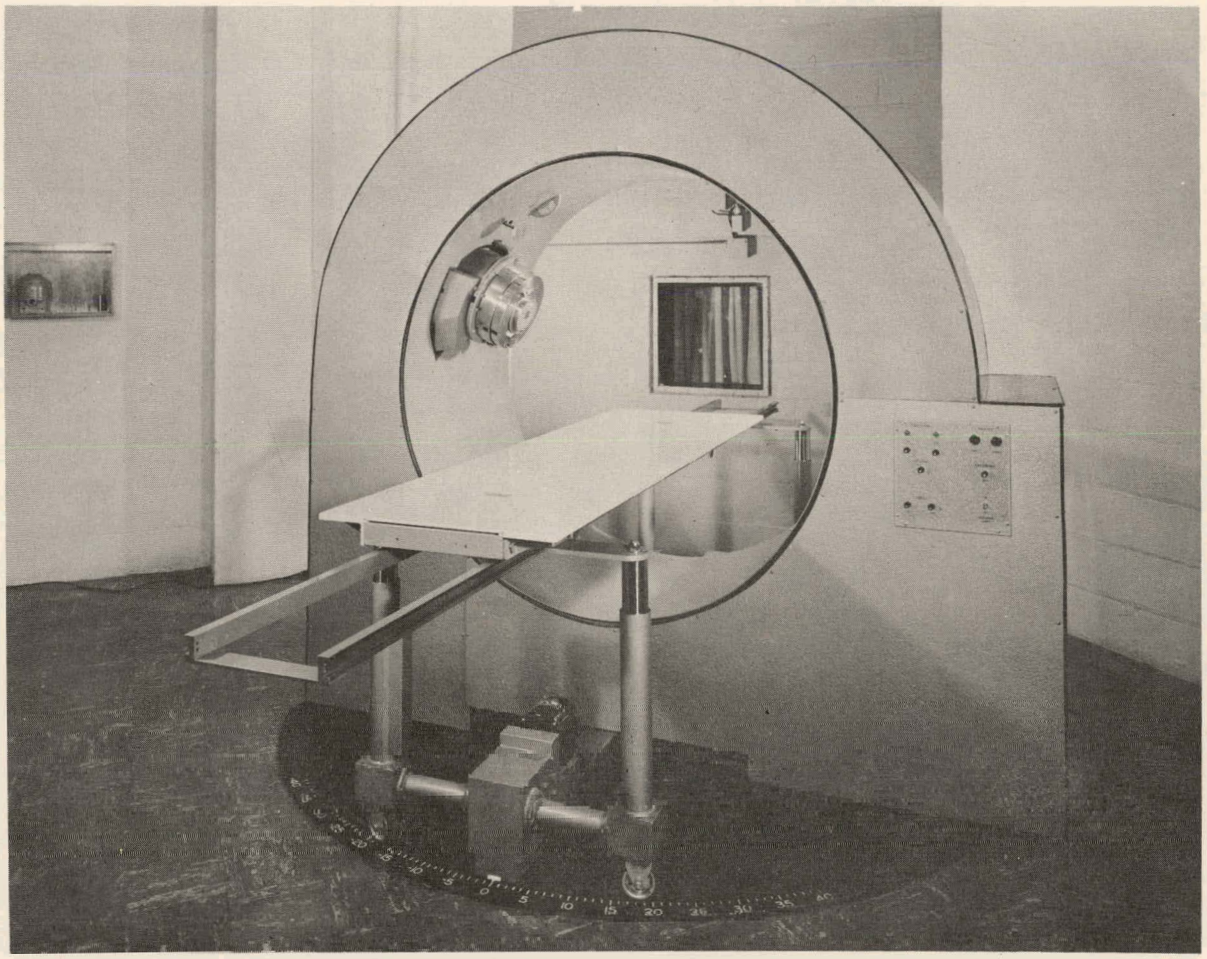

Figure 1. Photograph of the revolving Co^{60} unit for radiation therapy showing the main shield, treatment cot and support, and one of the auxiliary control panels.

The cobalt source is 0.75 cm in diameter and 3.00 cm long. Figure 7 is a photograph, taken before activation, of the piece of cobalt encased in aluminum, along with its cooling spider. A 15-cm scale is included in the picture for size comparison.

Figure 8 is a schematic drawing of the transfer shield in position for loading a source into the main shield. The leveling and adjusting screws are needed to align the axis of the port in the transfer shield with a plug hole in the main shield.

ACKNOWLEDGMENTS

The authors wish to thank Professor Lester S. Skaggs for the suggestion to build the cobalt unit, as well as for his participation and helpful advice in numerous discussions. They also wish to acknowledge the assistance of other staff members of The University of Chicago, Argonne National Laboratory, and the Materials Testing Reactor.

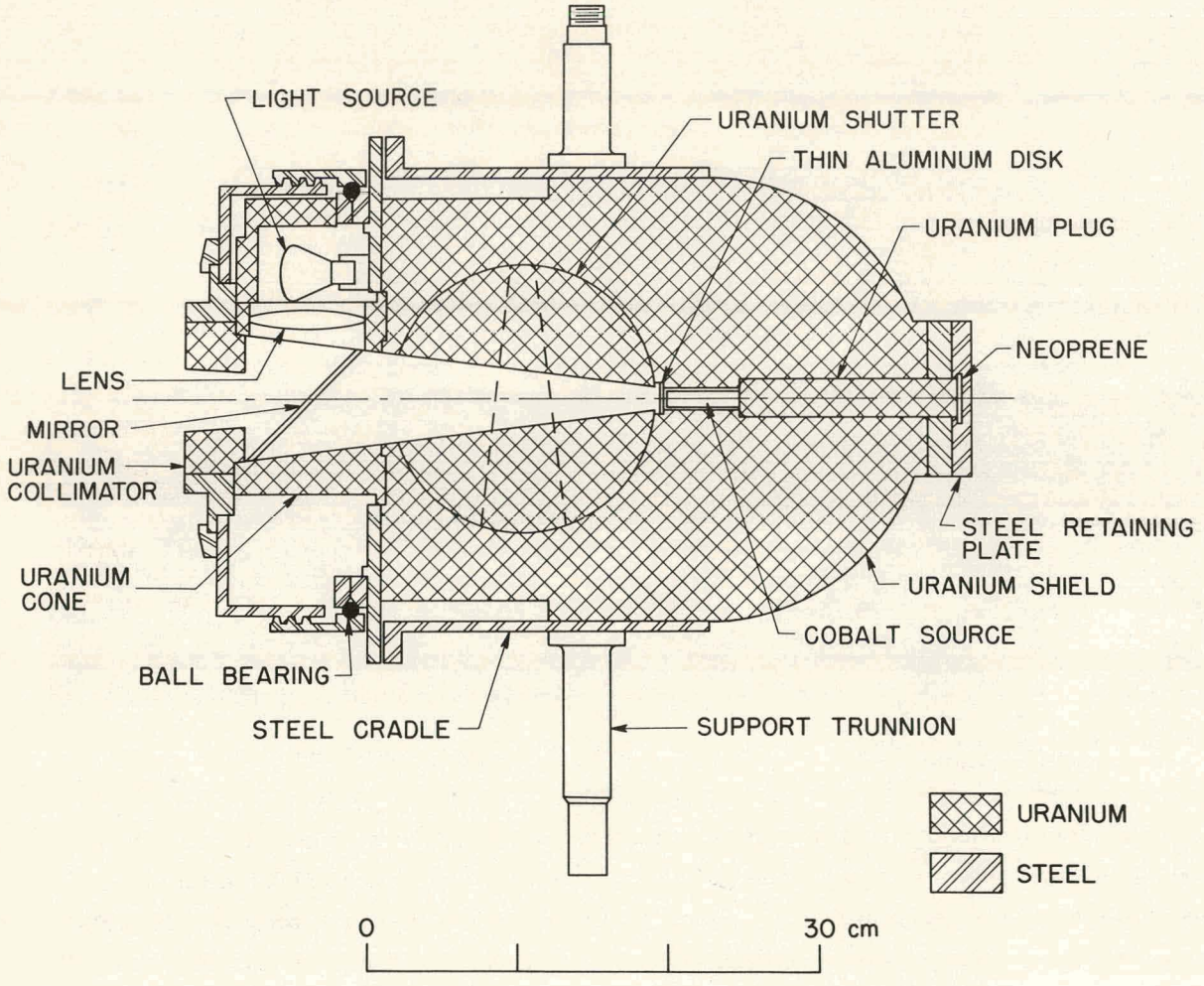

Figure 2. Schematic cross-section of the main shield of the revolving Co^{60} unit.

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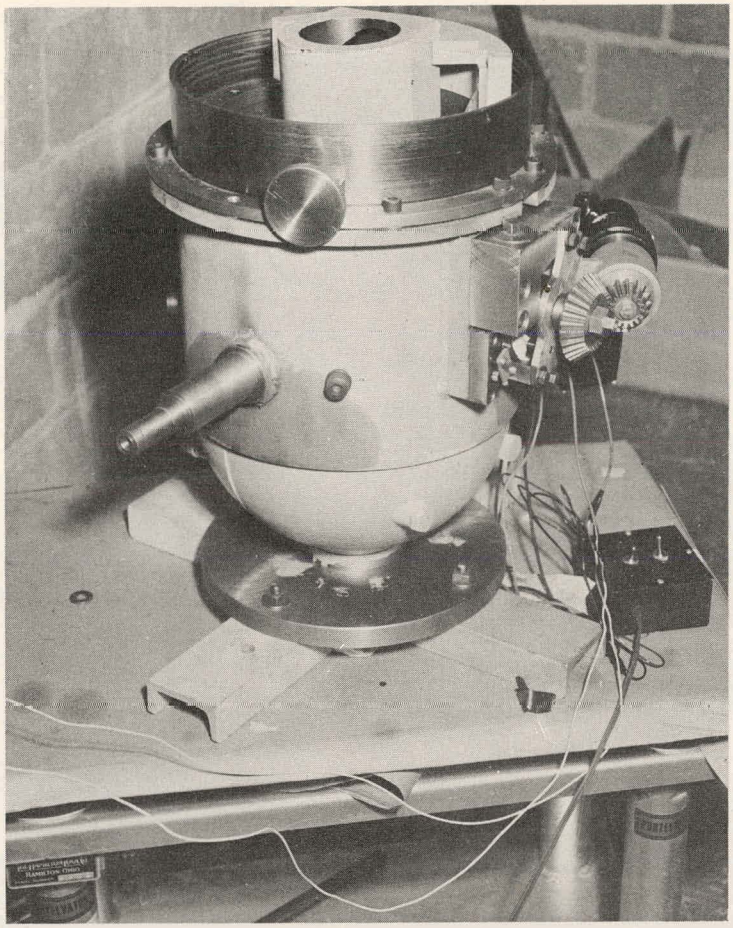

Figure 3. Photograph of the shield (without collimator) within a steel cradle.

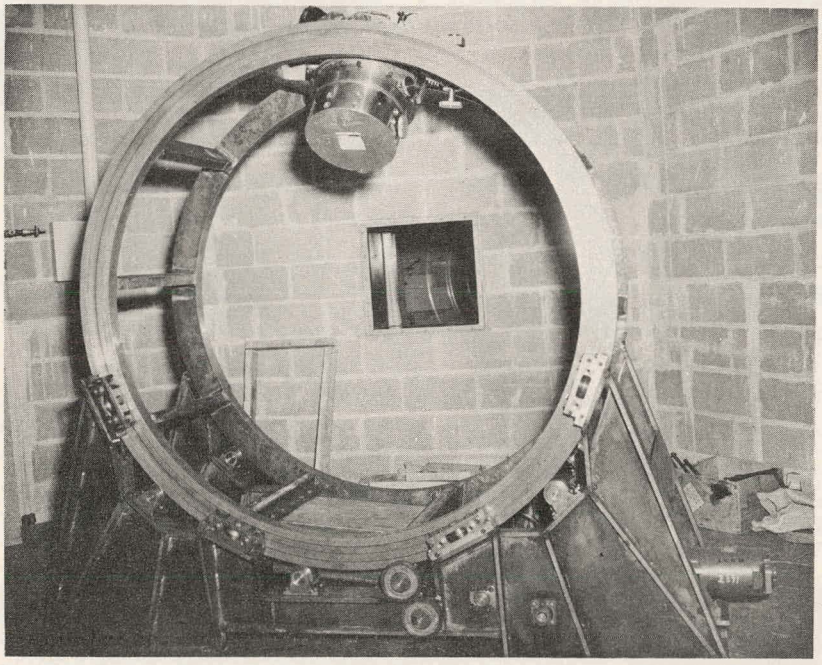

Figure 4. Photograph of the main shield of the revolving Co^{60} unit showing the variable collimator, counterweight, side rollers, drive rollers, large rings, and a part of the drive motor.

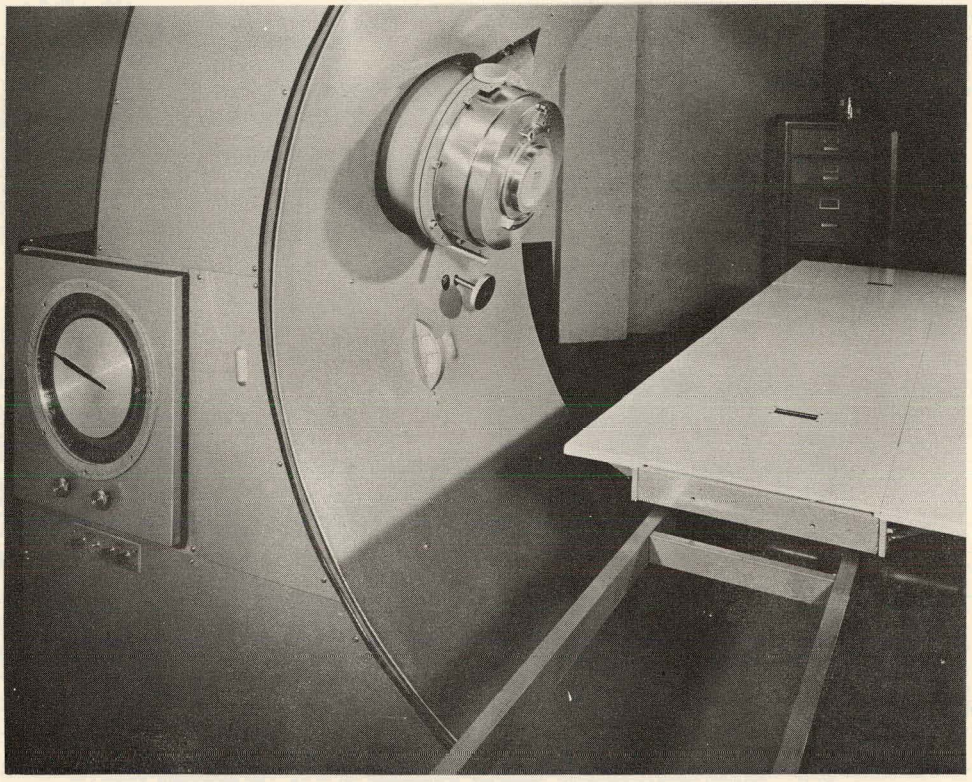

Figure 5. Photograph showing dual angular position controls, main shield with angulation controls and indicator, and treatment cot.

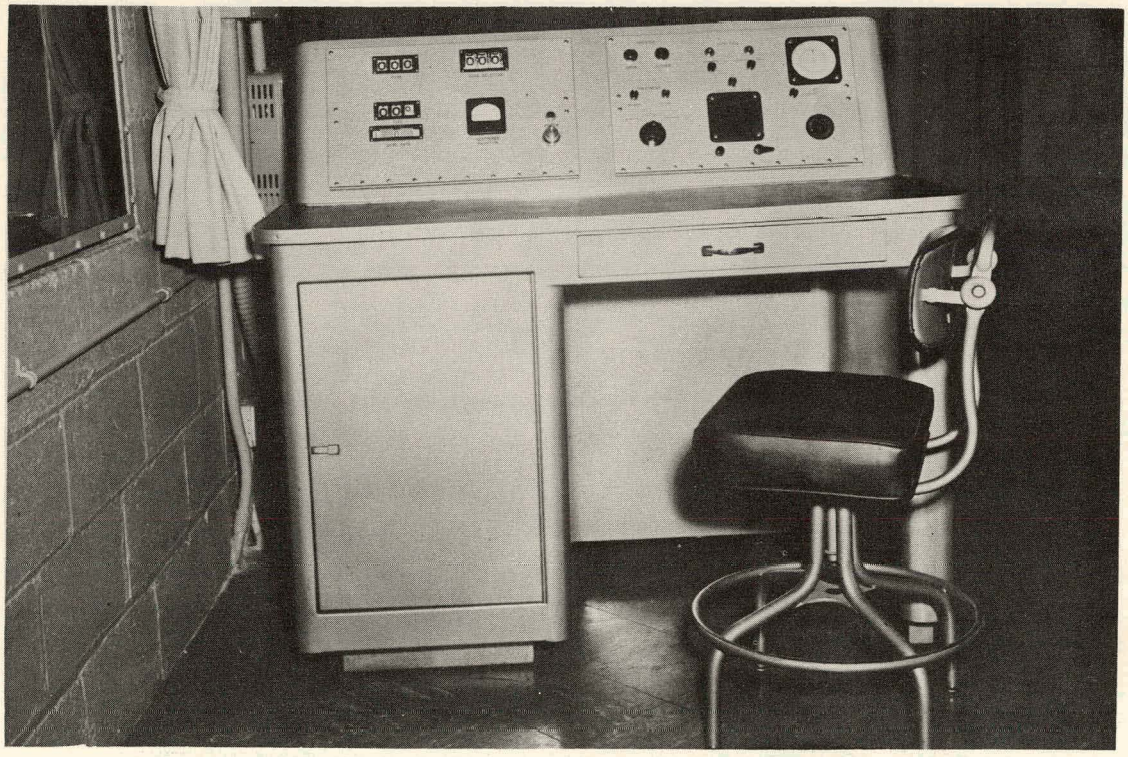

Figure 6. Photograph of the two-panel control console. Left panel contains the power key switch, scattered radiation meter, dose selector, and computer. Right panel contains the shutter, revolution controls, and an intercommunication unit.

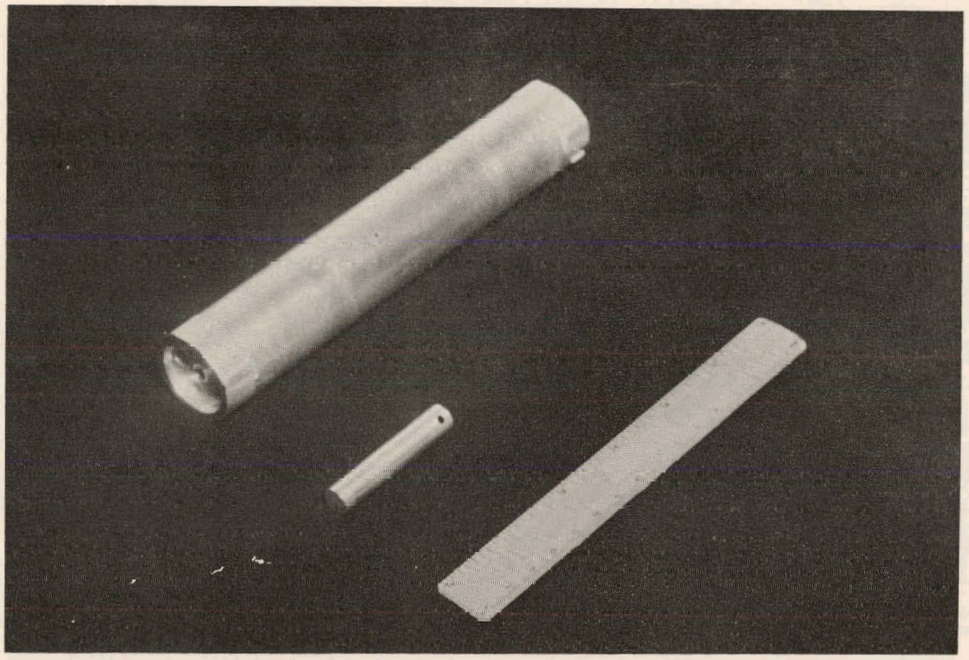

Figure 7. Photograph of the aluminum-encased piece of cobalt (before activation) with its cooling spider.

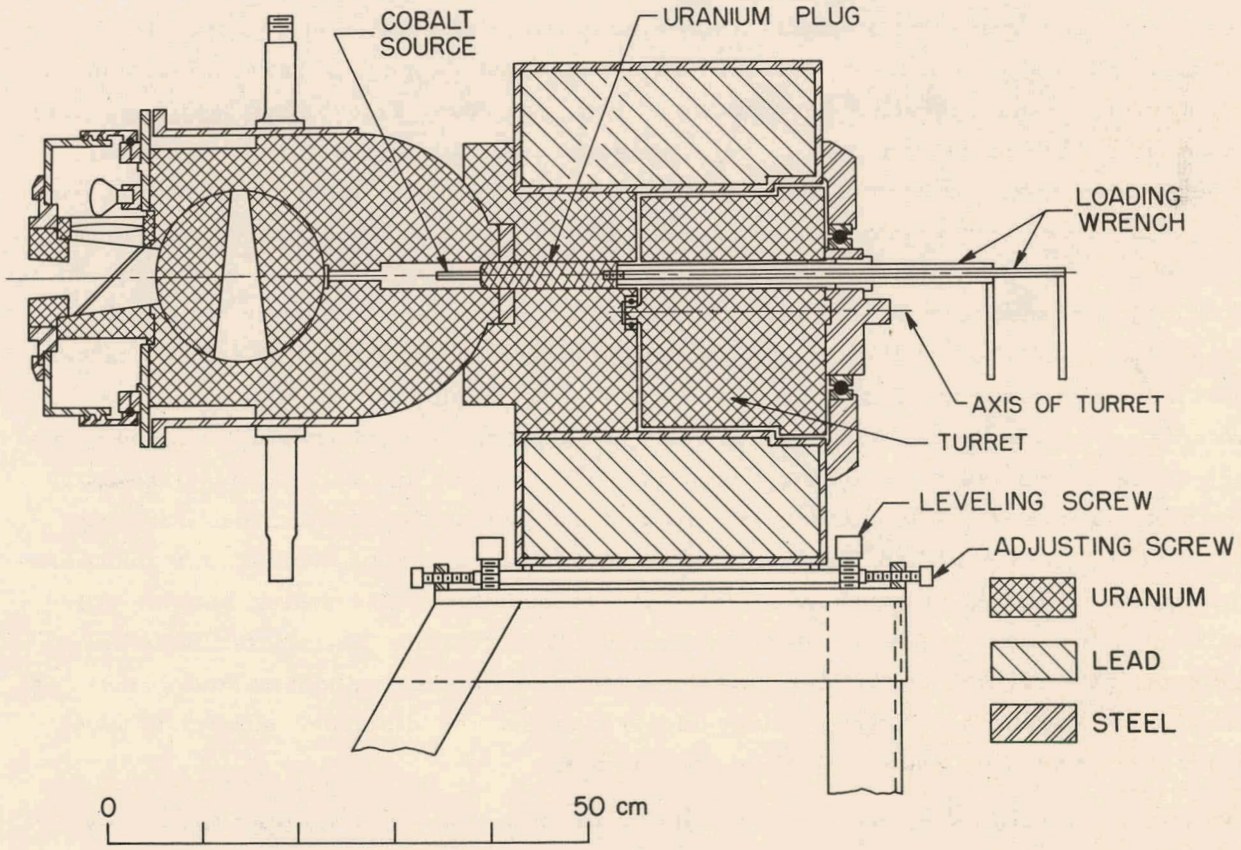

Figure 8. Schematic drawing of the transfer shield in position for loading source into the main shield.

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ISOTOPE THERAPY FOR INTRA-ABDOMINAL TUMORS*

By

P. V. Harper and K. A. Lathrop

The radium volume implant technique developed by Paterson and Parker[1] produces in the treated region a relatively uniform localized field of radiation. The development and clinical trial of a flexible extension of this method forms the basis for this report.

The use of radium, cobalt, or radon implants for intra-abdominal tumors has certain inescapable disadvantages. Plans for having the isotope available must be made well in advance of its use; hence, unexpected situations usually cannot be dealt with at the primary operation. The operator is subjected to a substantial radiation dosage in the course of making the implantation and closing the abdomen. It is necessary to remove the implant, at the termination of treatment (except for radon seeds), and the possibility of loss and the consequent dangerous search must be considered. Post-operative complications such as wound disruptions, which require surgical intervention, can lead to very uncomfortable situations for the surgeon.

In order to circumvent these difficulties, the following technique was devised. Very fine polyethylene tubing (Clay Adams PE-10, outer diameter 0.61 mm, inner diameter, 0.28 mm), which is kept sterile and available at all times in the operating room, is threaded around and through the tumor, following as closely as possible the Paterson-Parker radium rules. Three-quarters of the tubing is evenly distributed on the periphery of the tumor and one-fourth is placed evenly through the volume. The tubing is spaced about 1 cm between turns, and careful measurements are made of the length of tubing comprising the implant and of the ends leading from it. The ends of the tubing are brought out through the wound and left long, one end being longer than the length of tubing in the implant. During this process the tumor is disturbed and handled as little as possible. The tubing may be filled with mercury, and the distribution checked by X ray before closing the abdomen. This permits careful, painstaking placement of the implant without exposing the surgeon to radiation. Nearby vulnerable organs may be displaced surgically away from the implant to whatever extent is feasible.

* Accepted for publication in Surgical Forum, Vol. V. An extended version will be submitted to the editors of Annals of Surgery.

After the patient has recovered sufficiently from the operation, roentgenograms of the implant with mercury in the tubing are made, and from these, the volume of tissue to be irradiated is calculated.

I^{131} is used as a source of γ rays. The tubing wall filters out a portion of the β radiation, and the tissue immediately adjacent to the tubing filters out the rest. This tissue is exposed in any case to a very intense γ-ray field so that little additional damage is done by the β radiation. Radioactive iodine was chosen because it is cheap, readily available, and has suitable chemical properties for concentration to the necessary small volume, and because the short half-life makes removal of the isotope unnecessary.

The air dose rate at 1 cm from a 1 mc point source of I^{131} is 2.28 r per hr* as compared with 8.3 r per hr for 1 mg of radium shielded by 0.5 mm of Pt. Thus, 3.65-mc hours of I^{131} correspond to 1 mg hr of radium. The linear absorption coefficients in tissue for radium and I^{131} γ rays are both about 3 per cent per cm[3], so that this same proportion should be valid at various distances from the sources in tissue. On this assumption, 1 mc of I^{131}, allowed to decay completely, gives the same tissue dose as 76-mg hrs of radium. Using this factor, the Paterson-Parker radium tables may be used directly to calculate the number of mc that are needed to produce the desired total dose of radiation.

A quantity of I^{131} that is about 20 per cent in excess of the required amount (usually 5 to 10 cc of solution) is placed in a conical centrifuge tube. One or 2 mg of KI are added as carrier, and the iodine is precipitated as AgI with a 50 per cent excess of $AgNO_3$. The solution is acidified with HNO_3, digested at 70° for an hour to flocculate the precipitate, and then centrifuged. The supernatant is decanted, and the precipitated AgI is dried with a stream of air in the dark at room temperature. The supernatant is surveyed to check the completeness of the precipitation, which is usually about 99 per cent. The centrifuge tube containing the isotope is handled at all times in a lead-shielded container to minimize exposure. The total-body exposure to the operator, determined by a pocket dosimeter, during the processing of 200 mc of I^{131}, without taking extraordinary precautions, was 7 mr.

The silver iodide is prepared for placement in the implant by dissolving it in a sufficient volume of saturated KI to give the correct number of mc per cm of tubing. This is accomplished with sufficient accuracy by filling an appropriate length of the polyethylene tubing with saturated KI and discharging it onto the AgI precipitate, which dissolves readily.

The isotope is then brought to the patient's bedside. The tubing in the patient is cleaned with distilled water and dried with alcohol, ether, and air and filled with clean dry mercury. Particular care is taken to displace all air bubbles.

* Calculated from the data of Bell and Graham (2), using The Method of Marinelli et al. (3)

The tubing is marked as shown in Figure 1, AB = CD, DE = FG. The distance DE is measured at the time of surgery, and CD is the difference of AD measured at surgery and AC. The long end of the tubing, on which the distance FG is marked, is placed in the centrifuge tube containing the isotope solution, which is drawn up by suction into the tubing until the solution reaches point F. The end of the tubing is then withdrawn from the centrifuge tube, and the isotope is drawn into the implant until the head of the column of isotope solution appears at point C beyond the tumor. The syringe is then removed from the tubing and the mercury is allowed to drop back to point B, forcing the isotope solution back to point D. When this procedure is carried out properly, the isotope is in the tubing within the tumor while the two leads going down to the implant contain air or mercury. The ends of the tubing are sealed with heat and pressure and left long. This entire procedure usually takes about a minute, and exposure to the operator is of the order of 10 mr.

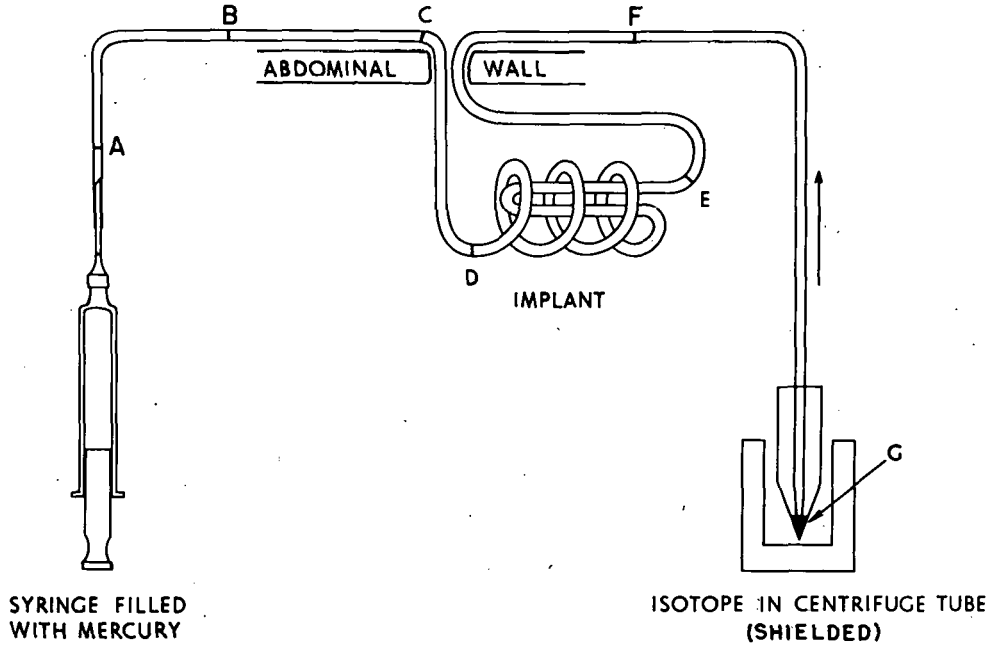

Figure 1. Apparatus for placing isotope in implant.

If it is necessary to re-explore the patient, the isotope may be removed and later replaced without difficulty. Similarly, the isotope can be removed prior to autopsy.

The tubing is left in place after the decay of the isotope. The ends are cut short, sealed, and allowed to retract beneath the skin, where they cause no difficulty.

Several lines of investigation are in progress in connection with this method. First, an effort is being made to check independently the calculated radiation dosage. Measurements with Sievert chambers in a phantom indicated approximate agreement

between the measured and calculated dose. However, since the Sievert chamber is energy sensitive, the calibration for the I^{131} γ rays was in some doubt.

Measurements have been made in a water phantom with X-ray film that had been calibrated against a standard Victoreen chamber. Preliminary results indicate that the measured dose is somewhat lower than the calculated dose.

The treatment of carcinoma of the pancreas has been undertaken as a clinical experiment using this method. Radical surgical treatment of this disease has proved to be ineffective, and the palliative and possible curative effects of properly applied local irradiation are being explored. The procedure has been attempted in 7 cases, successfully in 5. The following are case abstracts in chronological order:

1. A. S. Number 57-76-16: This 56-year-old man had a 150-g tumor of the body of the pancreas which was first discovered at operation. This was wrapped with tubing as described above. In the process, the hepatic artery was perforated twice. The tubing was withdrawn and bleeding ceased promptly and there were no apparent ill effects. A cholecystogastrostomy was performed. The patient at first did well after the operation, but soon became severly depressed, refused to eat, and finally died of inanition 3 weeks after the operation. No autopsy was obtained. No isotope had been placed in the tubing.

2. L. M. Number 58-65-08: This 55-year-old woman had a mass in the pancreas that was discovered in May, 1953, during cholecystectomy. She was re-explored at the Mayo Clinic in August, 1953, for continued back and epigastric pain, and the tumor was found to have extended around the root of the mesentery. She was again re-explored at this hospital on November 5, 1953. At operation, a large tumor, weighing about 135 g, in the head and body of the pancreas was wrapped with tubing. It was impossible to place the core of the implant symmetrically because the superior mesenteric artery and portal vein traversed the implant. The common duct was transplanted to the greater curvature of the duodenum, and the pylorus was transsected and an anterior gastroenterostomy was performed. These procedures removed the bile duct and stomach from the field of irradiation. One week after operation, 135 mc of I^{131} were placed in the tubing. This gave a calculated dose of 5000 r to the lowest point in the implant. The patient tolerated this well, without evidence of radiation sickness. Her appetite remained good. Four weeks after isotope administration she developed a severe anemia (hemoglobin of 80 g per cent) and leucopenia (white count, 1900). This responded during the next 3 months to repeated transfusions and supportive therapy. The patient's epigastric pain and back pain were much improved so that she was able to discontinue completely the use of narcotics. Her tumor mass decreased slightly in size, and the skin over it became tanned from radiation coming from the implant. Six months after treatment she began to fail, then numerous abdominal masses became palpable, and the patient became severely jaundiced and died on June 8, 1954. No autopsy was obtained.

3. M. S. Number 58-90-08: This 66-year-old woman had a history of back pain, weight loss, and anorexia for 9 months and had a moderate obstructive jaundice for 2 weeks. She was explored on December 5, 1953, and a small tumor (15 g) of the pancreas or distal common duct was wrapped with tubing. The common duct was transplanted to the greater curvature of the duodenum to relieve the obstruction. On December 10, 10.6 mc of radioactive iodine were placed in the tubing, and 5 days later, this was removed and replaced with 21.3 mc which gave a calculated total radiation dose of 9200 r. The patient's jaundice disappeared but she continued to have some pains in her back. She did fairly well for 3 months. There was no apparent adverse effect from the radiation. She was hospitalized again on January 9, 1954, when she complained of nausea and vomiting. Roentgenograms of the duodenum adjacent to the implant did not reveal any abnormal conditions. Further studies revealed a marked uremia. The patient expired on March 23, 1954. At autopsy, the cause of death was attributed to chronic pyelonephritis. Grossly, there was not much evidence of tumor, but microscopic studies revealed that tumor was infiltrating the peripancreatic tissues. In the region of the implant, the tumor showed marked regression. There was some evidence of fibrosis in the duodenum adjacent to the implant.

4. H. T. Number 59-30-17: This 42-year-old man gave a 5-month history of abdominal and back pain and a 50-pound weight loss. The character of his stools had changed to an oily amber liquid that frequently floated on top of the water. Physical examination was not remarkable. Laboratory data showed 1+ reduction in the urine, and the glucose tolerance curve was markedly diabetic. Roentgenograms were negative. On February 1, 1954, an exploratory laparotomy was done at which time a carcinoma was found that involved almost the entire pancreas and the surrounding tissue. There was some local lymph node involvement and a few small metastases to the under surface of the liver. A gastrojejunostomy, cholecystoduodenostomy, and splanchnicectomy were done, and the tumor was implanted with polyethylene tubing. The patient made an uneventful recovery. On February 11, 1954, 176 mc of iodine were placed in the tubing. The radiation dose in the implant was difficult to estimate because of the peculiar elongated shape of the tumor. The volume was estimated at about 150 cc, and the radiation dose at about 8600 r. The patient was discharged on February 19, 1954, and returned to work 3 weeks later. His back pain was nearly gone, and his only complaints were persistent diarrhea and inability to gain weight. Four weeks after the isotope administration he developed marked leucopenia and anemia, which required no particular treatment and regressed spontaneously. He continued to do well until about May 1, 1954, when he was readmitted for splanchnic blocks because of the recurrence of pain. At this time, a hard mass was palpable in the epigastrium. Splanchnic block gave some relief from pain. The patient expired suddenly on June 19, 1954. An autopsy re-

vealed complete destruction of the pancreas in the region of the implant, without significant damage to any nearby structures. The portal vein was patent. The tumor, however, had spread extensively to the liver and to the gastrocolic omentum. The cause of death was apparently biochemical.

5. R. H. Number 59-93-87. This 50-year-old man had a carcinoma of the head of the pancreas that was discovered at laparotomy. The pancreas at this time was extensively mobilized, and cholecystjejunostomy was performed. Four weeks later, he was re-explored at this hospital. The operation presented extreme technical difficulty because of the extensive fresh adhesions from the previous surgery. While the pancreas was being mobilized for the second time, the portal vein was torn, and the resulting hemorrhage could not be controlled. The patient expired and no autopsy was obtained.

6. J. B. Number 60-10-62: This 59-year-old man complained of severe burning abdominal pain for 1 year and lost 25 pounds in the 6 months prior to admission. He also had recently developed a steatorrhea, and in the course of his work-up, he was found to be diabetic. Physical examination was otherwise negative, and roentgenograms revealed a pyloric lesion of undetermined nature. On May 21, 1954, the patient was explored, and a large tumor mass of about 120 g was found in the head of the pancreas. Anterior gastrojejunostomy following transsection of the pylorus, choledochojejunostomy, and cholecystectomy were performed, and the tumor was implanted with polyethylene tubing. Following this operation the patient became jaundiced and was re-explored 1 week later, at which time an obstruction of the choledochojejunostomy was relieved, and a transjejunal catheter was left in the common duct. The patient made a good recovery from the second operation except for a slight wound dehiscence, and on June 2, 1954, 97.3 mc of iodine were placed in the implant giving a total dose of 8900 r. The core of this implant was displaced somewhat by the portal vein and superior mesenteric vessel (Figure 2). The patient got along fairly well and did not show evidence of radiation sickness. He developed a leucopenia to the extent of 1900 cells per cu mm 4 weeks following the administration of the isotope but recovered spontaneously.

Following removal of the common duct catheter 1 month after operation, a persistent jejunal fistula remained, which subsequently communicated with the opening in the abdominal wound. The patient had several febrile episodes that responded to Gantrisin and were interpreted as being caused by cholangitis. He gradually became weaker and became jaundiced on August 14, 1954, and died on August 16, 1954. Throughout his postoperative course he was free from pain. Autopsy revealed marked regression of the tumor with no evidence of further spread or radiation damage to other organs. The fistula from the jejunum communicated with the implant which was infected, and the cause of death was a septic thrombophlebitis of the portal vein.

7. T. D. Number 60-43-91: This 49-year-old woman complained of weakness for 3 months, a 20-pound-weight loss in the previous 2 months, anorexia for 6 weeks, and increasing jaundice for 6 weeks. Physical examination was negative except for jaundice, and laboratory examination revealed an obstructive type of jaundice. On July 2, 1954, the patient was explored and a carcinoma of the distal common duct was found to be causing the obstruction. A gastrojejunostomy and cholecystjejunostomy were performed, and the tumor mass was implanted with polyethylene tubing. The patient made a rapid recovery, and on July 8, 1954, 28.5 mc of iodine were placed in the tubing. She has had no adverse effect from the radiation, continues to do well, and has been gaining weight. The total dose delivered to the tumor was 7400 r.

CONCLUSIONS

As a result of these clinical studies, it appears that radiation may be delivered to intra-abdominal tumors in amounts that are intolerable to the patient if the same doses were delivered externally. This is probably because of the relatively small volume of tissue irradiated, and because it is possible to move vulnerable organs away from the field of radiation. In all the cases in which pain was a problem, some relief was achieved. Cases 3 and 6 were done unexpectedly without prior planning. The great need for being able to do the implantation at the primary exploration is illustrated by case 4. It seems feasible at the present time to at-

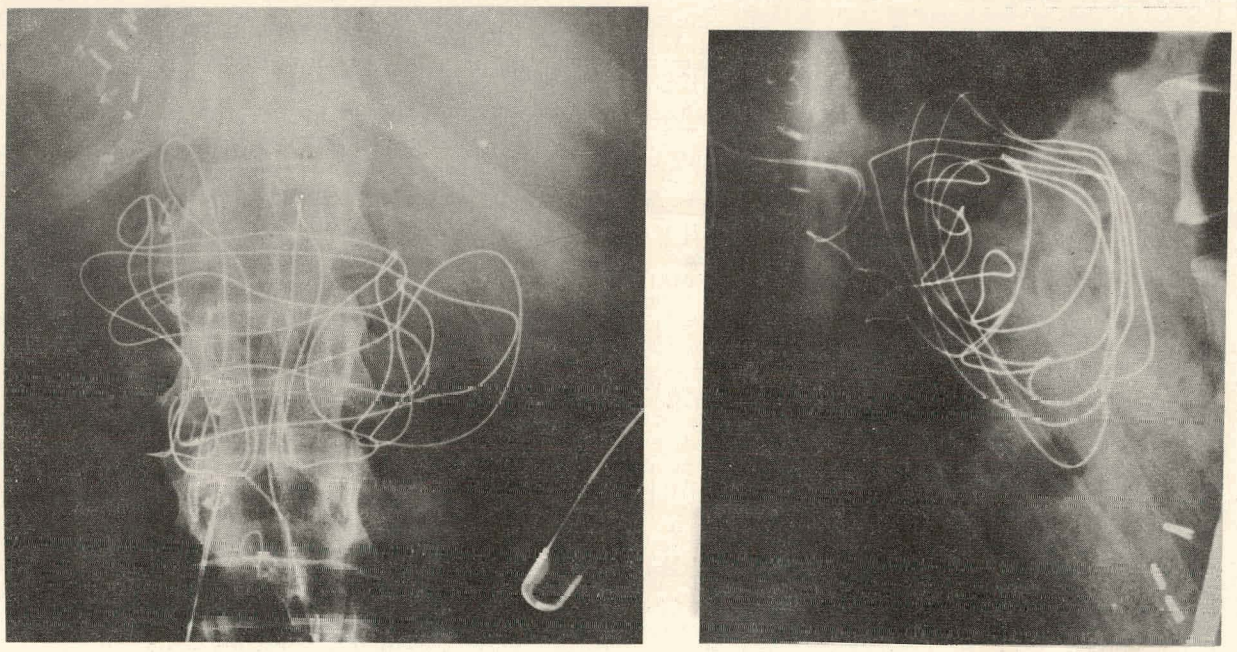

Figure 2. A–P (A) and lateral (B) views of the typical implant that was placed in patient J.B. The volume is 121 cc and 97.3 mc of I^{131} gave a calculated dose of 8900 r. The core of this implant is displaced somewhat by the portal vein and superior mesenteric vessels.

tempt to deliver cancericidal doses to early small carcinomas of the pancreas since the results from such treatment can certainly be no worse than those from radical surgery. Small implants of dose levels ranging from 20,000 to 40,000 r are being made in experimental animals to investigate the dangers and limitations of the application of this method.

The use of isotopes other than I^{131} is also being explored and will form the basis for later reports.

SUMMARY

An extension of the conventional radium implant technique is presented that permits much more flexible application of the method and the use of various artificial radioactive isotopes.

Clinical experiments using this method are yielding encouraging results.

ACKNOWLEDGMENT

We wish to express our gratitude to Dr. J. W. J. Carpender and his staff for their advice and encouragement in this project.

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THE FEASIBILITY OF USING Cs^{131} K RADIATION IN THERAPY

By

P. V. Harper and K. A. Lathrop

It is possible, using γ rays, to produce in any desired volume of tissue an approximately uniform, fairly well localized radiation field, using the isotope distribution worked out by Paterson and Parker [1] for radium. Substituting other γ-ray emitters for the radium has led to an increased flexibility of this technique.[2,3] One serious limitation, however, is the capacity of the patient to tolerate the total-body irradiation produced by the highly penetrating γ rays. This limitation can be removed by substituting an isotope emitting soft X rays for the γ emitter, the uniformity of the radiation field being maintained by appropriate modification of the Paterson-Parker distribution.

Cesium 131 appears to be a suitable isotope for this purpose, and the investigation of its properties from this point of view forms the basis for this report.

Cs^{131} decays by K capture to xenon 131 with half-life of 10.2 days. It thus emits the characteristic fluorescent X radiation of xenon, the principal components of which the K_α and K_β lines have energies of about 30 kv, and a half-value depth in tissue of about 2 cm as calculated from the mass absorption coefficients.

The air dose at 1 cm from a 1-mc source of such radiation has been calculated by Marinelli, Quimby, and Hine[4] to be 1.1 r per hr. From this, it may be estimated that as much as 200 to 300 mc would be required for a large implant.

Cesium 131 is formed from barium 130 in the following raction:

$$Ba^{130} \xrightarrow{n\gamma} Ba^{131} \xrightarrow[T\ 1/2 = 12\ days]{K\ capture} Cs^{131} \xrightarrow[T\ 1/2 = 10.2\ days]{K\ capture} Xe^{131}$$

The natural abundance of Ba^{130} is 0.101 per cent, and the generally accepted cross section[5-7] for the $Ba^{130} \xrightarrow{n\gamma} Ba^{131}$ reaction with thermal neutrons is the value given by Yaffe[8] of about 30 millibarns. While the low natural abundance and small cross section of Ba^{130} appeared to make production of the isotope in sufficient quantities impossible, we were led to reinvestigate this problem because of the earlier report by Katcoff[9] of the much higher cross section of 6 barns.

Katcoff based his estimate of the cross section on the assay of cesium 131 by measuring the K radiation, assuming a counting efficiency of 1 per cent. A soft component of the radiation was interpreted by Katcoff as L radiation, and no evidence at all of any γ-ray activity was found.

The smaller cross section found by Yaffe[8] was based on the earlier observation of Fu-Chun Yu et al.[10] that the soft component of the radiation from Cs^{131} represented conversion electrons of a highly converted (97 per cent) 145-kv γ ray. Basing his assay on this decay scheme and apparently measuring the soft radiation on the assumption that it was conversion electrons, he found a cross section smaller by a factor of 200 than that found by Katcoff.

The crux of the matter appeared to lie in whether the soft component of the Cs^{131} radiation was composed of the very soft L X rays or of conversion electrons.

A detailed analysis by Cork et al.[11] of the decay scheme of Ba^{131}, using an enriched Ba^{130} sample for activation, revealed no evidence whatever of the 145-kv γ ray found by Fu-Chun Yu[10], and a report by Kondaiah[12] indicates that the conversion electrons believed to originate in the Cs^{131} actually came from the parent Ba^{131}.

EXPERIMENTAL

Our measurements were performed on a Cs^{131} sample separated from $Ba(NO_3)_2$ that had been irradiated with thermal neutrons at Oak Ridge National Laboratory.* The barium was removed by precipitation with H_2SO_4, and the supernatant containing the cesium was evaporated to dryness. Measurements of radioactivity were made with a thin-window Geiger tube. The absorption curve in aluminum is almost identical with that published by Yaffe[8] (Figure 1). The soft component has a mass absorption coefficient in aluminum of 320, which corresponds to a wave length of about 3 Å. The absorption coefficient of the soft component in polyethylene sheeting (86 per cent carbon) gives a carbon mass absorption coefficient of about 25 (Figure 2), which corresponds to a wave length of 2.7 Å. This observation can be explained only on the basis that the radiation is electromagnetic rather than conversion electrons. The characteristic L radiation lines of xenon lie between 2.3 Å and 2.6 Å, which is in fair agreement with the absorption measurements. Passing the radiation from Cs^{131} sample through a 300 gauss magnetic field did not remove the soft component of the radiation (Figure 1). Carbon 14 β rays which are in the same energy range as the conversion electrons in question were completely suppressed under these circumstances, the counting rate being reduced from 190 cts per sec to background. These findings appear to eliminate any possibility of the soft radiation being conversion electrons. It was also found that 1.92 g per cm^2 of lead absorber reduced the counting rate of the Cs^{131} sample from 377 cts per sec to background, which confirms Katcoff's observation[9] that there was no evidence of γ-ray activity.

* Oak Ridge Cat. Ba^{131} – I – Irradiated Unit.

The conclusion seems inescapable that Yaffe's cross section is incorrect and that Katcoff's is more nearly correct. Using Katcoff's figure, the irradiation of 1 mole of natural barium for 4 weeks at a flux of 2×10^{13} neutrons per cm^2 sec^{-1} should produce about a curie of Cs^{131}. The absorption cross section of natural barium is sufficiently low (1.38 barns) to avoid serious flux depression even with this large mass of material, and no other noxious isotope is formed in significant quantities; hence, the production of the isotope seems practical.

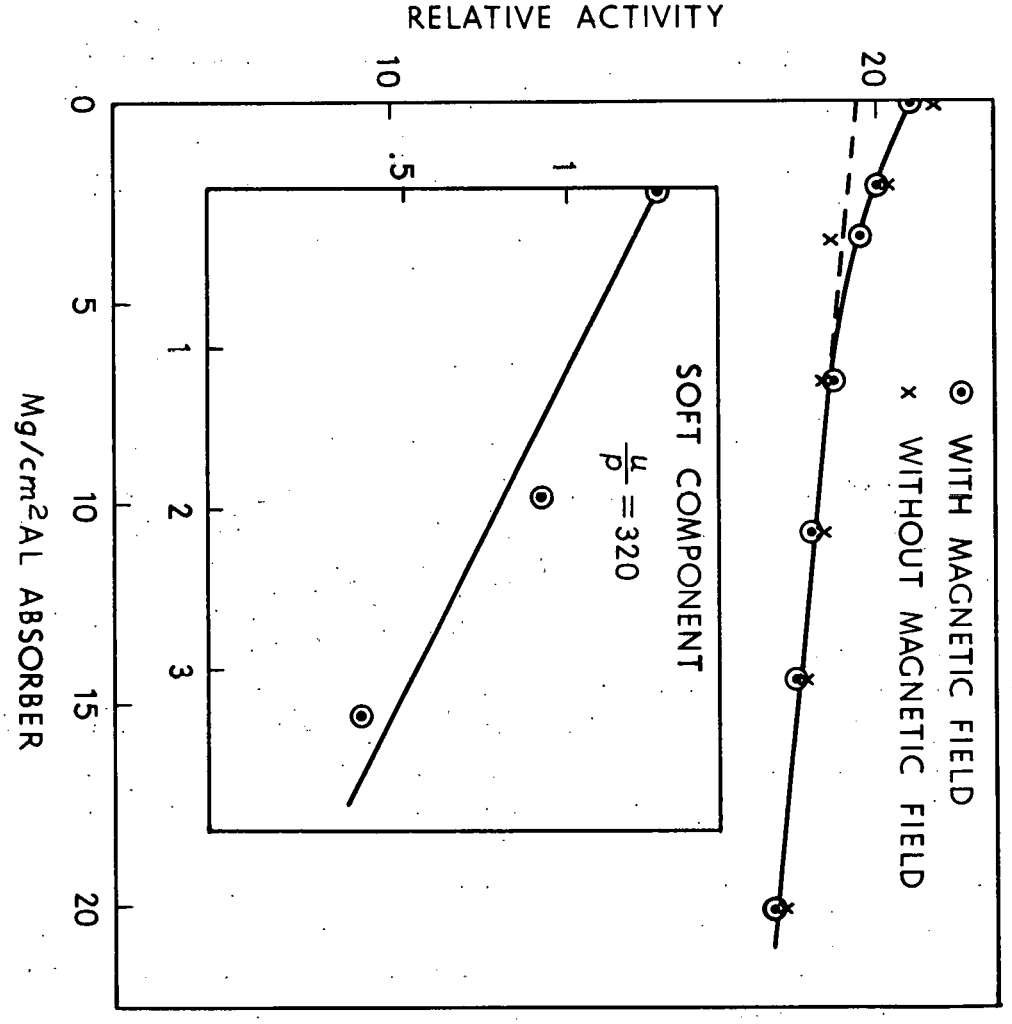


Figure 1. Absorption curve in aluminum of Cs^{131} radiation.

Since it appears definite that the radiation from Cs^{131}, at least as measured with a thin-window Geiger tube, consists solely of K and L radiation, it seemed feasible to utilize K-L coincidence measurements as a method of assay. The L radiation is completely separated from the K radiation by 20 mg per cm^2 of Al, while the mica window of the tube (2 mg per cm^2) shields out the M and N radiation and Auger electrons. It is necessary to make additional corrections since only 80 per

cent of the K transitions (Kα) are accompanied by coincident L transitions.[13] In addition, 29 per cent of the K photons are converted into photoelectrons (Auger electrons) most probably from the L shell* and a similar conversion of 75 per cent of the L photons likewise takes place.[14] This decay scheme of excited Xe^{131} is

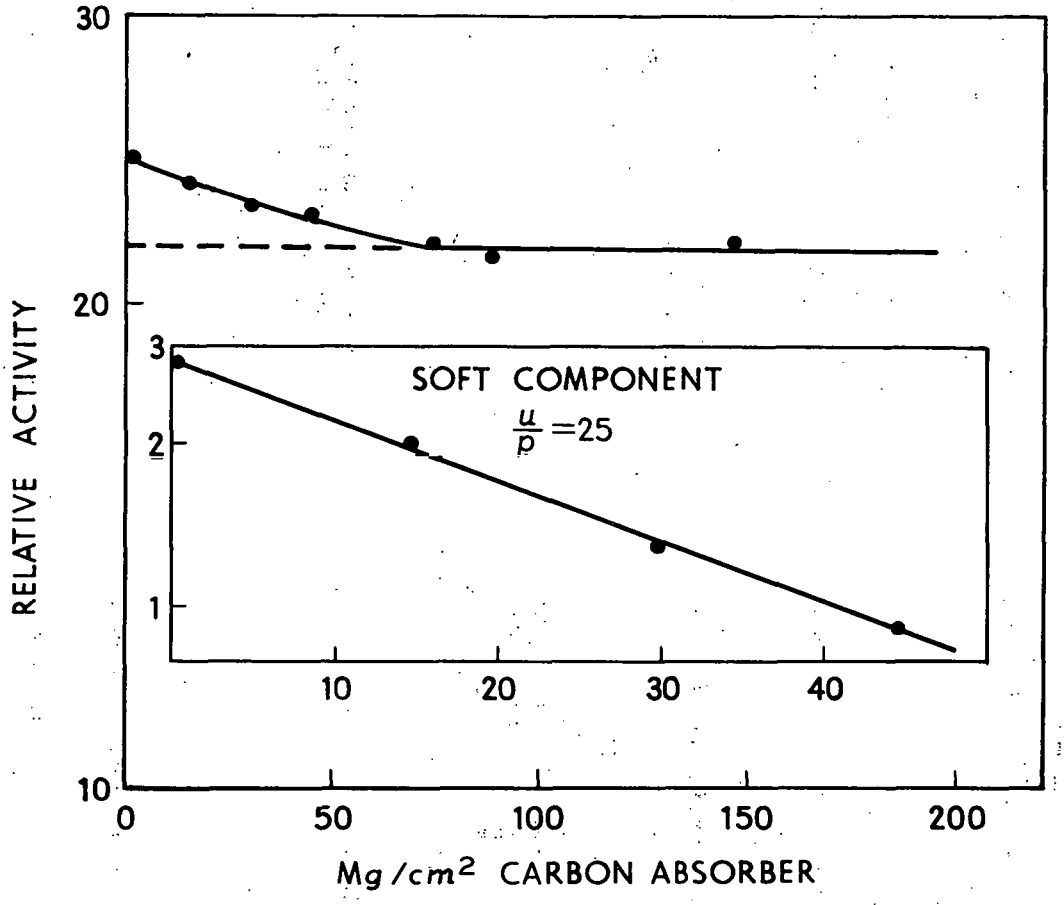


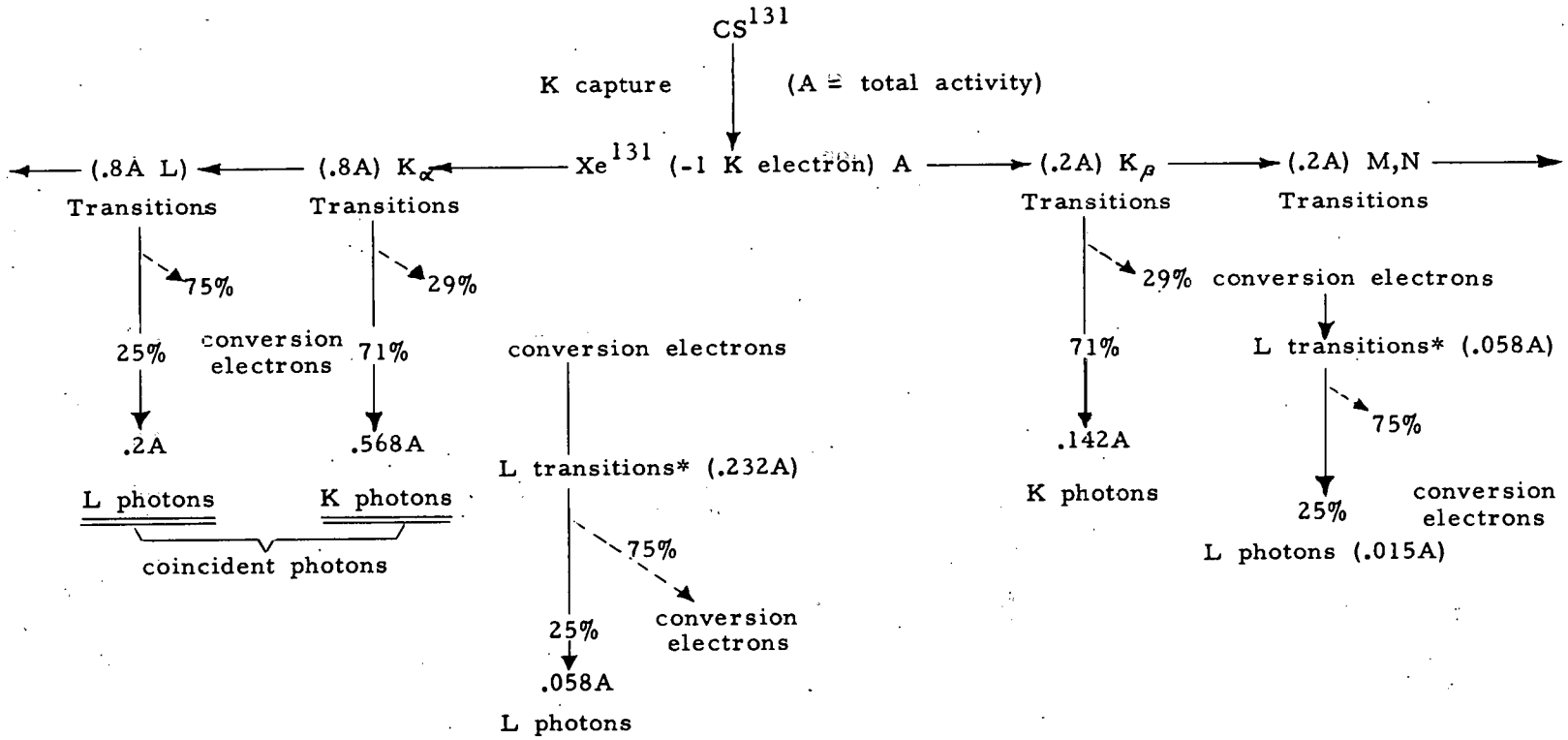
Figure 2. Absorption curve in carbon of Cs^{131} radiation.

shown in Figure 3 with the coincident transitions indicated. Using this method of assay, preliminary measurements indicate a cross section for the $Ba^{130} \xrightarrow{n\gamma} Ba^{131}$ reaction of the same order as that found by Katcoff.

DISCUSSION

While the therapeutic use of Cs^{131} is apparently feasible, several problems remain before clinical experiments can be undertaken. Foremost is the curie level isotope separation, which presents no theoretical difficulties, since barium and

* It is assumed that the $L/_{M,N}$-ratios for this conversion are large. Neglecting this factor entirely introduces an error of less than 30 per cent.



Total K photons

.568A
.142A
.71A

Total L photons

.2A
.058A
.015A
.273A

* Assuming conversion electrons come from L shell.

80% of K counts and 73% of L counts from coincident photons.

Figure 3. Radiation scheme of Xe^{131} following Cs^{131} K-capture.

cesium possess such divergent chemical and physical properties. The dosimetry in tissue surrounding a point source of Cs^{131} must be worked out so that calculations of the necessary modifications of the Paterson-Parker distribution rules may be made. Depth dose measurements with soft X rays present considerable difficulties because of the changing energy of the radiation with scattering and rescattering, since conventional methods of measurement are quite energy sensitive in this range. The ferrous sulfate method appears promising in this connection.

Handling the isotope should present little in the way of exposure problems, since lead glassware should provide complete shielding.

In addition to the conventional types of applicators, which rely entirely on geometrical attenuation for the protection of nearby structures from the effect of the γ rays, the use of the K X rays will permit the use, for instance, of large plane applicators in the bed of a tumor, in which the attenuation by absorption in tissues would be the principal means of protection of nearby structures, geometrical attenuation under these circumstances being insufficient.

SUMMARY AND CONCLUSION

The characteristic 30-kv fluorescent X radiation emitted by Cs^{131} during its decay by electron capture offers definite advantages for radiation implant therapy.

An investigation of the physical properties of this isotope reveals that its production at curie level is feasible.

ACKNOWLEDGMENT

We wish to express our appreciation for the cooperation and help of Dr. Lester Skaggs and his staff without whose suggestions this work would not have been possible.

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BLOOD LEVEL STUDIES OF C^{14}-DIGITOXIN IN HUMAN SUBJECTS WITH CARDIAC FAILURE* **

By

G. T. Okita, P. J. Talso†, J. H. Curry, Jr., F. D. Smith, Jr., and E. M. K. Geiling

SUMMARY

The lack of a sensitive method for the assay of micro amounts of digitalis and its derivatives has been the primary obstacle which has prevented earlier investigators from obtaining data from in vivo human studies.

Using biosynthetically labeled C^{14}-digitoxin(1), it is not only possible to isolate and detect submicrogram quantities of the unchanged drug (by the isotope dilution method), but also to follow conversion products of the parent compound.

In this communication is described the rate of disappearance of unchanged digitoxin and its conversion products from the blood stream of cardiac patients suffering from congestive heart failure.

Eight subjects with congestive heart failure were divided into two groups. Those in one group were given a single intravenous injection of 0.5 mg of radioactive digitoxin, and those in the other, 1.2 to 1.5 mg. Prior to this injection, digitalis medication had been withheld for from 14 to 34 days and none was given afterwards. The specific activity of the drug was between 0.36 and 0.65 μc per mg.

Ten- to 20-ml blood samples were withdrawn by venipuncture at intervals ranging from immediately after to 96 hours after injection. The samples were transferred to heparinized glass stoppered tubes and were frozen for storage.

The extraction procedure employed for the isolation of unchanged digitoxin in blood was a modification of one that has been described previously.(2) The radioactivity of the various extracted fractions, with the exception of the original blood residue, was determined by means of an internal gas-flow Geiger counter.(3) The blood residue was combusted to carbon dioxide in a vacuum combustion line and was counted in an ionization chamber by means of a vibrating reed electrometer.(4) All counting times were long enough to give a standard error of less than 10 per cent.

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Table 1

BLOOD LEVEL OF UNCHANGED DIGITOXIN AND TOTAL C^{14} AFTER SINGLE INTRAVENOUS ADMINISTRATION OF 0.5 MG AND 1.2 TO 1.5 MG C^{14}-DIGITOXIN

Time after administration	Dose - 0.5 mg C^{14}-digitoxin				Dose - 1.2 to 1.5 mg C^{14}-digitoxin			
	Unchanged digitoxin		Total C^{14}		Unchanged digitoxin		Total C^{14}	
	µg %	% in vasc. system	µg eq. %	% in vasc. system	µg %	% in vasc. system	µg eq. %	% in vasc. system
0.2 min	3.8 (3.5-4.2)	40.5 (37.2-44.4)	7.9 (6.8-8.6)	84.1 (72.8-91.5)	12.1 (10.8-12.9)	43.2 (38.6-46.2)	19.6 (16.8-21.1)	72.3 (62.2-76.3)
5 min	1.8 (1.5-2.0)	19.5 (16.0-21.4)	5.5 (4.6-6.0)	58.5 (48.9-63.7)	6.3 (5.8-7.2)	22.4 (20.6-25.7)	14.5 (13.8-15.7)	52.7 (48.9-56.5)
15 min	1.1 (0.9-1.2)	11.6 (9.6-11.8)	3.8 (3.3-4.0)	40.6 (35.0-42.8)	3.3 (3.0-3.5)	11.8 (10.9-12.7)	9.8 (9.5-10.1)	35.8 (33.7-38.2)
30 min	0.9 (0.8-1.2)	9.6 (8.5-11.8)	3.1 (2.7-3.5)	33.2 (28.7-37.3)	2.2 (1.9-2.4)	7.9 (6.8-8.6)	7.7 (6.9-8.2)	27.8 (25.4-30.0)
1 hour	0.6 (0.4-0.8)	6.4 (4.3-8.4)	2.3 (2.2-2.8)	24.3 (23.2-27.5)	1.6 (1.4-1.7)	5.7 (4.9-6.2)	6.5 (6.2-6.9)	23.5 (20.4-26.0)
2 hours	0.35 (0.2-0.5)	3.7 (2.1-5.3)	2.1 (1.8-2.2)	22.0 (18.7-23.0)	1.1 (0.8-1.3)	3.9 (2.8-4.6)	5.5 (5.2-5.8)	19.8 (18.0-22.1)
6 hours	0.25 (0.1-0.3)	2.6 (1.1-3.2)	1.8 (1.5-1.9)	19.5 (16.1-20.6)	0.9 (0.7-1.1)	3.2 (2.5-3.9)	5.0 (4.7-5.6)	17.8 (16.7-20.4)
12 hours	0.25 (0.1-0.3)	2.5 (1.2-3.2)	1.6 (1.3-1.8)	17.0 (13.8-19.5)	0.8 (0.5-0.9)	2.8 (1.8-3.2)	4.6 (4.2-5.1)	16.4 (15.2-18.8)
24 hours	0.17 (0.1-0.2)	1.8 (1.1-2.1)	1.0 (0.8-1.1)	10.6 (8.5-12.0)	0.7 (0.5-0.9)	2.5 (1.8-3.2)	2.9 (2.4-3.3)	10.4 (8.6-11.8)
48 hours	0.15 (0.1-0.2)	1.6 (1.1-2.1)	0.6 (0.5-0.7)	6.5 (5.3-7.4)	0.5 (0.3-0.6)	1.8 (1.1-2.1)	1.3 (0.9-1.6)	4.7 (3.2-5.8)
72 hours	0.10 (0.1-0.1)	1.1 (1.1-1.1)	0.3 (0.2-0.5)	3.2 (2.2-5.4)	0.25 (0.1-0.3)	0.9 (0.4-1.1)	0.7 (0.4-0.9)	2.5 (1.4-3.2)
96 hours	tr (tr-0.1)	tr (tr-1.1)	0.25 (0.1-0.5)	2.5 (1.1-5.3)				

Using a known amount of C^{14}-labeled digitoxin as a standard, recoveries of 97 ± 4 per cent were obtained.

Radioactivity values for all fractions except that of the unchanged compound were combined and taken to represent the radioactivity of the conversion products. Table 1 gives the data on blood levels of unchanged digitoxin and total C^{14} at various times following the intravenous injection of 0.5 or 1.2 to 1.5 mg of C^{14}-digitoxin.

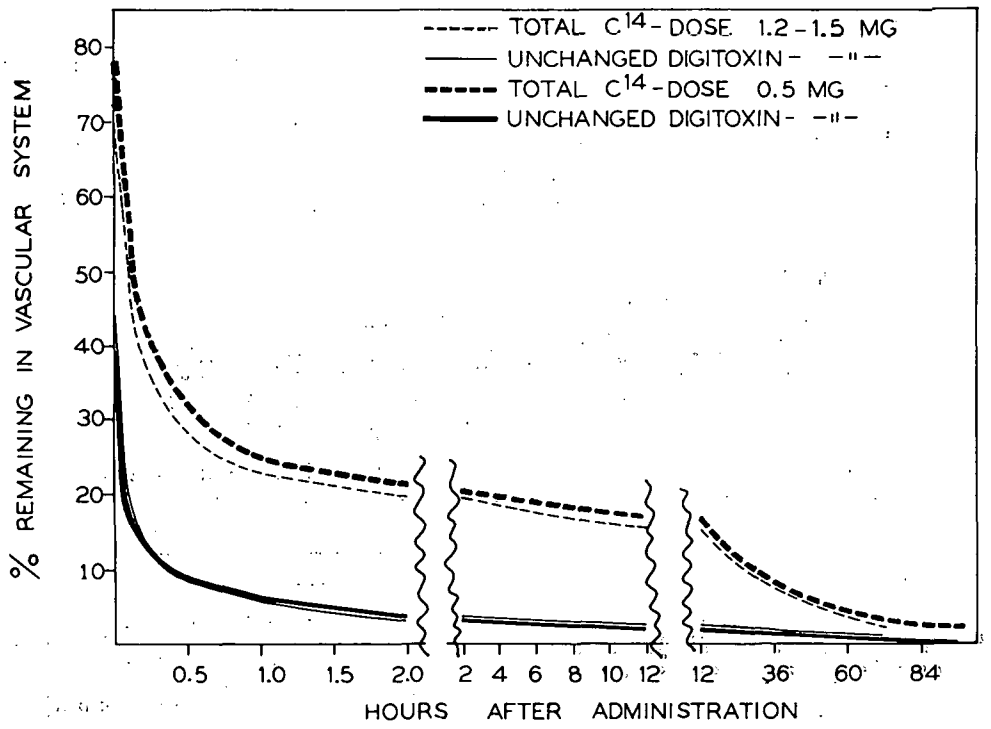

Figure 1. Blood level curves showing per cent of dose remaining in vascular system as unchanged digitoxin and total C^{14} after a single intravenous administration of biosynthetically labeled C^{14}-digitoxin.

The curves in Figure 1 indicate that contrary to the findings of earlier investigators, digitoxin can be found in the blood stream as long as 96 hours after its intravenous administration. The curves also suggest that, on the basis of the per cent of injected dose remaining in the blood, there is no appreciable difference between the two dose levels. Figure 2, however, shows that there is a marked difference in the concentration of both the unchanged drug and total C^{14} on a microgram or microgram equivalent per cent basis between the two doses used. Figure 3 indicates that the rate of disappearance of unchanged digitoxin from the blood stream is not dose dependent. Hence, the concentration on a microgram equivalent per cent basis appears to be dose dependent, whereas the rate of disappearance and concentration on the basis of the per cent of injected dose remaining in the blood is not.

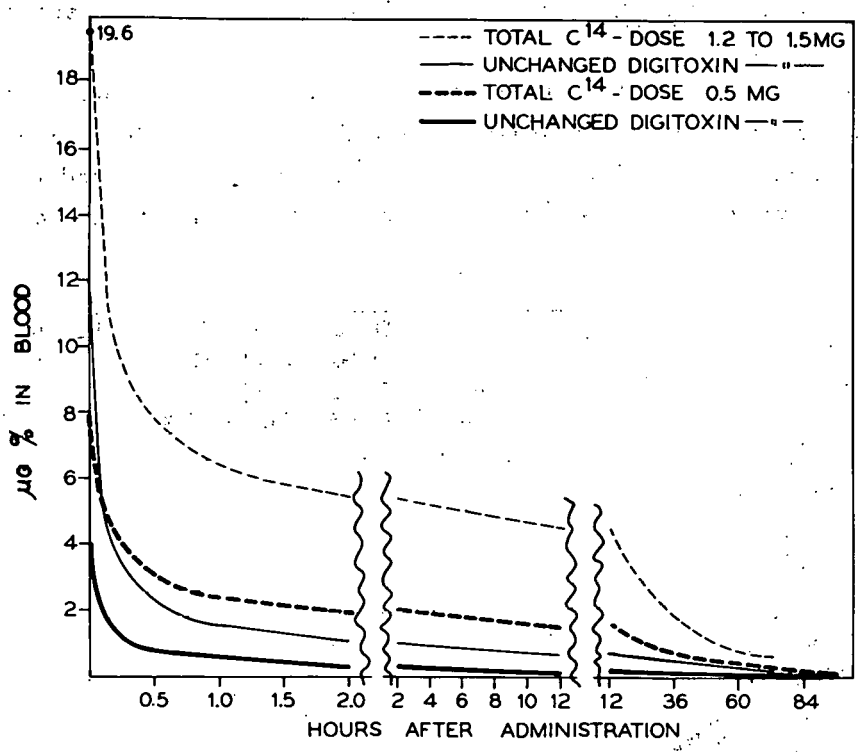

Figure 2. Blood level curves showing µg per cent of unchanged digitoxin and total C^{14} in blood after single intravenous administration of biosynthetically labeled C^{14}-digitoxin.

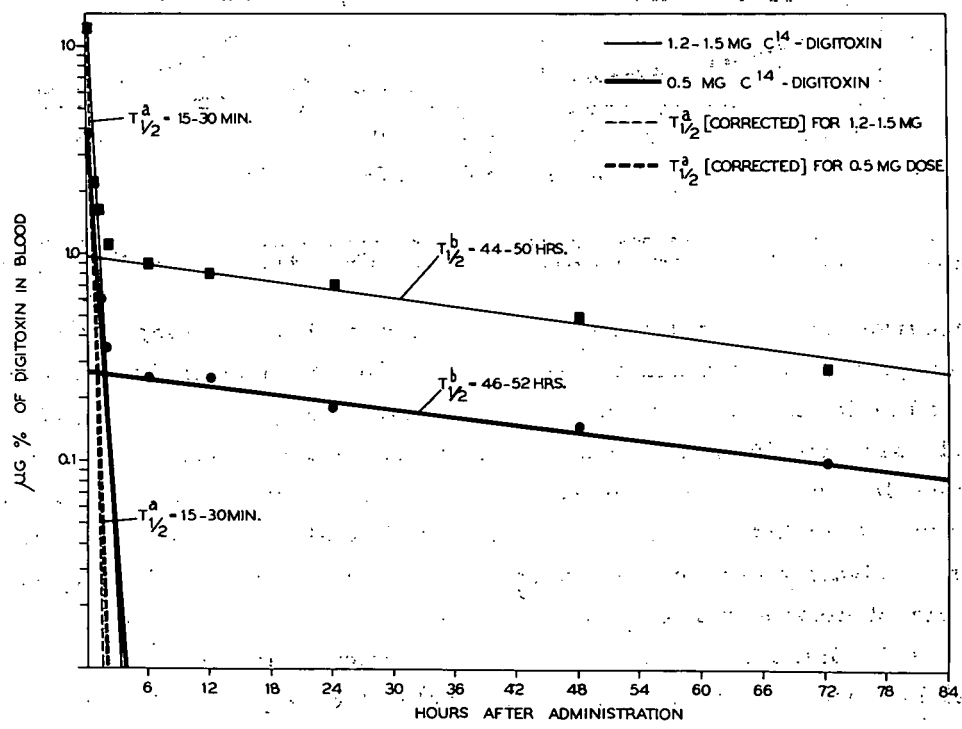

Figure 3. Semilogarithmic plot showing disappearance rate of unchanged digitoxin in blood after intravenous administration of C^{14}-digitoxin.

During the 96-hour observation period, two exponential rate components were observed for unchanged digitoxin. The first component had a biological half-life of 15 to 30 minutes, while the second had one of 48 to 54 hours. There is some evidence that there is at least one other component with a very long half-life that may represent the true turnover time of the drug.

Although unchanged digitoxin was found in the blood up to 96 hours after a single intravenous injection of the glycoside, it is believed that micro amounts are present in the blood as long as 40 days afterwards as evidenced by its presence in the urine.(2)

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PROTEINS IN MULTIPLE MYELOMA
III. ORIGIN OF BENCE-JONES PROTEIN

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By

F. W. Putnam and S. Hardy

SUMMARY*

The profuse synthesis and diverse nature of the proteins elaborated by patients suffering with multiple myeloma present an interesting problem to the biochemist. The disease, which is of unknown etiology, is believed to affect the plasma cells that are involved in globulin synthesis and antibody production. Several aberrations in protein metabolism are associated with multiple myeloma. These are: 1) the production of anomalous serum globulins, 2) the excretion of the Bence-Jones urinary proteins, and 3) paramyloidosis.[1]

The origin of the Bence-Jones protein has not been determined but it has been ascribed to renal cleavage [2,3] or degradation of tissue proteins. It has been established [4-6], however, that there are several types of myeloma globulins and of Bence-Jones proteins but their relationships to each other and to normal serum proteins have not been elucidated. Thus, the rate of synthesis and the possible precursor relationships of myeloma globulins and Bence-Jones proteins have been investigated by means of isotopic tracers [7,8].

Labeled glycine ($NH_2CH_2C^{13}OOH$) was obtained in a yield of 55 per cent by the method of Sakami et al.[9]

A total of 5.41 g of the compound was given orally in divided doses over an 11-hour period to a patient, who was suffering from multiple myeloma. Blood samples were withdrawn at 8:00 A.M., and 24-hour urine collections were made.

The serum proteins were isolated by salt fractionation. The procedure yielded a preparation that was 85 per cent β globulin but also contained all of the serum γ globulin, which, initially, comprised only 3 per cent of the total serum protein. Electrophoretic analysis also revealed the presence of a small amount of serum albumin.

During ultracentrifugation, the β globulin split into two major components. The ratio of the sedimentation rate of the sharp fast peak to the slow one was 1.6, which was not in agreement with the electrophoretic distribution. This ratio could be altered by further fractionation or by dialysis against water to remove the euglobulin. The myeloma globulins of the β type may contain two sedimenting components [4]; one with the s_{20} or normal γ globulin (6.6 S), the other with an s_{20} of 9 S. Neither, component however, has the mobility of γ globulin at pH 8.6.

* Summary of a paper that appears in the Journal of Biological Chemistry, 212:361 (1955).

Serum albumin was obtained from the supernatant of a 1.8 M ammonium sulfate solution that was adjusted to a concentration of 2 M. The precipitate appeared to be free of globulin when it was examined electrophoretically, but a small amount of a more rapidly sedimenting component appeared during ultracentrifugation.

To isolate the Bence-Jones protein, the urine was adjusted to pH 5.2 and solid ammonium sulfate was added with stirring until the concentration reached 3 M. The precipitate was dialyzed against water until free of the sulfate ion and then was lyophilized. Throughout the pH stability range (pH 5 to 9), the protein migrated with a single component during electrophoresis and ultracentrifugation. In pH 8.6 veronal buffer, 0.1 ionic strength, it had an s_{20} of 3.08 S, and it migrated with a mobility of -3.37×10^{-5} cm^2 $volt^{-1}sec.^{-1}$ compared with a mobility of $-3.07 \times 10^{-5} cm^2 volt^{-1} sec.^{-1}$ for the myeloma globulin. Although their mobilities were similar, the two pathological proteins could be distinguished by their sedimentation behavior and their N-terminal amino acid groups. The abnormal serum globulin contained both N-terminal aspartic and glutamic acids (1.3 and 1.7 moles, respectively, per 160,000 g), whereas the Bence-Jones protein had 2 moles of N-terminal aspartic acid per 40,000 g with only traces of N-terminal glutamic acid, glycine, and threonine.

Isotopic analyses indicated a high initial C^{13} concentration of the glycine of the Bence-Jones protein followed by a rapid decline. The rate of decline of the concentration of C^{13} in the Bence-Jones glycine was almost identical with the decline in N^{15} concentration of urinary urea when N^{15} glycine was fed to a normal subject [10,11].

Since the Bence-Jones protein is an excretory product and the body pool of this protein is small, the isotopic decline is not a direct measure of turnover but rather an expression of synthesis, release into the circulation, renal clearance, and the activity of the glycine pool.

The C^{13} abundance of the Bence-Jones protein glycine was at first 6 times greater than that of the serum protein glycine, but later it was 50 per cent lower. These data suggest that the myeloma globulin is not broken down in the kidney or anywhere else to form Bence-Jones protein. Comparison with isotopic studies of other workers indicates that the Bence-Jones protein could not arise from the degradation of normal tissue protein, rather, it seems to be synthesized de novo. [6]

Specific activity curves for the Bence-Jones protein and for the myeloma globulin reach a maximum at about the same time, which is about 5 days prior to their intersection. Thus it would seem that the syntheses of the Bence-Jones protein and of the myeloma globulin are independent processes. It is possible however, that there may be a common precursor, or an additional precursor, or a diluent of the product.

The half-time of the myeloma globulin is 17-20 days, a length of time that might indicate that hyperproteinemia is due to an accumulation of myeloma globulin by slow turnover rather than by rapid synthesis [8]. The data, however, are insufficient to support this conclusion.

The experimental results do reveal that the Bence-Jones protein arises by de novo synthesis and is apparently derived directly from the nitrogen pool rather than from any plasma or tissue protein precursor.

Some experiments with a patient who had different pathological proteins and graver clinical condition have given further evidence fot the direct interaction of the Bence-Jones proteins with the metabolic pool of nitrogen [12].

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PROTEINS IN MULTIPLE MYELOMA
IV. INTERACTION WITH METABOLIC NITROGEN*

By

S. Hardy and F. W. Putnam**

SUMMARY***

In a previous study of plasma protein formation in a patient with multiple myeloma, no relationship could be found between the synthesis of a heterogenous abnormal β globulin and a urinary (Bence-Jones) protein [1]. A patient who excreted a different Bence-Jones protein and whose abnormal serum protein was a physically homogeneous cryoglobulin was chosen for the investigation that is outlined in this summary. The cryoglobulin had the mobility and molecular weight of normal human γ globulin but differed by the absence of glutamic acid as an amino end-group.

The patient, a 70-year-old male, who was suffering from multiple myeloma, was given 20.94 g of the labeled glycine in divided doses on the first day of the experiment. Blood samples were withdrawn daily at 8:00 A.M. for a period of 2 weeks. Urine samples were taken for 27 days, but 24-hour pooled specimens were not collected because the patient was incontinent.

The method of Schoenheimer and Ratner [2] was used to prepare the labeled glycine ($N^{15}H_2CH_2COOH$) in a yield of 89 per cent from potassium phthalimide, which contained 33 atom per cent excess N^{15}. The serum proteins were fractionated, and the urinary protein was isolated as described previously [1] except that the γ globulin was precipitated by dialysis against 1.6 M ammonium sulfate instead of 1.8 M. The supernatant fraction, which contained α and β globulins and albumin was precipitated at 2 M, and representative samples were analyzed after electrophoresis and ultracentrifugation. The proteins were hydrolyzed, and the glycine was estimated colorimetrically [3,4]. Nitrogen content and nitrogen partition of the urine were determined by the Kjeldahl method. A modification of Sanger's method [5] was used for N-terminal amino acid analysis, and the abundance of N^{15} was determined by means of the mass spectrometer.

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*** Summary of a paper that appears in the Journal of Biological Chemistry, 212:371 (1955).

When the serum was refrigerated a precipitate formed, which dissolved when the serum was warmed to room temperature. This property, which is a characteristic of cryoglobulin and found in only a few pathological sera, was also exhibited by a concentrated solution of the isolated protein in buffer. The cryoglobulin had the mobility and sedimentation constant of the γ globulin prepared by salt fractionation. Although the α and β globulins comprised 15 per cent of the "albumin" fraction, the purified cryoglobulin was extremely homogeneous in electrophoretic and ultracentrifugal properties. In veronal buffer, it had a sedimentation constant (s_{20w}) of 6.33 S, a value, which, when extrapolated to infinite dilution, corresponds to that reported for normal human γ globulin (6.6 S) [6]. In one sample, there was 5 per cent of a heavier component with a sedimentation constant of 9.65 S. The electrophoretic mobility in pH 8.6 veronal buffer was $-1.0 \times 10^{-5} cm^2 sec^{-1} volt^{-1}$; in 0.1 ionic strength cacodylic buffer, pH 6.8, the mobility was $-0.75 \times 10^{-5} cm^2 sec^{-1} volt^{-1}$.

The Bence-Jones protein also was quite homogeneous upon electrophoresis and ultracentrifugation.[6] Approximately 97 per cent of the salt-precipitated protein migrated with a mobility of $-2.2 \times 10^{-5} cm^2 sec^{-1} volt^{-1}$ in veronal; the sedimentation constant (not corrected for protein concentration) was 2.14 S. From 20 to 25 per cent of the urinary N was found to be protein, and it was estimated that the patient excreted daily about 10 g of protein.

The cryoglobulin and the Bence-Jones protein contained only aspartic acid in the N-terminal position. The cryoglobulin had 2.0 moles of N-terminal aspartic acid per 160,000 g and was devoid of other amino end-groups. The Bence-Jones protein contained 1.0 mole of N-terminal aspartic acid per 26,000 g of protein.

The isotopic data for the glycine of the Bence-Jones protein and of the cryoglobulin and the N of urinary urea are plotted in Figure 1.

The high initial isotopic content of the urinary protein glycine, which must have closely approached that of the glycine pool [7], and the nearly constant relationship between the N^{15} abundance of the Bence-Jones glycine and urinary area N are evidence that nitrogen flows almost directly from the metabolic pool into the Bence-Jones protein. Since the concentration of N^{15} in the Bence-Jones glycine was only double that in the urinary urea, there was a rapid equilibration of the labeled glycine with the body pool of N. The relative constancy of the ratio suggests that glycine peptides are not preferential intermediates in the biosynthesis of the Bence-Jones proteins.

Neither the Bence-Jones protein nor the cryoglobulin is the sole immediate precursor of the other, if the criteria of Zilversmit *et al.* [8] and Reiner [9] are applied to the data obtained from this experiment. Furthermore, it can be concluded that the urinary protein is not derived by the renal degradation of the circulating cryoglobulin. Indeed, it is quite likely that the Bence-Jones protein is synthesized independently of the plasma proteins.

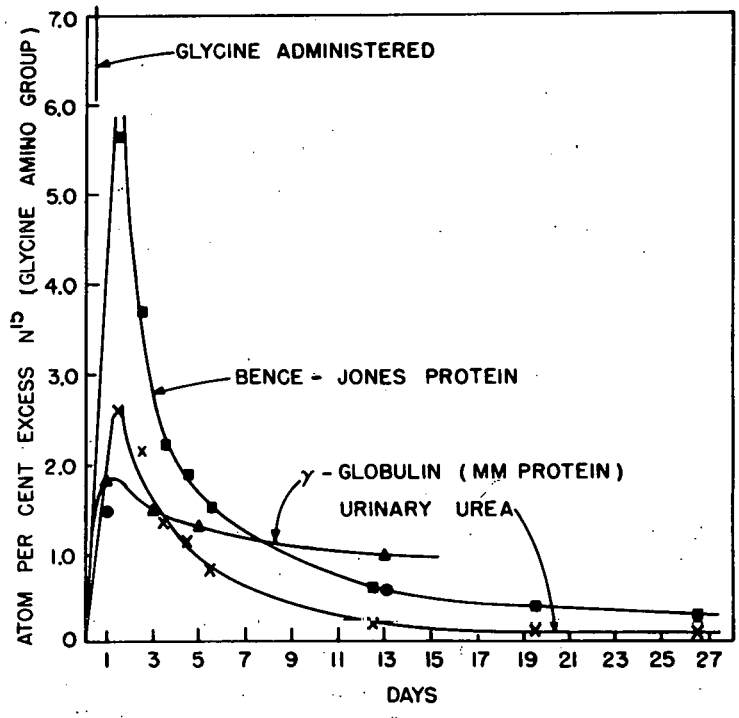

Figure 1. N^{15} concentration of urinary urea, of the glycine protein, and of the glycine of the serum γ globulin (cryoglobulin) as a function of time. The two closed circles refer to the glycine of the albumin fraction.

In this experiment, the half-time of isotopic decline in the Bence-Jones protein glycine was about 1.8 days, double the half-time found for the labeled glycine in an earlier study [1]. The half-time of decline in the N^{15} of the urinary non-protein-nitrogen was, if anything, somewhat greater, i.e., about 2.5 days. This retarded excretion was associated with uremia, and there was clinical evidence of kidney damage.

The turnover time of the cryoglobulin could not be determined precisely because the patient's condition did not permit complete blood sample collections. The data, however, suggest that the turnover rate was within the limits that have been reported for plasma proteins. [10]

The first subject, who excreted less than 2 g of Bence-Jones protein daily, was in nitrogen balance, whereas the subject of this investigation, who excreted up to 25 per cent of his urinary N as protein N, was apparently in negative nitrogen balance.

The pathological globulins of the two patients were distinguishable by their physical properties and their content of N-terminal amino acids; the urinary proteins, by their physical constants. Nevertheless, plots of the isotopic data, which were done on a scale that allowed for the greater dose of isotope in the second case, indicate that the curves for the urinary proteins are almost identical and those for the pathological globulins are closely alike.

In conclusion it can be stated that no evidence was found for the origin of the urinary protein from serum proteins or for any precursor relationship between the two pathological proteins. The parallel decline of the N^{15} in the glycine of the urinary protein and in the urinary urea, ammonia, and total nitrogen indicates a direct interaction with the metabolic pool of nitrogen in the synthesis of Bence-Jones protein rather than the intervention of serum or tissue protein precursors.

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CHOLESTEROL METABOLISM: THE USE OF C^{14}-LABELED ACETATE TO STUDY CHOLESTEROL METABOLISM IN MAN*

By

R. G. Gould†, G. V. LeRoy, G. T. Okita, J. J. Kabara, P. Keegan†, and D. M. Bergenstal

SUMMARY

These studies were undertaken to determine the rate at which cholesterol-C^{14} appeared in the plasma of human subjects after administration of C^{14}-labeled acetate and to determine the relationship between the dose of acetate and the specific activity of the radioactive cholesterol. The specific activity-time curves for cholesterol-C^{14} activity in the plasma of dogs, which had been given single doses of the precursor acetate, have been reported.[1,2] Hellman and his associates[3] have described the buildup and decline of radioactivity in the plasma free and ester cholesterol in patients who had been fed 2-C^{14}-acetate.

The subjects of this study were patients in the Argonne Cancer Research Hospital.

Tracer doses of 100 or 200 μc of carboxyl-labeled sodium acetate (1-C^{14}-acetate) were used throughout this investigation. These were administered either orally or parenterally but for some patients, both routes were used.

The specific activity of the labeled compound was 1.0 mc/mM. The 100-μc doses contained 8.2 mg of sodium acetate with no additional carrier. A dose of 100 μc was chosen so that as many as 5 doses could be given over a period of months to a patient without exceeding the maximum permissible dose for man. Since our studies revealed that about 56 per cent of the radiocarbon was eliminated during the first 24 hours in the expired air, we assumed that after a 100-μc dose, not more than 25 μc of C^{14} would be retained in the slowly exchanging fat compartments of the body. The maximum permissible dose for such retention is given as 250 μc.[4]

Ten-ml samples of blood were obtained by venipuncture at intervals ranging from 30 minutes to several weeks after the tracer dose was given. The samples were placed in heparinized tubes for centrifugation. The plasma fraction was transferred as completely as possible and its volume recorded.

The total lipid extract was prepared by boiling the plasma sample in 20 to 25 times its volume of 1:1 alcohol-acetone in three portions and filtering it through a sintered glass funnel. Any precipitate that formed overnite in the combined total-lipid extract was filtered off and the volume was again recorded.

* This paper has been submitted for publication in the Journal of Laboratory and Clinical Medicine.

† Los Alamos Scientific Laboratory of the University of California.

An aliquot estimated to contain 0.5 to 1.0 mg of free cholesterol was analyzed by the Sperry and Webb[5] modification of the Sperry-Schoenheimer procedure. The rest of the extract was treated with a slight excess of digitonin, and the precipitated free cholesterol digitonide was assayed for C^{14}.

One of the filtrates from the free cholesterol digitonide preparations was used for esterified cholesterol analysis, and the other for assay of C^{14}.

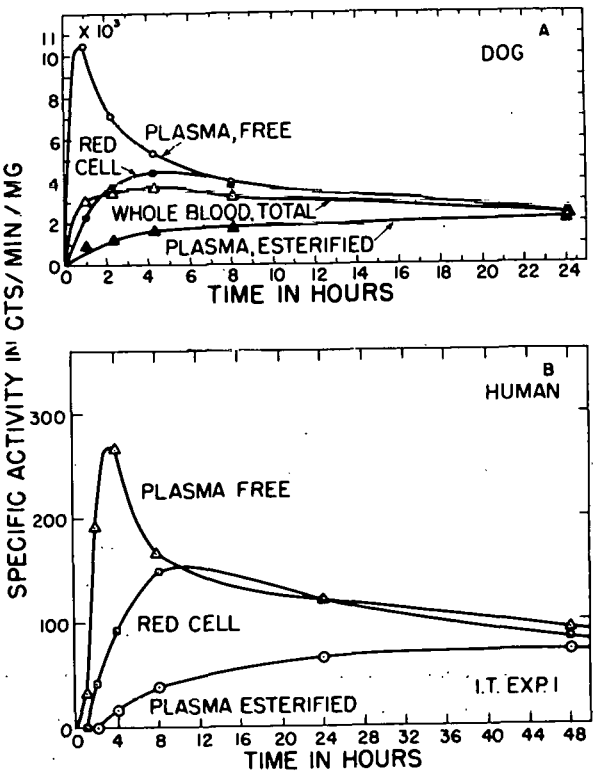

Figure 1. A. Specific activity-time curve for blood cholesterol-C^{14} in the dog after oral administration of 1-C^{14}-acetate. B. The specific activity time-curve for euthyroid patients after 100 μc of labeled acetate orally.

Red cell samples were thoroughly extracted by boiling with 20 to 25 times the red cell volume in a 3:1 mixture of alcohol ether in 3 portions and were filtered through a sintered glass funnel. The volume of the combined extract was recorded.

Aliquots that were estimated to contain 1 and 10 mg of cholesterol were taken respectively for cholesterol analysis and for C^{14} assay. No attempt was made to isolate the red cell esterified cholesterol fraction.

In some experiments, the cholesterol digitonide was counted as an infinitely thick sample by means of a thin-window Geiger counter[2]; in others, by a liquid scintillation counter.[6]

The specific activity of plasma free cholesterol reached peak values at 3 to 4 hours after oral doses of the labeled acetate. The specific activity of the ester

fraction over a 48-hour period. It was usual for these to become essentially equal and then to decrease at comparable rates beginning on the second day. The peak values for red cell free cholesterol were reached in about 8 to 12 hours. Equilibration between the plasma free and red cell fractions took place rapidly and was usually complete when the red cell value was at its peak. Typical specific activity-time curves for cholesterol-C^{14} in a euthyroid subject are shown in Figure 1. The results of a comparable study in a dog are also shown. It appears that the most useful times for obtaining blood samples are 2, 4, and 24 hours after the tracer

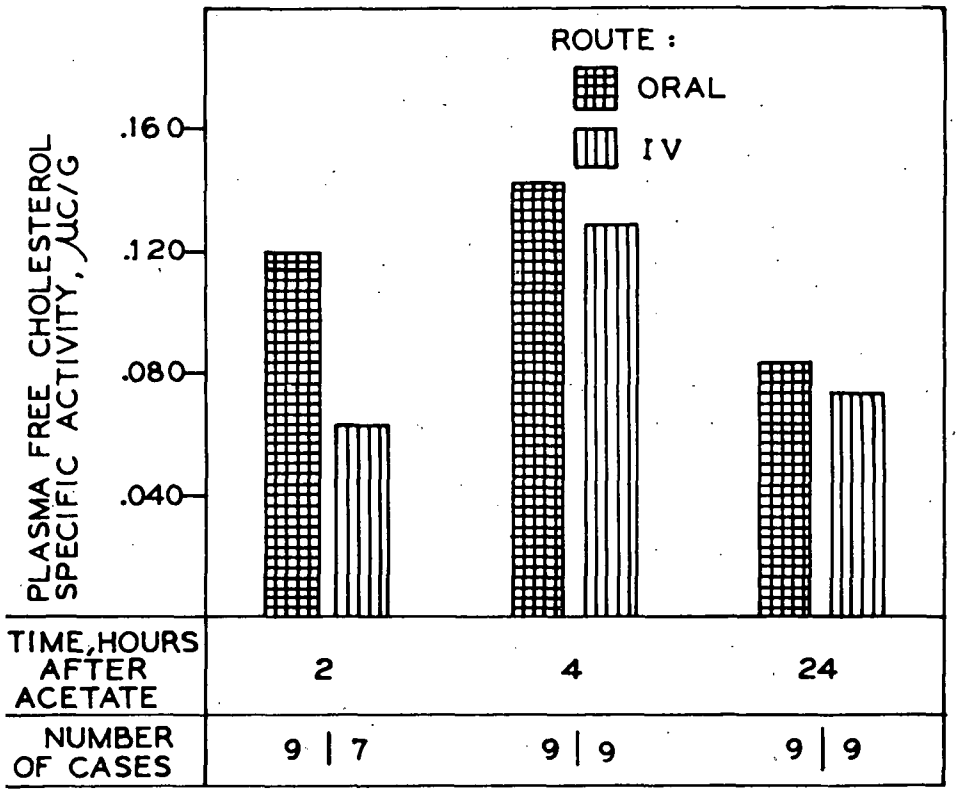

Figure 2. Comparison of specific activity of cholesterol-C^{14} at various times after oral and intravenous administration of acetate.

dose. In general, the maximum value for specific activity will be seen in the 4-hour sample. The effect of the route of administration was investigated and the results are shown in Figure 2. Except for the 2-hour sample, there is little difference. However, we prefer the intravenous route since zero time is known accurately.

When our data are compared with those from studies on dogs[2] (Figure 1), the principal difference appears to lie in the fact that the plasma free value reached a peak in about 1 hour in dogs and 3 to 4 hours in man; the red blood cell values,

at about 4 to 6 hours in the former, and 8 to 12 hours in the latter. On the basis of experiments on laboratory animals, it can be assumed that in human subjects, the liver free cholesterol-C^{14} reached a higher peak and sooner than that of the plasma free fraction. It is also likely that the two fractions reach equilibration at about the time that the plasma free value is at its peak. It is evident that the cholesterol molecules in liver and blood interchange much faster than the reported turnover times of 9 to 12 days.

In human beings, the rate of movement of cholesterol across the liver-blood boundary is calculated to be about 0.2 g per hour in each direction. This lability of individual molecules may be part of the regulatory mechanism to maintain the level of plasma cholesterol, whereby changes in plasma concentration may be reflected by changes in liver concentration resulting in alteration in the synthesis of hepatic cholesterol.

The ascending portion (3 to 4 hours) of the specific activity-time curves has clinical application in that it can be used as a relative expression of the rate of synthesis of cholesterol in liver, of movement of labeled molecules from liver to the plasma free fraction, and of movement of the plasma free fraction into the red cells and other body tissues. For practical purposes, the peak of the curve (the 4-hour value) can be considered as equivalent to the rate of synthesis.

The next part of the curve (4 to 24 or 36 hours) represents the distribution of labeled cholesterol molecules throughout the rapidly exchangeable cholesterol pool of the body and is probably not useful for clinical purposes.

Starting at 24 to 48 hours, subsequent measurements can be used to estimate the turnover time of plasma cholesterol. If the size of the rapidly exchangeable

Table 1

COUNTING DATA FROM TWO REPRESENTATIVE SUBJECTS TREATED WITH C^{14}-LABELED CHOLESTEROL

Patient	Method	Total counts	Time (min)	Weight of sample (mg)	Net cpm/mg	Background (cpm)	Efficiency (%)
12	Liquid scintillator	9330	30	1.08	146	153	58
8	Flow gas Geiger	4210	20	1.2	170	30	54

cholesterol pool is known, the turnover rate in serum equivalent units[7] may be estimated. Pool size can be approximated by determining the isotopic dilution[7,8] following the injection of labeled cholesterol. Values of approximately 30 g have been obtained for normal human beings.

The data from our experiments demonstrate the feasibility of giving repeated small doses of labeled acetate to study cholesterol synthesis in human subjects since the level of specific activity in the various blood fractions, after oral or intravenous administration, is high enough to permit reasonable counting accuracy. The counting data for two representative cases are given in Table 1. With low doses, the counting time must be extended because the ratio between background and net count is not great. One way to increase the accuracy of the count is to draw larger samples of blood so that there are larger amounts of cholesterol available. Another, is to give larger doses (200 μc) of the precursor acetate.

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CLINICAL RESEARCH USING CARBON-14 AND TRITIUM

By
G. V. LeRoy

The isotopes of hydrogen and carbon have been used extensively in biological research. A great many biochemical systems in which carbon or hydrogen are critical elements have been explored using stable or radioactive tracers. Clinical investigations with these isotopes are not nearly so numerous as they should be considering their potential value to medicine. When only the stable isotopes, C^{13} and H^2, were available, the difficulties of measurement and the large dilution factors that occur in vivo were important deterrents to large scale clinical applications. The present availability of long-lived C^{14} and H^3 should encourage exploitation of these useful nuclides. At the present time (1954) there appear to be only four procedures involving C^{14} or H^3 that can be considered routine diagnostic studies:

1. Measurements of total-body water, using T_2O or THO;(1)
2. Measurements of uric acid pool size and turnover rate in patients suspected of having gout, using C^{14}-labeled uric acid or glycine;(2)
3. Determination of specific activity-time curves for free and ester T-cholesterol of serum after oral administration of tritium-labeled cholesterol;(3) and
4. Estimation of the rate of cholesterol synthesis, using C^{14}-labeled acetate.(4)

There are three important reservations regarding the use of these radioisotopes that seem to have some bearing on the lag in clinical interest. They are the mass effect, the potential radiological hazard, and the difficulty in instrumentation. 1). In the case of tritium, the atomic mass, 3, is significantly greater than that of the abundant stable isotopes of mass 1. On theoretical grounds, this mass difference should modify physical chemical reactions to an appreciable extent, and this has been observed in concentrated in vitro systems. There is no evidence, however, for dilute systems, in vivo, using tolerable doses, that the mass effect modifies results to a significant extent. Studies of body water in man give comparable results regardless of whether the tracer is deuterium water, tritium water, or a solute such as antipyrine.

2). When the long-lived radioisotopes of carbon and hydrogen became available, concern was expressed about the potential hazard from their use in tracer studies. It was feared that incorporation of the isotopes in critical (e.g., genetic) structures would produce "hot spots" and delayed radiation injury to the subjects. It appears,

however, that the rapid rates of turnover of compounds containing principally carbon and hydrogen, and the large size of the metabolic pools, afford adequate assurance that long-term deposition is the exception. The data for calculating permissible doses of C^{14} and H^3, as given in the Bureau of Standards' Handbook 52, should be familiar to all investigators. The amounts cited are entirely adequate for most clinical procedures.

3.) Instrumentation of C^{14} and H^3 has been greatly improved as a result of the current interest in liquid scintillator systems. The efficiency of such instruments is potentially very great, and it is certain that instrument makers will improve them to the point where they are as available, and as convenient to operate, as the crystal scintillators are now. Gas counting systems, also, are steadily improving, although it is unlikely that they will ever be as convenient for routine work as are liquid scintillator systems.

At the University of Chicago and the Argonne Cancer Research Hospital, several research groups are committed to a program of applying C^{14} and H^3 to clinical research problems. Up to the present time, members of the groups have participated in the following clinical studies:

RESEARCH	NUMBER OF PATIENTS
1. Cholesterol-C^{14} synthesis after single dose of 1-C^{14}-acetate (100 μc)*	47
2. Turnover and space measurement after an intravenous dose of 4-C^{14}-cholesterol (10-30 μc)*	4
3. Turnover and space measurement after intravenous dose of 6,26-T_2-cholesterol (20-40 μc)*	6
4. Double-labeling study, using 6,26-T_2-cholesterol orally, and 1-C^{14}-acetate intravenously	6
5. Distribution and excretion study of digitoxin-C^{14}, biosynthetic (<1 μc)*	14
6. Excretion study of colchicine-C^{14}, biosynthetic (<1 μc)*	11
7. Excretion study of ring-labeled C^{14},colchicine (1-2 μc)*	3
8. Steroid hormone excretion study after 6,26-T_2-cholesterol (30-50 μc)	3
9. Steroid hormone excretion study after 4-C-^{14}-progesterone (30-50 μc)	2
10. Distribution of 6,26-T_2-cholesterol after oral doses in patients with cardiovascular disease (5 μc/day)	6
11. Distribution of C^{14}-labeled isonicotinic acid hydrazide (200-400 μc)	4
12. Protein synthesis studies after C^{14}-labeled glutamate (400 μc)	2
Total number of patients studied using C^{14} and H^3	108

* This is the average dose that was given.

Clinical research, by its very nature, presents problems to the investigator that do not arise in studies with small animals. The difficulties associated with the use of C^{14} and H^3 are no exception to this generalization, however, as the tabulation given above suggests, very satisfactory studies can be carried out, and the results are worth the effort required.

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STUDIES ON THROMBOPLASTIN GENERATION*

By

C. L. Spurling and P. D. W. King

SUMMARY

Animal tissues contain a potent thromboplastin that, under certain conditions, rapidly converts prothrombin to thrombin, and it is quite generally agreed that the precursors of a substance of similar action are present in the circulating blood.[1] The combination or the release of the precursors to produce spontaneous thromboplastin seems to be initiated by the disruption of vascular continuity. The identity, nature, and mode of action of these substances have not been established, and in view of the well-defined differences of opinion, a re-evaluation of the first stage of coagulation was undertaken.[2-10] The recent reports of Christmas disease[11] and similar disorders[12-14], and of plasma thromboplastin antecedent (PTA) deficiency[15] are additional stimuli for this research since, in these diseases, there is defective thromboplastin formation and the deficient factors are not antihemophilic globulin (AHG).

An excellent means for investigating the first stage of coagulation is provided by the thromboplastin generation test of Biggs et al.[16,17] Thus, using comparable techniques, thromboplastin generation was studied to evaluate the various opinions that are currently held and to learn the role of the Christmas factor[11] (plasma thromboplastin component (PTC)[14]).

Unless otherwise stated, all glassware and plastic with which the platelets came in contact prior to final use were coated with General Electric Dri-film #SC-87, rinsed 20 or more times with distilled water, and dried. Needles and syringes were coated with silicone oil.

For the preparation of platelet-poor plasma, normal blood was withdrawn by venipuncture, using the two-syringe technique. The blood was added to 0.1 M sodium oxalate in a plastic tube in a ratio of 9 parts blood to 1 of oxalate. The tube was capped with Parafilm, inverted gently twice, and centrifuged for 10 minutes in an angle centrifuge at 1500 rpm. Most of the platelet-rich plasma was transferred to a smaller plastic tube and centrifuged at 4000 rpm for 15 minutes. The clear supernatant was then separated from the platelet button. Plasma to be used on the day of its preparation was refrigerated at 5°C; otherwise it was frozen for storage.

* The complete text appears in the Journal of Laboratory and Clinical Medicine, 44:336 (1954).

Whole platelets were prepared by washing the platelet button twice with 2 ml of 0.85 per cent NaCl. The final button was suspended in an amount of saline equal to one-third of the original volume of platelet-rich plasma. Optimum results were obtained when the platelets were handled in a room maintained at 5°C.

With one variation, the method of preparing whole platelets was used to prepare triturated platelets. After each washing, the platelets were transferred to a glass homogenizer and triturated for several minutes. Since frozen platelet particles seem to have the same activity as fresh material, the final platelet button was frozen. When it was to be used, it was thawed and again triturated in an amount of saline that was equal to the original volume of the platelet-rich plasma.

PTC was obtained from normal human serum by adsorption on barium sulfate, subsequent elution with sodium citrate, and the adjustment of pH to destroy SPCA and prothrombin.[14] The preparation was dialyzed against 0.85 per cent saline, adjusted to the original serum volume, and frozen in 0.5-ml aliquots. No other known coagulation factors have been detected in this preparation. Sera from patients with PTC deficiency gave preparations that were inert for thromboplastin generation.

AHG was prepared from normal human barium sulfate adsorbed plasma by one-third saturation with ammonium sulfate.[4] The final preparation was adjusted to the original plasma volume, and 0.5-ml portions were frozen for storage.

Serum was obtained by placing normal human blood in a glass tube and allowing it to clot. As the blood clotted, the tube was agitated on a slow rotor and rimmed twice with an applicator stick to obtain maximal prothrombin consumption. After 1 hour, the serum was separated and incubated for 20 minutes at 37°C to allow for thrombin destruction. The serum prothrombin time was found always to be greater than 60 seconds.

A basic reaction mixture was prepared by mixing in the following order equal parts of PTC, AHG, triturated platelets, and calcium chloride (0.025 M) in a 13- by 100-mm glass tube held in a 37°C water bath. A timer was started at the moment that $CaCl_2$ was added.

Just before the basic reaction mixture was prepared, 0.1-ml aliquots of platelet-poor plasma were pipetted into 13- by 100-ml glass tubes and were then refrigerated at 5°C. One minute before use, a tube of plasma was removed from the refrigerator and placed in the water bath. At various intervals, 0.1 ml of the reaction mixture and 0.1 ml of $CaCl_2$ were added to 0.1 ml of oxalated platelet-poor plasma, and the clotting time was measured by means of a stop watch. The mixture was thus used as a source of thromboplastin in a one-stage prothrombin determination.[1]

Powerful thromboplastin was generated by the basic ingredients as evidenced by the fact that within 4 minutes it was sufficiently active to clot platelet-poor plasma in less than 10 seconds (Figure 1A). It may be assumed that no appreciable

amount of thrombin was present in the mixture because more than 60 seconds were required for it to clot a fibrinogen solution.

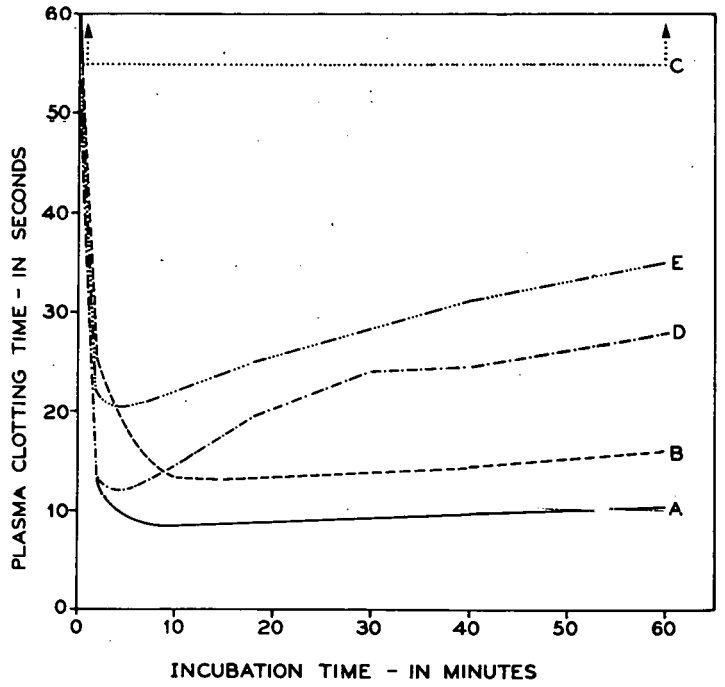

Figure 1. Activity of spontaneous thromboplastins.
A. Basic reaction mixture.
B. Basic reaction mixture without AHG.
C. Basic reaction mixture with saline replacing platelets, PTC, or calcium.
D. Basic reaction mixture with serum replacing PTC.
E. Basic reaction mixture without AHG and with serum replacing PTC.

No thromboplastin was generated in the absence of PTC, platelets, or calcium. The replacement of one of these by saline before the mixture was added to plasma resulted in a clotting time that was always greater than 55 seconds (Figure 1C). Thromboplastin was generated in the absence of AHG (Figure 1B) but the yield was considerably less and a longer time was required for its production. Thus it may be concluded that PTC, platelets, and calcium are necessary and probably sufficient for the generation of thromboplastic activity. For some reason, AHG increases the yield and rapidity of formation.

A "thromboplastin unit" was established by measuring the time that was required for multiple dilutions of the basic reaction mixture at maximum activity to clot platelet-poor plasma. Undiluted mixtures usually gave an 8.3-second clotting time; whereas 100-fold dilutions in saline, a 60-second time. Therefore, if the undiluted mixture is said to contain 100 thromboplastin units (per 0.1 ml), then a mixture that has a 60-second time has 1 thromboplastin unit, etc.

To learn whether the clotting reaction occurred in two stages, platelets, PTC, and calcium were incubated together for 5 minutes and then divided into two equal

parts. AHG was added to one of these, and the thromboplastic activity of both parts was followed. At the same time, the activity of a basic reaction mixture of PTC, platelets, AHG, and calcium was followed. The addition of AHG at zero time was found to eliminate the lag phase, and an activity curve (A) with a slope equal to the maximum slope of the basic reaction mixture (B) resulted immediately (Figure 2). The lag phase was not eliminated when mixtures of AHG, platelets, and calcium or of AHG, PTC, and calcium were incubated prior to the addition of the omitted factor. From the data it appears that PTC and platelets react together in the presence of calcium to form a weak thromboplastin and that a second reaction occurs in which AHG reacts rapidly with thromboplastin to increase its potency.

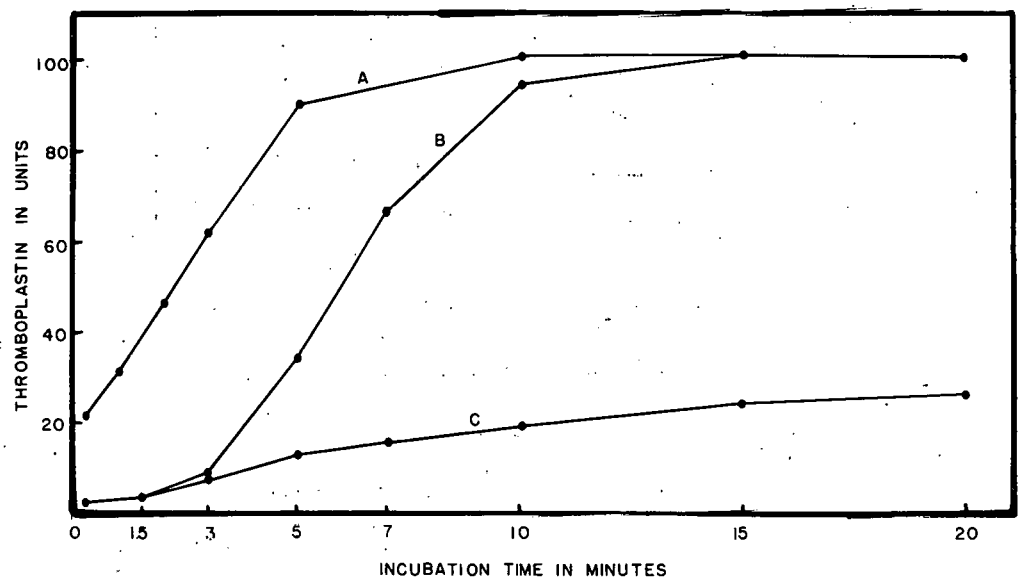

Figure 2. Elimination of lag phase.
A. Curve obtained upon addition of AHG to a mixture of PTC, platelets, and calcium after 5-minute incubation period.
B. Curve obtained at the same time as A with a basic reaction mixture.
C. Curve obtained from the incubation of PTC, platelets, and calcium.

Various concentrations of the basic ingredients were tested to provide additional information on the kinetics of the clotting mechanism. Analysis of the findings is complicated by the probability that at least two reactions are involved. The data are most suggestive of an enzymatic action of PTC on a platelet substrate. It is possible that the reaction product of PTC and platelets may combine stoichiometrically with AHG to form thromboplastin or one may serve as a cofactor to the other in prothrombin conversion.

When the quality of the surface was varied (Figure 3), the rate of production and amount of thromboplastin formed were increased by increasing surface contact with glass. Since the other factors were constant and had already been exposed to glass surfaces, it appears that this surface effect is primarily upon the platelets.

In addition, Quick[18] and Conley[6] have reported that AHG (thromboplastinogen) is "activated" by rough surfaces.

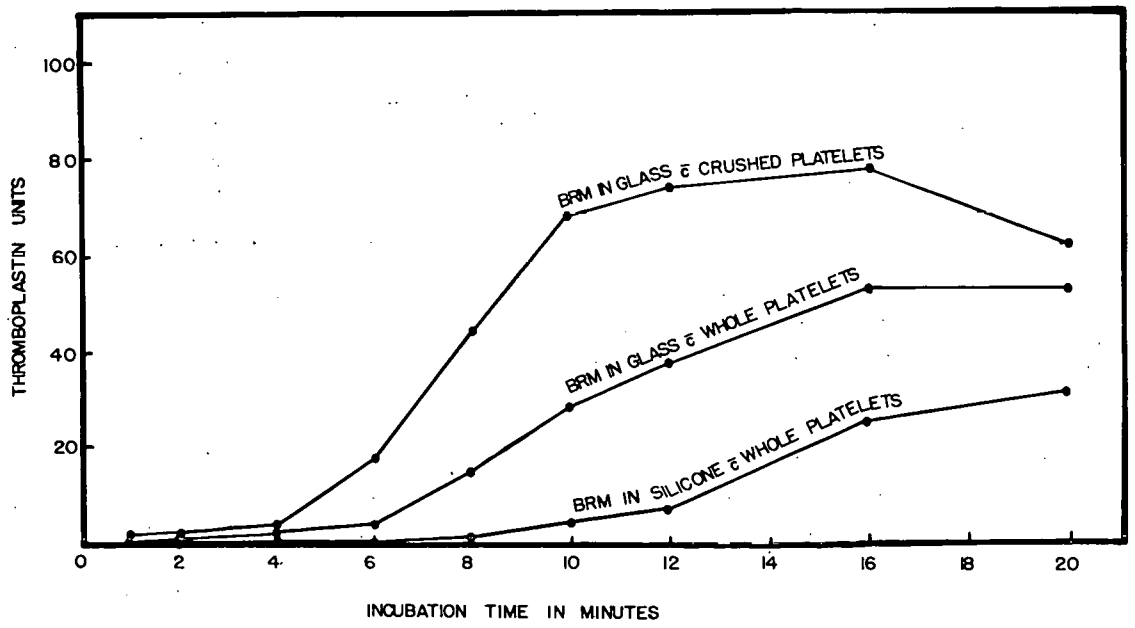

Figure 3. Effect of surface on thromboplastin generation.

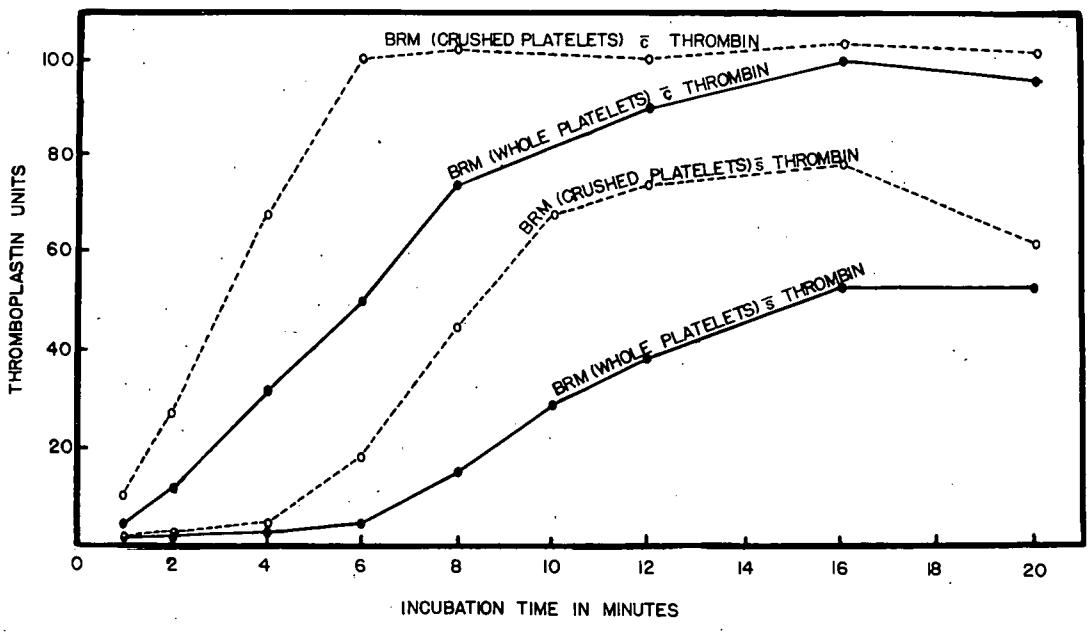

Figure 4. Effect of thrombin on thromboplastin generation.

When a small amount of thrombin was added to the basic reaction mixture, thromboplastin production was enhanced and the lag phase was eliminated (Figure 4). This was found to be true with either whole or triturated platelets, and the

enhancement was of the same magnitude. It has been reported that thrombin has a labilizing effect on platelets[19,20] and that thrombin activates AHG.[21] Desforges and Bigelow[22] have reported that thromboplastic activity develops from thrombin and platelets that are intact or disintegrated. It is not inconceivable that thrombin like PTC can react enzymatically with platelets to produce thromboplastic activity.

When serum was used as the source of PTC in the basic reaction mixture, a powerful thromboplastin evolved quickly, with or without AHG, and then disappeared steadily (Figure 1, D and E). We assumed that the effect was due to an antithromboplastin in the serum because the thromboplastin that is generated in the absence of serum is relatively stable. To test the assumption, serum was incubated with spontaneous and with commercial tissue thromboplastin (Simplastin), and with each preparation there was a rapid loss of thromboplastic activity.

In another series of experiments in which serum was used as a source of PTC and antithromboplastin, thromboplastin was generated in a reaction mixture and allowed to disappear. At that point, by re-adding singly and in combination the ingredients of the raction mixture and testing for reappearance of thromboplastic activity, evidence was obtained that a large amount of PTC remained in the reaction mixture after thromboplastin had formed (and disappeared) and that AHG and platelets were consumed in the reaction. These findings lend support to the concept that PTC acts enzymatically on a platelet substrate and that the reaction product combines with AHG to form thromboplastin. The process may be described briefly as follows: As platelets release their factor(s), PTC acts enzymatically on the factor(s) in the presence of calcium to form a weak thromboplastin. Then AHG reacts with the weak thromboplastin to produce a "complete" thromboplastin, or one serves as a cofactor for the other. Thrombin catalyzes the reaction, perhaps by its action on the platelet factor(s). Tissue thromboplastin also acts as a catalyzer by causing thrombin formation. The amount of thromboplastin is limited by antithromboplastin(s). It is quite possible that surfaces may also have effects on components other than the platelets.

It is not known whether the activities of thromboplastin generated with and without AHG differ qualitatively and quantitatively. In the thromboplastinogen activity test of Quick[23], a labile component is destroyed when tissue thromboplastin is heated, and the resulting preparation will correct the decreased prothrombin consumption of thrombocytopenic but not hemophilic blood. A concept of "complete" and "partial" thromboplastins has been presented by Langdell, Wagner, and Brinkhous[24], who have shown that the "prothrombin time" of hemophilic plasma is prolonged if partial thromboplastins are used.

The identity of the platelet cofactor of Johnson[25,26] also requires further investigation. This substance, which can be extracted from either normal or hemophilic plasma or serum, seems to act as a cofactor with platelet particles to develop thromboplastic activity.

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FACTORS AFFECTING THE SUCCESSFUL TRANSFER OF LEUKEMIA P-1534 IN MICE

By

E. L. Simmons and L. O. Jacobson*

INTRODUCTION

The use of lymphatic leukemia P-1534, to which the DBA/2 mouse is specifically susceptible, serves as a useful means of identifying the presence of functional cells in treated cell suspensions where the viability of the product is under question. In mice of other strains that normally resist leukemia P-1534, sublethal exposures to X radiation overcome the regulatory forces of the normal host and death ensues. Advantage has been taken of this finding to study the role of the spleen in resisting the successful proliferation of injected cells.

MATERIALS AND METHODS

The DBA/2 female mice were obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, as was the original group of mice inoculated with leukemia P-1534, which was transplanted weekly thereafter in our laboratory. The CF No. 1 female mice used were obtained from Carworth Farms, Inc., New City, New York.

The routine procedure used for transplanting leukemia was as follows: A leukemic mouse, which had been inoculated 7 days previously, was killed by cervical fracture. The spleen and a small wedge of liver tissue were ground with a porcelain mortar and pestle and diluted with 4 ml of sterile isotonic saline. A 0.4-ml injection was given intraperitoneally. Cell counts showed that such injections contained of the order of 30×10^6 cells.

A G. E. Maxitron X-ray machine, operated at 250 kvp and 30 ma, with 1/4-mm copper and 1-mm Al filtration, was the source of radiation. Leukemic mice were irradiated in pairs, unanesthetized, in corked celluloid tubes. The target to center of mouse distance was 36 cm, and the exposure rate in air averaged 300 r per min. Operated mice, which were to be injected with leukemic cells, were anesthetized with Nembutal^R and irradiated in a 6-inch circle on a Lucite board. The target distance was 57 cm, and average exposure in air was 70 r per min.

* With the technical assistance of Joan Barr.

EXPERIMENTAL OBSERVATIONS

Use of leukemia to estimate cell count: Lymphatic leukemia P-1534 may be transferred readily in DBA/2 mice by intraperitoneal injections of leukemic cell suspensions of macerated spleens. The results of such injections are always fatal, although the length of time required for death varies with the number of cells injected. The day on which 50 per cent mortality occurs is plotted against the number of cells injected (Figure 1). This is seen to vary from 12 days, when a massive injection of 30×10^6 cells is employed, to 21 days when only 25,000 cells are given.

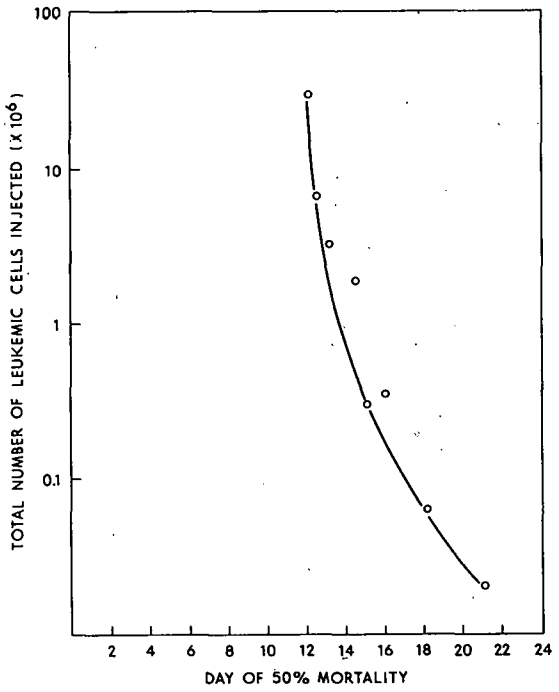

Figure 1. Relationship of number of leukemic cells injected into DBA/2 mice to day of 50 per cent mortality.

For other studies in progress in our laboratory, it is often necessary to destroy the spleen cells to obtain cell products. Treatments such as homogenization, incubation with enzymes, decompression, and use of sonic vibrations are among the methods employed. Because of the cellular debris that results, it is often impossible, by visual cell counts, to determine whether functional cells still remain, and their number. A means of estimating the number of leukemic cells that survive any of the above-mentioned treatments may be estimated by injecting the treated cell suspension into intact DBA/2 mice and then observing the subsequent mortality.

The use of this test procedure assumes, of course, that the intact cell is the causal agent in the transmission of leukemia in the mouse, rather than a virus, cell fragment, or cell product. As Furth[1] points out, this view is now generally accepted. In our laboratory all attempts to induce leukemia with suspensions from

which the leukemic cells have been removed or have been killed have been unsuccessful in the production of the disease. The treatments that have been used thus far include repeated rapid freezing and thawing with dry ice, lyophylization, injection of the supernatant fluid following centrifugation on the ultracentrifuge (10,300 G), less intense centrifugation on an Adams Safety-Head centrifuge at 1855 G, passage through a Seitz filter, and subjection of the leukemic cells to sonic vibrations for 5 minutes or more.

Table 1

SURVIVAL IN DBA/2 MICE FOLLOWING INJECTION OF SPLEEN CELLS FROM IRRADIATED LEUKEMIC MICE

	Exposure to leukemic donor mice (r)							
	No X ray	1000	2000	3000	4000	5000	6000	8000
Treatment to recipient	Survival of recipient mice (%)							
No X radiation	0	0	71	100	100	100	100	100
500 r	0	0	31	50	79	75	75	87

Sensitivity of the leukemic cell to X irradiation: DBA/2 mice that had been inoculated with leukemia 7 days previously were given a graded series of total-body exposures to X rays ranging from 1000 to 8000 r. Four hours later, they were sacrificed, and suspensions of spleen cells were prepared for passage to recipient DBA/2 mice that had either been given no treatment whatsoever, or 500 r total-body irradiation. The results in Table 1 show that following 3000 r the leukemic cell fails to take in nonirradiated mice. However, when the recipients were weakened with 500 r, some fatalities continued to appear even with higher doses to the leukemic cell.

Transfer of leukemia to CF No. 1 mice: Leukemia P-1534 is strain specific to the DBA/2. Injections of leukemic cells into normal, nonirradiated mice of other strains does not result in death. If mice of other strains are X-irradiated, however, inoculation with leukemic cell suspensions proves fatal, the extent of the mortality varying with the dosage of radiation that is administered. This phenomenon has been well known since the work of Krebs in 1930.[2] An excellent review may be found in "Leukaemia in Animals" by Engelbreth-Holm.[3]

Kaplan has shown[4,5] that a striking inhibition of lymphoid tumor induction in irradiated C-57 black mice can be brought about by injections of bone marrow cell suspensions, and Lorenz and his associates have also shown[6] that spleen-shielding inhibits leukemogenesis in this strain. In order to determine whether protection of the spleen during irradiation of a different strain would subsequently afford protection against inoculation with DBA/2 leukemic cells, groups of CF No. 1 mice were given graded exposures from 100 to 600 r, with and without protection of the exteriorized spleen. As shown in Table 2, spleen-shielding afforded protection against leukemia. Additional groups of recipient CF No. 1 mice were injected with leukemic spleen suspensions from DBA/2 mice that had been given 3000 r. When these donor mice were given 3000 r before injection, spleen-shielding of the recipients was found to afford complete protection so that the CF No. 1 mice, in effect, reacted in the same fashion as normal mice.

Table 2

SURVIVAL IN CF NO. 1 MICE INOCULATED WITH LYMPHATIC LEUKEMIA P-1534 FROM DBA/2 MICE

Exposure to CF No. 1 recipients (r)	Exposure to leukemic donors (DBA/2) (r)			
	No X radiation		3000	
	Survival (%)		Survival (%)	
	Total-body	Spleen-shielded	Total-body	Spleen-shielded
0	100	100	100	100
100	100	100	100	100
300	67	100	100	100
400	0	100	25	100
500	0	83	25	100
600	0	83	13	100

Preliminary studies show that leg shielding is also efficacious in preventing the take of leukemia. Since Jacobson et al.[7] have shown previously that injections of spleen, liver, or bone marrow cells to the CF No. 1 mouse prevent acute radia-

tion death, studies are now in progress to determine whether such treatments protect when the irradiated mice are inoculated with leukemia.

Even though inoculation of the otherwise untreated CF No. 1 mouse with leukemic cells does not kill, the reaction of the body in resisting the invading cells may be observed in the marked and maintained increase in spleen size following a single injection. As shown in Figure 2, spleen hypertrophy reaches a peak 5 days after injection. Even 4 months after the injection, the size of the spleen is twice that of control animals.

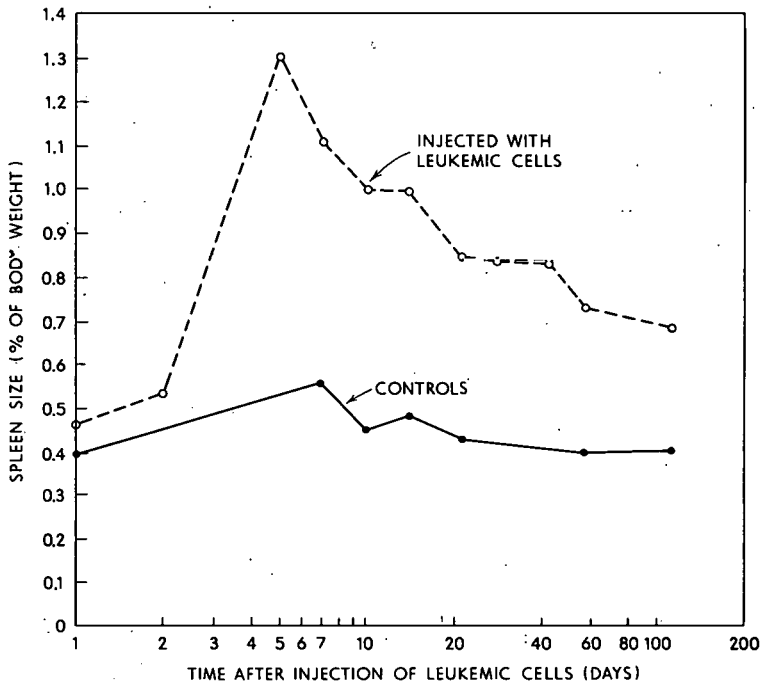

Figure 2. Effect of leukemia from DBA/2 mice on spleen size of CF No. 1 mice.

Finally, since the DBA/2 mouse is extremely sensitive to the presence of cells of leukemia P-1534, tests were performed to learn whether inoculated CF No. 1 mice can transmit the disease back to normal DBA/2 mice. Inoculations were given to 3 groups of CF No. 1 mice: 1) nonirradiated, 2) 500 r total-body X irradiation, and 3) spleen-shielded during 500 r. At daily intervals, mice from these groups were sacrificed. Their spleens were macerated and were injected into DBA/2 mice. The capacity of such spleens to transmit leukemia indicates either the continued presence of the original DBA/2 leukemia cells that migrated into them, or that some change has occurred in the splenic tissue that is then able to induce leukemia in the receptive DBA/2 mouse.

When leukemic cells were injected into normal CF No. 1 mice, subsequent injections of suspensions made from their spleens to DBA/2 mice resulted in deaths during the first 4 days only. When CF No. 1 mice were given 500 r before the

leukemic cells were injected, passage of their ground spleens to DBA/2 mice resulted in death. This continued as long as donor mice were available to permit such transfers. This has been observed as late as 17 days. The terminal picture following the injection of leukemic cells is one of development of secondary infections. Thus, when spleen preparations from the inoculated irradiated CF No. 1 donor mice were being passed back to untreated DBA/2 mice, control injections were also given to nonirradiated CF No. 1 mice to test whether the deaths observed were not due to the causal agents of secondary infections. None of the controls died from secondary infections.

When the CF No. 1 mouse is given 500 r while the spleen is protected, inoculation with leukemia followed by subsequent passage of the spleen suspensions to DBA/2 mice resulted in deaths during the first 4 days, but not beyond. This is the same pattern observed in the nonirradiated mice, and again shows that the capacity of a strain other than the DBA/2 to resist leukemia P-1534 resides in the spleen, and that the effect of irradiation in causing such strains to become susceptible to leukemia is due to damage to the spleen.

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THE EFFECT OF HYPOPHYSECTOMY AND GROWTH HORMONE ON RADIOACTIVE SULFUR UPTAKE IN CARTILAGE*

By

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SUMMARY

It has been shown that radiosulfur, S^{35}, is fixed readily in connective tissue[1-5] of the human being, rat, and mouse. This is especially true for cartilage, in which the rate of fixation is many times greater than that of the other tissues. That the S^{35} is incorporated into chondroitin sulfate, the essential polysaccharide, was demonstrated by Dziewiatkowski[6] and Boström.[7]

In view of the influence of the pituitary on the growth of cartilage[8], knowledge of the effects of the growth hormone on the metabolism of cartilage is essential. Thus, this investigation was undertaken to examine the uptake of injected S^{35} into the xiphoid and costal cartilages and the tibial cap of normal and hypophysectomized rats with and without stimulation by growth hormone.

Young female rats, hypophysectomized and normal, were used as the test animals. Hypophysectomy was performed when the animals were 22 days old. Litter mate rats served as the controls. Growth hormone was given, which contained thyrotrophin (TSH) at the level of 0.04 ± 0.02 USP units per mg. Since growth hormone‡ was used at the level of 100 μg per dose, it was contaminated with 0.004 USP units of TSH. The thyrotrophin** that was administered to the animals contained no appreciable impurity, and 3 mg were equivalent to 1 USP unit of TSH. Hence, as a control for growth hormone, 0.004 USP units of 0.012 mg were given per dose. The controls received comparable volumes of physiologic saline.

Ten μc of S^{35}, as sodium sulfate, were given intraperitoneally each day for 8 days to both normal and hypophysectomized rats to test the capacity of cartilage to metabolize this material. Simultaneously, some of the rats from both groups were given 100-μc doses of growth hormone. The animals were sacrificed by etherization on the day after the last injection, and representative tissues were removed, ashed, and sulfur-precipitated with $BaSO_4$. Radioactivity was measured by means of a windowless gas-flow counter using helium or Q gas. The reliability of sampling is shown in Table 1.

* The complete text has been submitted for publication in Endocrinology.

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‡ Armour, lot number R491132.

** Armour, lot number J11002.

Table 1

RELIABILITY OF SAMPLING

(As illustrated by the weights and counts of the right and left tibial caps from representative animals)*

Group	Weight*		Tibial cap	
	Body (g)	Tibial caps (mg)	cpm† of S^{35}	cpm/100 mg
H	59	38.3	745	1900
		37.0	705	1900
	58	36.4	800	2180
		33.3	600	1770
H + GH	70	42.7	2020	4740
		41.2	1965	4780
	73	37.0	1260	3420
		38.2	1340	3520
N	110	54.5	760	1390
		54.2	725	1340
	107	60.2	950	1600
		56.7	900	1600
N + GH	119	67.0	830	1250
		68.5	960	1400
	119	57.7	740	1300
		58.2	720	1250

H = hypophysectomized rat receiving S^{35}
H+GH = hypophysectomized rat receiving S^{35} plus growth hormone
N = normal rat receiving S^{35}
N+GH = normal rat receiving S^{35} plus growth hormone

* Weight of individual animals at end of experiment.

† cpm = counts per minute.

Table 2

EFFECT OF HYPOPHYSECTOMY AND GROWTH HORMONE ON CONNECTIVE TISSUES OF THE RAT

Tissue	Group 1				Group 2			
	H	H+GH	N	N+GH	H	H+GH	N	N+GH
	Ave. cpm/100 mg							
Xiphoid cartilage	(3) 1975	(4) 4200	(3) 1420	(2) 1250	(3) 3190	(5) 4650	(4) 3000	(3) 1945
Costal cartilage	(8) 1000	(8) 3275	(6) 1920	(4) 1910	(10) 1075	(13) 4250	(10) 3330	(16) 2700
Tibial cap	(8) 1500	(8) 2600	(6) 865	(4) 890	(8) 1980	(9) 3750	(6) 1275	(12) 1350
Meniscus	(8) 685	(8) 1460	(6) 465	(3) 440	-	-	-	-
Ear	-	-	-	-	(1) 390	(3) 510	(2) 260	(3) 260
Eye coats	-	-	-	-	(1) 530	(4) 815	(3) 370	(1) 210
Lymph node	(4) 180	(4) 230	(2) 90	(2) 55	(3) 195	(1) 185	(1) 255	(2) 0
Pituitary	-	-	(2) 190	(2) 175	-	-	-	-
Ovary	-	-	(2) 95	(2) 125	-	-	-	-
Skeletal muscle	(1) 50	(1) 80	(1) 50	(1) 15	-	(1) 75	(1) 50	(1) 25
Cardiac muscle	-	-	-	-	(1) 100	(1) 140	(1) 40	(1) 30

Numbers in parentheses designate number of samples.

Note: H = hypophysectomized rat
H+GH = hypophysectomized + growth hormone
N = normal rat
N+GH = normal + growth hormone

Table 3

S^{35} FIXATION IN CARTILAGE OF HYPOPHYSECTOMIZED RATS AGED 46 AND 26 DAYS

Tissue	cpm/100 mg	
	46 days*	26 days†
Costal cartilage	7080	3330
Xiphoid cartilage	10,000	10,000
Tibial cap	5000	5200

* Average of 4 animals.

† Average of 3 animals.

Table 4

WEIGHT GAIN AND S^{35} FIXATION IN RATS TREATED WITH GROWTH HORMONE

Tissue	H	H+GH	N	N+GH
	cpm/100 mg			
Costal cartilage	650	3200	1750	1450
Xiphoid cartilage	1550	5840	1600	1100
Tibial cap	1150	2600	850	650
	g			
Weight gain*	5	1	27	21

H = hypophysectomized rat
H+GH = hypophysectomized + growth hormone
N = normal rat
N+GH = normal + growth hormone

* One animal in each group.

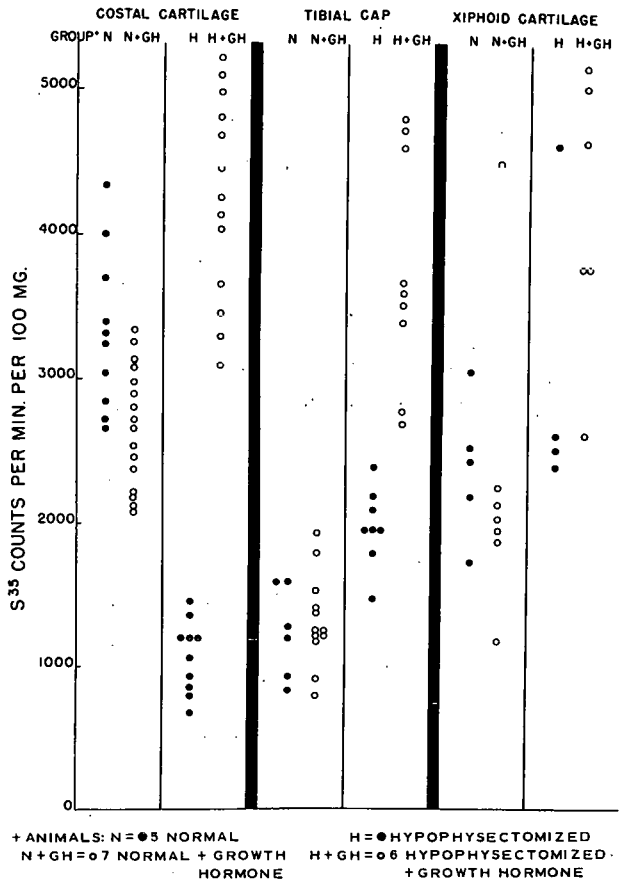

Figure 1. Effect of growth hormone on S^{35} fixation in cartilage of normal and hypophysectomized rats.

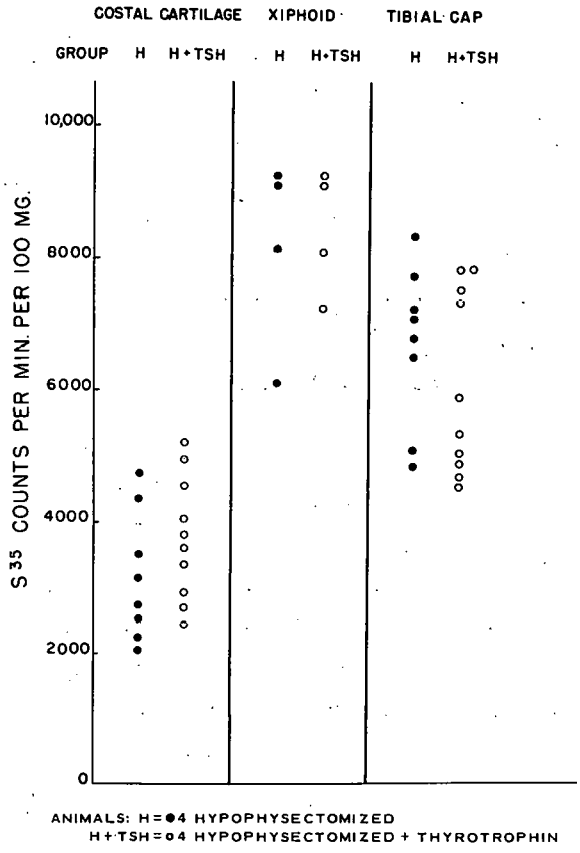

Figure 2. Effect of thyrotrophin on S^{35} fixation in cartilage of hypophysectomized rats.

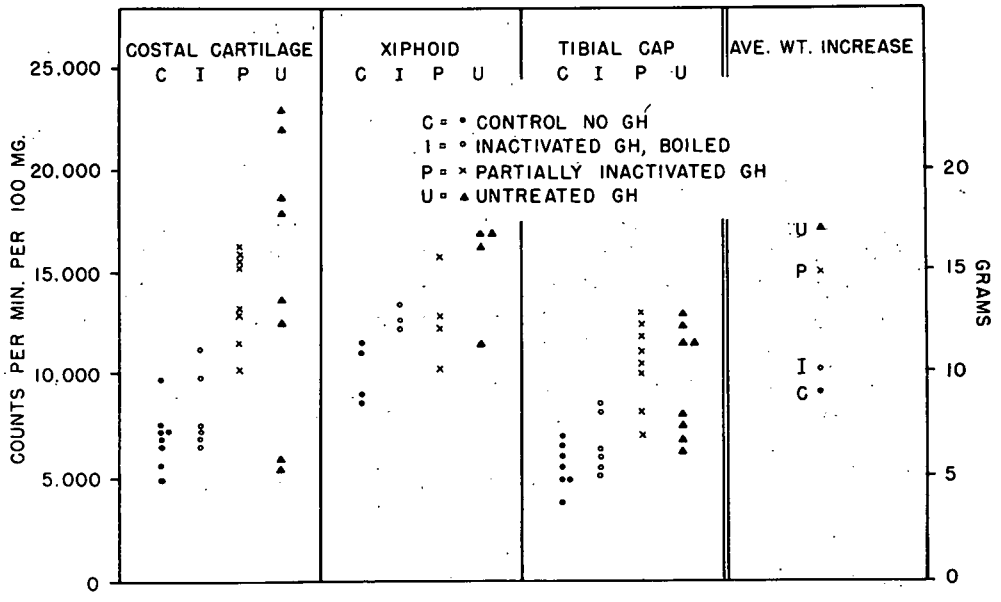

Figure 3. S^{35} fixation and weight increase in hypophysectomized rats receiving growth hormone, inactivated, and partially inactivated growth hormone.

Hypophysectomized rats that were given only S^{35} had 33 to 50 per cent less fixation of the isotope in the costal cartilage than nonhypophysectomized animals (Figure 1 and Table 2). The capacity of the cartilage of hypophysectomized rats to fix sulfur, although reduced, was still significant and was found to be present in animals that had been operated upon 24 days prior to administration of S^{35} (Table 3).

There was an increase in the fixation of S^{35} in the costal, xiphoid, and tibial cap cartilages of hypophysectomized rats that were also given growth hormone. In fact, the costal cartilage uptake was increased 3- to 4-fold by the administration of 100 μc per day for 8 days. Growth hormone also caused greater S^{35} fixation in hypophysectomized rats than in normal rats or normal rats that were given the hormone. Factors inimical to weight gain did not prevent stimulation of S^{35} fixation (Table 4).

When growth hormone was given to normal rats in doses that were comparable to those that caused increased S^{35} fixtion in hypophysectomized rats, no increased uptake was observed. In fact, the data suggest a reduction, which may be explained by an alteration in the sensitivity of the mechanism involved in the uptake by the cartilage of the hypophysectomized rat, or, perhaps, a hormone, which is present in the normal animal, has an inhibitory effect upon the action of the growth hormone. No significant effects on fixation were seen in normal or hypophysectomized rats that were given thyrotrophin in amounts that were equivalent to the quantities that contaminated the doses of growth hormone (Figure 2).

Partial inactivation of growth hormone produced partial stimulation of S^{35} fixation and weight gain. Complete inactivation by heat abolished the stimulatory effect of growth hormone with respect to the fixation of S^{35} and increase in weight (Figure 3).

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THE PREPARATION, LOCALIZATION, AND EFFECTS OF ANTITUMOR ANTIBODIES LABELED WITH I^{131}. PRELIMINARY REPORT.*

By

R. W. Wissler, P. A. Barker, M. H. Flax, M. La Via, and D. W. Talmage

INTRODUCTION

The possibility of healing a malignant neoplasm with a specific immune serum has stimulated much thought and experimental work since immunologic methods first proved effective in controlling infectious diseases.(1-4)

Previous work has demonstrated that neoplastic cells frequently possess sufficient immunologic antigenicity and specificity so that cellular or humoral antibodies to tumor cells may frequently be fabricated by the animal bearing the tumor in such potency as to prevent the "take" of the reinoculated tumor.(1-4) Furthermore, a specific type of local inflammatory response has been described at the edge of some tumors which may indicate the development of spontaneous resistance to the neoplastic cells.(5,6)

Antibodies to neoplastic tissue in one species can be produced in another species. These antibodies can be demonstrated serologically(7-9) and under some conditions have been shown to alter the growth or appearance of neoplastic cells in vitro.(10-12) There is little evidence, however, that the growth of well-established neoplastic cells in vivo can be altered by immunological methods.

More recently, it has been shown that a specific heterologous antitumor serum can be labeled with I^{131} and still show some degree of in vivo specificity.(13) This suggests that an antitumor serum might become effective in retarding neoplastic growth if it were coupled with a radioactive substance in carcinocidal quantities.

With the ultimate aim of putting this hypothesis to the experimental test, the experiments that are described in this report were undertaken to study, both in vitro and in vivo, the localization and effects of antitumor sera in a number of transplantable tumors.

An investigation of this problem seems particularly timely, since progress in immunological methods makes it appear likely that: 1) a potent antitumor serum can be produced by using certain adjuvant procedures; 2) the potency and specificity of

* Supported in part by Institutional Grant No. 14E from the American Cancer Society.

antitumor antisera can be measured more accurately than previously by using newly developed procedures; 3) the fate of antitumor sera can be traced in vivo with the aid of radioactive labels; and 4) these radioactive substances might be used to localize therapeutic amounts of radioactivity in a neoplasm.

METHODS AND MATERIALS

Neoplasms and their maintenance: Three transplantable tumors have been employed in this study, the Flexner-Jobling rat tumor, the Jensen rat sarcoma, and the Ehrlich mouse ascites tumor. The two rat tumors were maintained by weekly intramuscular transplants of 0.2 ml of 1:2 saline homogenate of the neoplastic tissue injected into the anterior thigh muscles of recipient Sprague-Dawley rats that weighed about 200 g. The Ehrlich's ascites tumor was preserved by weekly passage of 0.1 ml of a 7-day growth of the ascites tumor that was injected intraperitoneally into CF No. 1 mice, weighing about 25 g.

Preparation of tumor for immunization and antibody combining capacity determinations: The rat tumors were removed aseptically one week after inoculation, and the grossly bloodless areas of tumor were dissected free, frozen quickly, and placed in sterile petri dishes over P_2O_5 in a vacuum until used. The mouse tumor used for immunization was a fresh 5 per cent tumor suspension, while washed lyophilized tumor was used for the in vitro experiments.

Production of antitumor serum: Antisera to each of the rat tumors were produced by injecting normal adult rabbits with a suspension of lyophilized, grossly bloodless tumor, which corresponded to a 5 per cent suspension of fresh tumor in saline. The Ehrlich's ascites tumor was used as a 5 per cent suspension of washed tumor cells and was never lyophilized. It was prepared once a week on the day of the first injection for the week. The rabbits were injected on 3 consecutive days of each week by 5 portals simultaneously, i.e., 1-ml portions were given intravenously, intramuscularly, intraperitoneally, subcutaneously, and intradermally. The intradermal injections were given in 5 different areas, 0.2 ml to an area, each day. The injections were repeated in this manner for 3 consecutive weeks. In some instances, the first injection of the first week was given intramuscularly in Freund's adjuvant. The antiserum was collected by withdrawing blood, using sterile precautions, from the hearts of the immunized rabbits on the 4th, 5th, and 6th weeks. Control serum from noninjected animals was obtained simultaneously. The antiserum was lyophilized and stored at $-20^{\circ}C$ in the dry form over P_2O_5 in a vacuum.

Preparation of gamma globulin: The γ-globulin fraction of the sera was separated by the method of Nichol and Deutch[14] at the time it was needed for further study.

Iodination of the antitumor serum: Iodine-131 was attached to the antitumor γglobulin by the method of Talmage et al.[15] In many experiments, this consisted of one or more iodinations in which 0.08 ml of 0.01 M KI was added to 10 mc of I^{131} in a glass vial standing in a lead pot that was, in turn, housed in a rapid-flow isotope hood behind a lead barricade. Most of the manipulations were carried out using long-handled forceps or remote control pipettes. Sufficient 0.5 M HCl was then added to acidify the solution (1 to 2 drops), one drop of 0.1 M $NaNO_2$ was added, and the vial was quickly stoppered to avoid escape of the volatile I_2^{131}, which was apparent as a slight yellow tint to the solution. The suitable γglobulin was added immediately as 0.5 ml containing 5 mg of protein in carbonate buffer (pH 9.7). The reaction vial was restoppered, agitated slightly and allowed to stand for 5 minutes after which time 1 drop of 1 M KI was added. After another 5 minutes the iodinated globulin was transferred dropwise to a 130- x 15-mm column of amberlite (Fisher IR-4B(OH)). This was followed by the dropwise addition of distilled water in 1-ml quantities. The fluid emerging from the column was collected in 1-ml amounts in glass tubes. Each tube was surveyed separately, and the washes containing the greatest amounts of radioactivity were combined (usually 4-ml yield). Suitable small samples of the iodination solution were removed before and after passage through the amberlite column to assay the degree of protein-binding by I^{131}. This was done by diluting the samples to 0.5 ml with distilled water, adding 0.5 ml of a 1:10 dilution of normal rabbit serum and 1 ml of 20 per cent trichloracetic acid, mixing well, and centrifuging. After washing the precipitates with 1:10 dilution of 20 per cent trichloracetic acid, the supernatants and precipitates were counted, and the per cent of original radioactivity in the protein precipitate was determined.

Elution of the labeled γglobulin: In order to further increase the specificity of the iodinated antitumor γglobulin, specific elution methods similar to those described by Talmage et al.[16] for purified antigen-antibody systems were studied and adapted to our conditions. In a typical elution, 25 mg of the appropriate lyophilized tumor was mixed with approximately 10 ml of the iodinated amberlite-treated antitumor γ globulin, and the mixture was shaken in the cold (4°C) for 3 hours. Following this, 1 ml of normal rabbit serum was added to the mixture that was then incubated for 5 minutes with shaking at 42°C. The suspension was then cooled in an ice bath, and 10 ml of chilled physiological saline added. The mixture was centrifuged at about 3000 rpm in a refrigerated centrifuge (4°C) for about 15 minutes. The supernatant solution was decanted, a second 1-ml volume of normal rabbit serum was added to the precipitate, and the incubation and centrifugation were repeated. To this washed precipitate was added 1 ml of appropriate whole antiserum along with 9 ml of physiological saline, and the mixture was shaken for 45 minutes at

42°C. The suspension was then centriguted, and the resulting eluted supernatant was stored in the cold until needed.

In vitro evaluation of the antitumor sera: Complement fixation[17] and/or the in vitro combination of the iodinated antitumor γ globulin with the powdered whole lyophilized tumor[16] were used to evaluate the potency and specificity of each antiserum before it was injected into the animals. Suitable controls for antibody (normal rabbit γ globulin) and antigen (various lyophilized normal tissues) were employed. Although the method of performing the latter test has varied to some extent, in general, it has consisted of placing either 10, 50, or 80 mg of lyophilized, pulverized tumor or control antigen in a test tube to which was added 0.5 ml of normal rabbit serum. After the tumor was thoroughly suspended in the normal serum, 0.5 ml of a suitable dilution (for counting) of the iodinated antibody γ globulin to be tested was added to each tube, and the tubes were shaken in the cold (4°C) for 1 hour. They were then centrifuged in the cold for 20 minutes, and 1.0 ml of a 1:10 dilution of normal rabbit serum was added to each precipitate, and the tubes were again shaken (20 minutes) and centrifuted in the cold.* The supernatants and the final precipitates were counted separately, and the results were expressed in terms of percentage uptake by the various antigens.

In vivo experiments: Eluted iodinated γ globulins, both normal and antitumor, have been given to animals bearing tumors of comparable size. In most instances the thyroids have been "blocked" by the prior feeding of Lugol's solution. The animals were sacrificed at varying intervals after injection. In general, the anti-Jensen and anti-Flexner-Jobling preparations were given intravenously and the anti-Ehrlich's ascites tumor preparations, intraperitoneally. At the time of sacrifice, the animals were anesthetized with ether and the thorax and abdomen were opened. A little heparin was introduced into the right ventricle, and a blood sample removed to determine radioactivity. (The ascites tumor was collected first in heparin-containing tubes.) The animals were then perfused with about 10 times their normal blood volume, using chilled saline at approximately 100 cm of gravity pressure, and representative organs and tissues were removed for weighing and counting.

Iodine-131 and counting procedures: The iodine was obtained from the Oak Ridge National Laboratory as carrier-free I^{131} and was used for iodination without further processing. It usually had a specific activity of from 8 to 20 mc per ml at the time of iodination, the higher specific activity samples, giving the more efficient iodination. Materials to be counted were placed in 8-ml plastic tubes, a maximum of 2 ml being added per tube, and were counted in a well-type scintillation counter using suitable iodine-131 standards for each experiment.

* In some tests the duration of this washing procedure was varied and the effects of this variation will be considered in experimental results.

EXPERIMENTAL RESULTS

Preliminary experiments: Various methods of immunization of rabbits against rat tissues were explored in two preliminary studies in which small groups of rabbits were injected by different routes and the potency of their antisera evaluated by complement fixation. These experiments demonstrated, among other things, that sera with a maximum complement fixing capacity were obtained 1 week after 3 consecutive weeks of immunization and that no further rise, and indeed some fall in the higher titer sera, was observed when two additional courses of immunization were given. The highest titers at the 4-week bleeding were observed in the sera of animals receiving the antigen by multiple portal injections (see Methods and Materials). These averaged 2 to 3 times the quantity of complement fixing antibody as

Table 1

COMPARISON OF ANTIBODY YIELDS FOLLOWING DIFFERENT COURSES OF IMMUNIZATION

Intraperitoneal with staphylococcus toxin	Intraperitoneal	Intramuscular $Al(OH)_3$ depots	Intramuscular Freund's adjuvant depots	Multiple portal	
4.28*	3.12	4.63	5.99	8.75	5.50
3.35	2.78	1.59	7.07	8.92	6.92
4.31	3.45	3.06	3.58	10.35	12.00
3.58	4.86	2.87	5.46	4.98	6.06
3.79	2.93	5.10	4.63	8.00	6.42
Ave. 3.86	3.43	3.45	5.35	7.79	

* All results expressed as 50 per cent complement fixation units using sera obtained 1 week after completion of 3 weeks of immunization.

compared to sera from animals receiving the same quantity of antigen with or without staphylococcus toxin[18], by the intraperitoneal route only[19], by intramuscular aluminum hydroxide depots[20], or by intramuscular Freund's adjuvant depots[21] (Table 1). The animals immunized with the antigen mixed with Freund's adjuvant yielded serum with the next highest titer. Since it seemed likely, theoretically, that the multiple portal schedule together with Freund's adjuvant would act in an additive fashion to stimulate greater antibody production than either one alone, the two types of immunization procedures have been combined in subsequent experiments. A comparison of the complement fixation titers for anti-Flexner-Jobling sera produced by

the same amount of antigen given by intraperitoneal injection alone, multiple portal injection alone, and in Freund's adjuvant followed by multiple portal injection revealed that the number of 50 per cent hemolytic units fixed by sera obtained by each of the 3 methods, respectively, were 2.2, 8.5, and 11.0. That potent antiserum was produced is evidenced not only by the comparative complement fixation results but also by the fact that, in a large number of γ-globulin preparations from these antisera, the immune sera have averaged 70 to 100 mg of γ globulin per 10 ml, while the normal rabbit sera have averaged about 40 mg (Table 2).

Table 2

YIELDS OF γ GLOBULIN PRECIPITATIONS
(in mg/10 ml of serum)

Anti-Jensen	Anti-Flexner-Jobling	Anti-Ehrlich's ascites	Normal
76.3	70.3	78.2	32.7
62.1	100.9	93.1	39.6
98.5	58.8	100.3	36.9
	60.4	115.8	36.8
	64.5	104.2	42.2
		106.6	41.9
		109.0	40.3
			46.0
			48.3
Ave. 79.0	71.0	101.0	40.5

In vitro studies using direct measurement of combining of iodinated antitumor γ globulin with lyophilized tumor: Although complement fixation has yielded considerable valuable data concerning the potency and specificity of antitissue antibodies, it is a cumbersome procedure that is difficult to apply accurately to a very complex antigen-antibody system such as this. Since the ultimate goal was to obtain antitumor sera that would combine to as great a degree as possible with tumor tissue in vivo, it seemed reasonable to try to use its combining power in vitro as a guide to the potency and specificity of the antitumor sera that were being studied.

Preliminary studies with γ globulin prepared from antirat kidney serum, anti-Jensen sarcoma serum, and normal rabbit serum showed that it was possible to obtain a measure of specificity and potency with this procedure (Figure 1). A study

of some of the characteristics of this reaction with noneluted antirat kidney serum (developed in rabbit) showed that the reaction was relatively constant, within the limits employed, when either the amount of antibody γ globulin or the time of the reaction was varied. But when the amount of antigen was varied with the quantity of antibody and raction time held constant, the per cent of radioactivity bound to the antigen steadily increased at a rapid rate until 5 to 10 mg of antigen were present and then increased at a slower rate (Figure 2). Subsequent study of this phenomenon using anti-Jensen γ globulin has indicated that a part of this increase

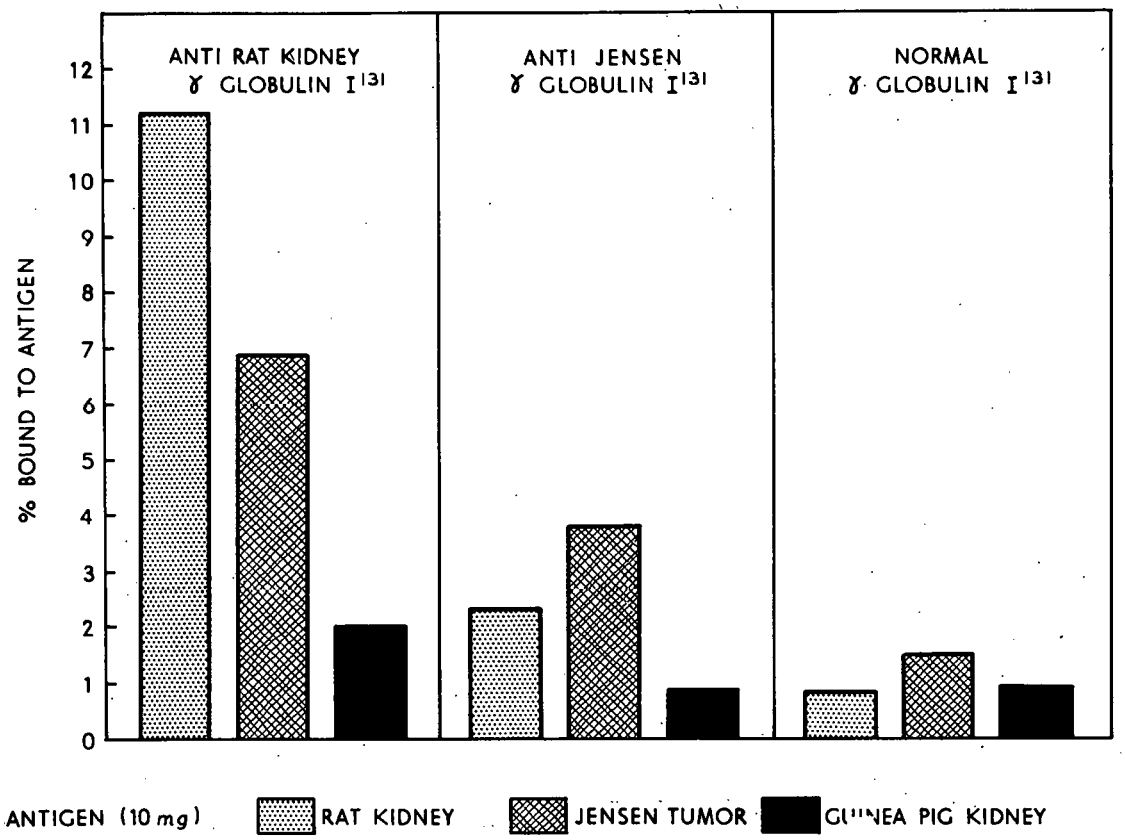

Figure 1. In vitro uptake of I^{131} labeled noneluted γ globulin by homologous and heterologous tissue.

was probably nonspecific absorption since iodinated normal rabbit globulin as well as specific antibody globulin was removed from the tumor by an overnight wash with noniodinated normal rabbit serum (Figure 3). A part of this increase in combining activity appeared to be specific, however, and suggested that the test was performed in a region of antibody excess. Figure 3 also illustrates the increase in specificity and potency that was obtained when the labeled γ globulin was purified by elution with additional specific antibody and mild heat. With the 80 mg antigen level, there was almost complete cross reaction between the eluted antitumor γ

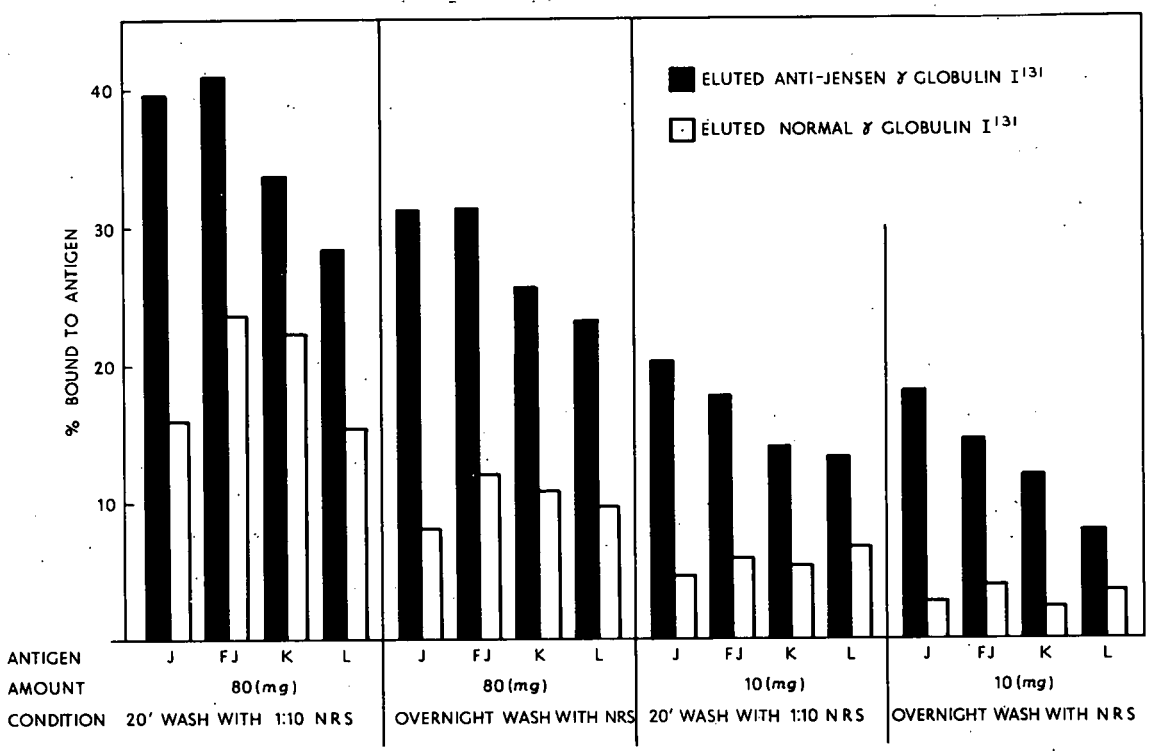

Figure 2. Effect of variation of antigen and antibody concentration and reaction time on the in vitro binding of I^{131} labeled noneluted antirat γ globulin by lyophilized rat kidney.

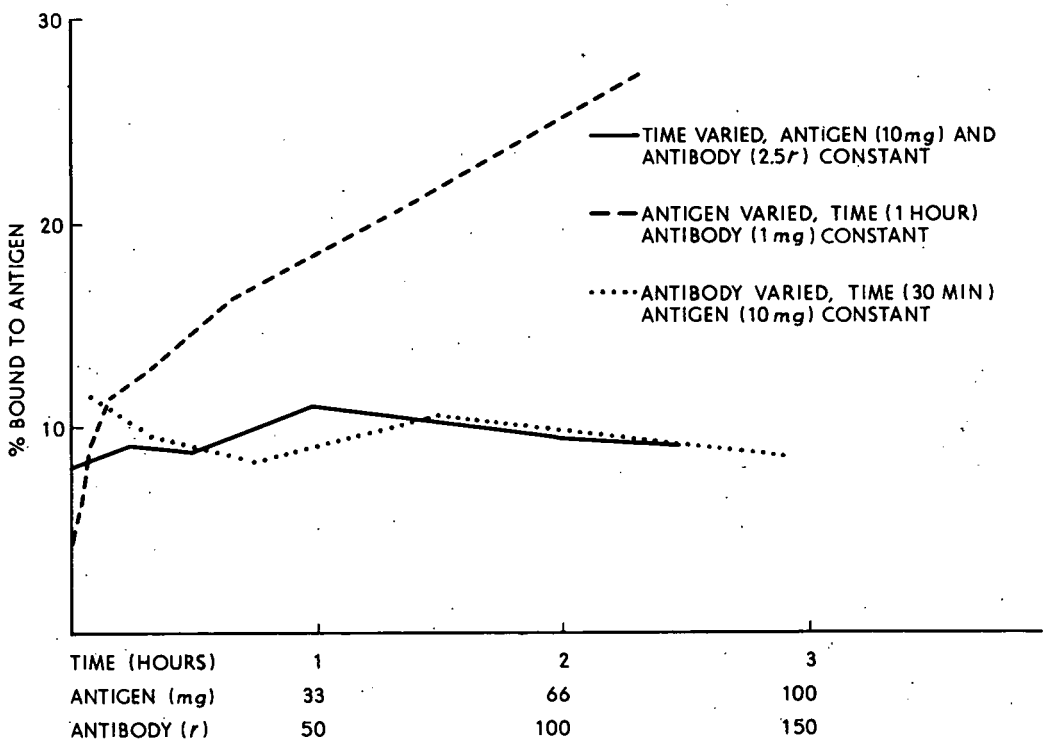

Figure 3. In vitro uptake of I^{131} labeled eluted anti-Jensen tumor and normal γ globulin by the corresponding tumor and other rat tissues under varying conditions.

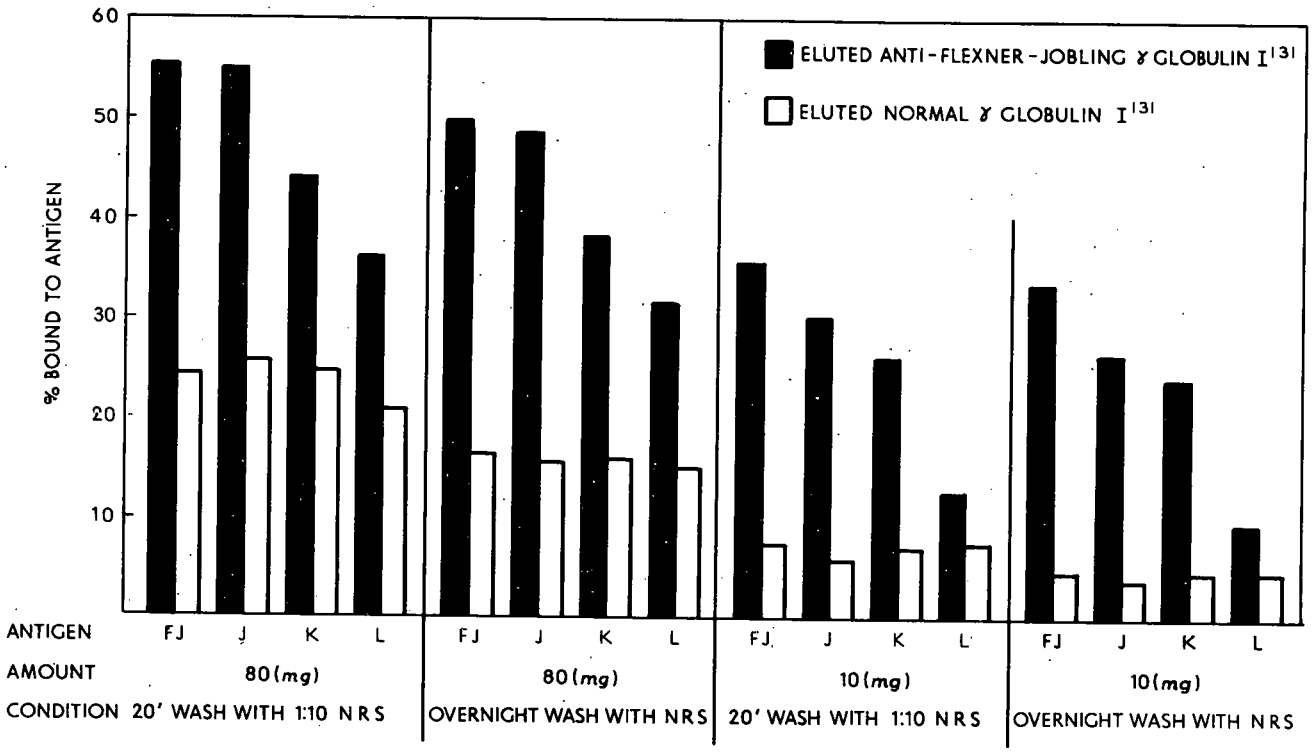

Figure 4. In vitro uptake of I^{131} labeled eluted anti-Flexner-Jobling and normal γ globulin by the corresponding tumor and other rat tissues under varying conditions.

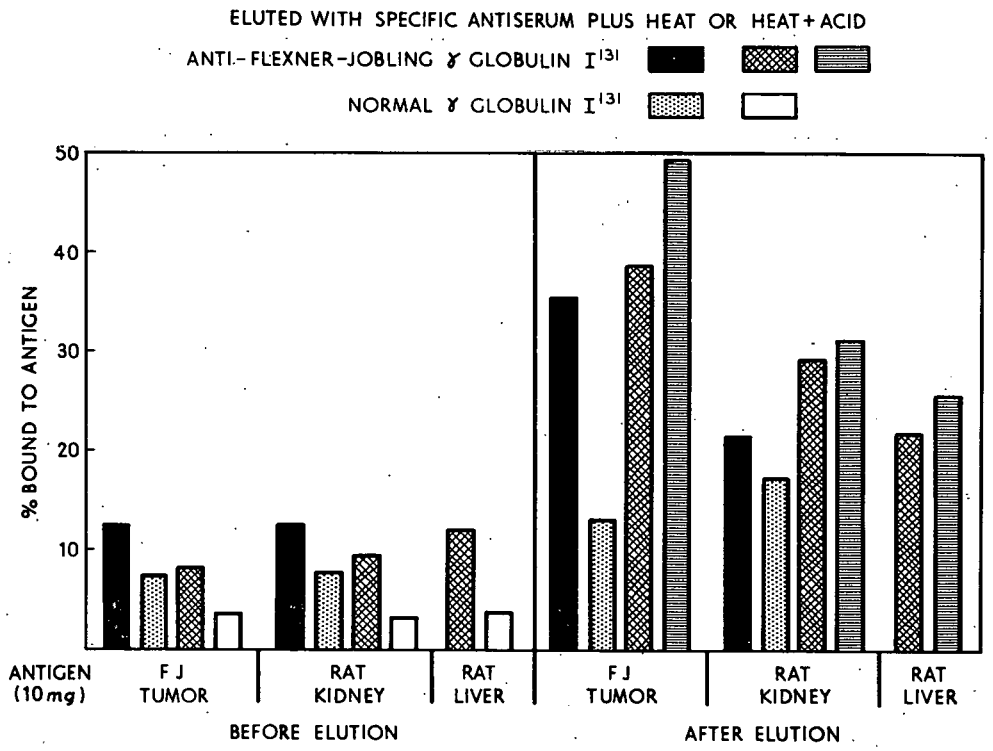

Figure 5. Comparison of the in vitro binding capacity of I^{131} labeled anti-Flexner γ globulin before elution and after elution by two different methods.

globulin and another malignant rat neoplasm as contrasted with the partial cross reaction with rat kidney or rat liver. At the 10-mg antigen level, some degree of specificity of the antiserum for the homologous tumor was demonstrable. Similar results for the eluted immune γ globulin I^{131} against the Flexner-Jobling tumor are shown in Figure 4. Figure 5 represents a comparison of two methods of elution that have been described by Talmage as effectively increasing the specificity of labeled antibody globulin in simpler antigen antibody systems.(16) The particular anti-Flexner-Jobling sarcoma γ globulin employed in this experiment showed no greater avidity for its tumor than for rat kidney before elution. After elution by heat plus specific antiserum, with or without acidification, the specificity of the eluate was quite evident. Although acidification during elution apparently increased the efficiency of the elution process, it resulted in little if any more specificity.

Table 3

COMPARISON OF SPECIFICITY ELUTED AND NONELUTED γ GLOBULIN

	Noneluted			Eluted		
	Liver	Kidney	Tumor	Liver	Kidney	Tumor
Anti-Ehrlich's ascites	4.4*	5.2	5.2	14.8	22.1	35.8
Anti-Jensen	3.8	5.1	4.1	9.9	14.3	18.9
Anti-Flexner-Jobling	9.3	9.5	8.1	21	26	41

* All results expressed as averages of several in vitro experiments performed with several different γ globulin samples eluted at different times. The numbers indicate the % of labeled γ globulin bound to the insoluble portions of the tissue.

The consistency with which elution by the specific unlabeled antiserum resulted in greater specificity of the labeled γ globulin antiserum may be seen in Table 3. This table summarizes the per cent of radioactivity that combined with the appropriate tumor, rat kidney, and rat liver, before and after elution in a series of in vitro tests (these samples of eluted I^{131} γ globulin were prepared for in vivo experiments to be described). The elution methods are admirably fitted for preparing material to be injected into animals since they involve only the addition of specific antiserum to the original labeled material. However, the low efficiency of the elution process for this type of antigen-antibody system makes it difficult to apply it to purification of antibody where one wishes to retain a therapeutically effective amount of radioactivity and still increase the specificity of the antitumor sera. In a number of experiments, the efficiency of elution with heat and unlabeled immune serum was determined. The data indicate that only 3 to 6 per cent of the original labeled γ globulin combined with the antigen during absorption and that from 20 to 50 per

cent of this combined labeled γ globulin was released from the antigen during elution. When the elution was performed at pH 3, somewhat more efficient elution was achieved.

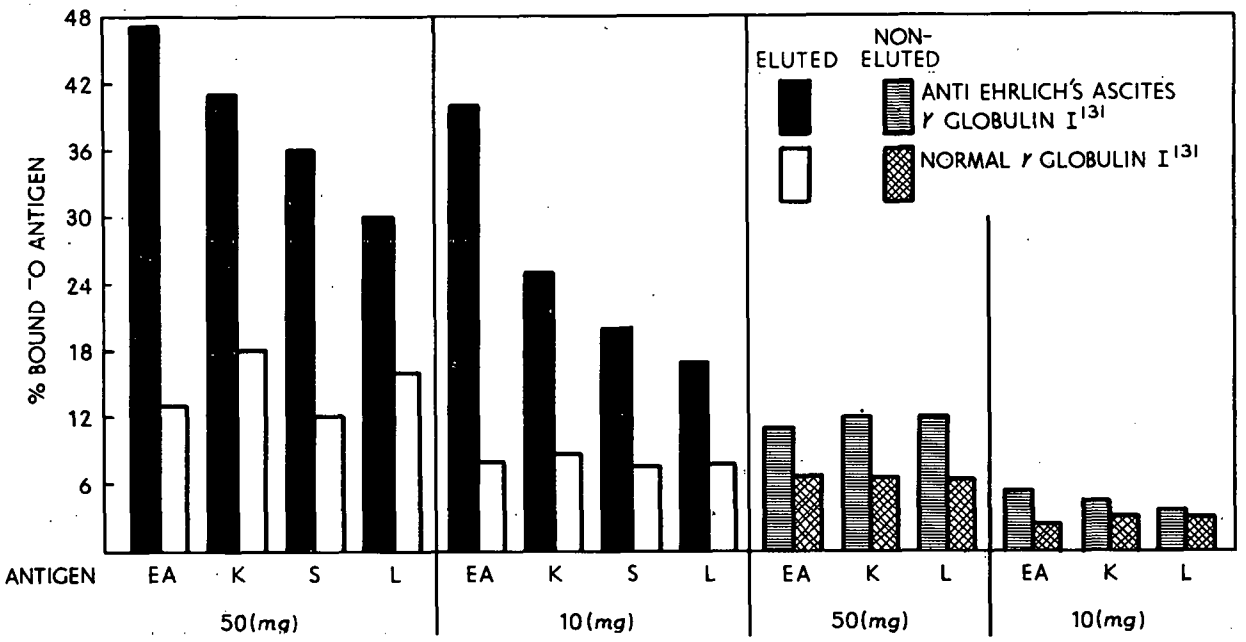

Figure 6. Comparison of the in vitro uptake of eluted and noneluted I^{131} labeled anti-Ehrlich's ascites γ globulin by the EA tumor and various normal mouse tissues.

That the in vitro combining test and the elution procedures were applicable to the Ehrlich's ascites tumor antiserum is illustrated by Figure 6, which shows a definite differential affinity of the labeled anti-Ehrlich's ascites γ globulin for the lyophilized Ehrlich's tumor that was not apparent before elution. Again, the kidney, in the mouse as in the rat, shows the greatest cross reaction with the eluted γ globulin. Since the ascites tumor has no stroma and is presumably a carcinoma in derivation, it seems very unlikely that this cross reaction was the result of a common connective tissue antigen as might be assumed in the case of the cross reaction between antirat tumor sera and rat kidney. The diminished difference between the affinity demonstrated with 10 mg of antigen and 50 mg of antigen and the greater contrast between the reaction of normal rabbit γ globulin and the reaction of the anti-Ehrlich's ascites tumor γ globulin suggest that there is less nonspecific absorption of labeled γ globulin by the ascites tumor than there was by the rat tumors.

In vivo experiments with rat tumors: A series of experiments was undertaken in order to ascertain whether the specific combining power of the eluted antitumor γ globulin for its antigen could be demonstrated in vivo. The eluted antirat tumor γ globulin was administered to the tumor-bearing animals 10 to 18 days after transplantation, and the animals were then sacrificed at intervals to determine the fate of the labeled antibodies.

Figure 7 summarizes the results of 1 of 7 experiments that have been performed with tumor-bearing rats. It is apparent that the eluted anti-Flexner-Jobling γ globulin (AFJ) (eluted in this instance with unlabeled antiserum plus heat and acid) that was injected intravenously into the rats bearing 17-day tumors in doses of 3.2 million counts per minute localized in the tumor to a greater extent than the normal rabbit γ globulin, which had similar counts per minute (3.7 million) and a similar protein content per ml. It is also evident that only a little of the injected radioactivity localized in the tumor in either group of animals and that there was much greater localization in the spleen, liver, and lungs of both groups. Furthermore, the localization in these organs was greater with specific antiserum than with control serum. Correspondingly, the rate of excretion of radioactivity in the urine was considerably faster in the animals receiving the control serum, while the labeled specific antitumor γ globulin disappeared somewhat more quickly from the blood stream. It is of interest that the tumors were the only tissues studied in which radioactivity increased during the interval from 1 to 24 hours and that the tumors apparently lost the radioactivity more slowly.

Similar results were obtained in three other experiments in which heat or acid plus heat-eluted AFJ antibodies were employed. When the noneluted AFJ globulin was utilized in a fifth experiment, the trends were the same, but the differences between the animals receiving the control serum and those receiving specific antiserum were less great and less consistent. No corresponding localization in the tumors was obtained in the single experiment in which eluted anti-Jensen sarcoma γ globulin was used on animals bearing Jensen sarcoma.

A chronic treatment experiment was performed in which doses of iodinated eluted AFJ serum were given tumor-bearing animals every other day for 16 days. Comparable tumor-bearing animals were given a similarly iodinated normal rabbit serum preparation. All animals were sacrificed at 1 hour after the final injection. The greatest differences in tissue localization of I^{131} between the two groups were again observed in the spleen and liver, the animals that had received the specific antiserum showing much more localization in these organs than those receiving NRS I^{131}. There were no consistent differences between the two groups in the per cent of injected radioactivity localizing in the tumors, and there was certainly no evidence for a progressively increasing concentration in the tumors at the expense of the other organs. However, the group receiving the specific antiserum had the smallest tumors and had slight but consistent histologic changes that suggested a deleterious effect on the tumor. In these animals, there was a slightly more prominent cellular reaction at the perimeter of the tumors.

All in all, the in vivo experiments with the rat tumors were disappointing. Although the in vitro studies indicated that a fairly specific antitumor serum was available, it would not localize effectively in the tumor when given intravenously.

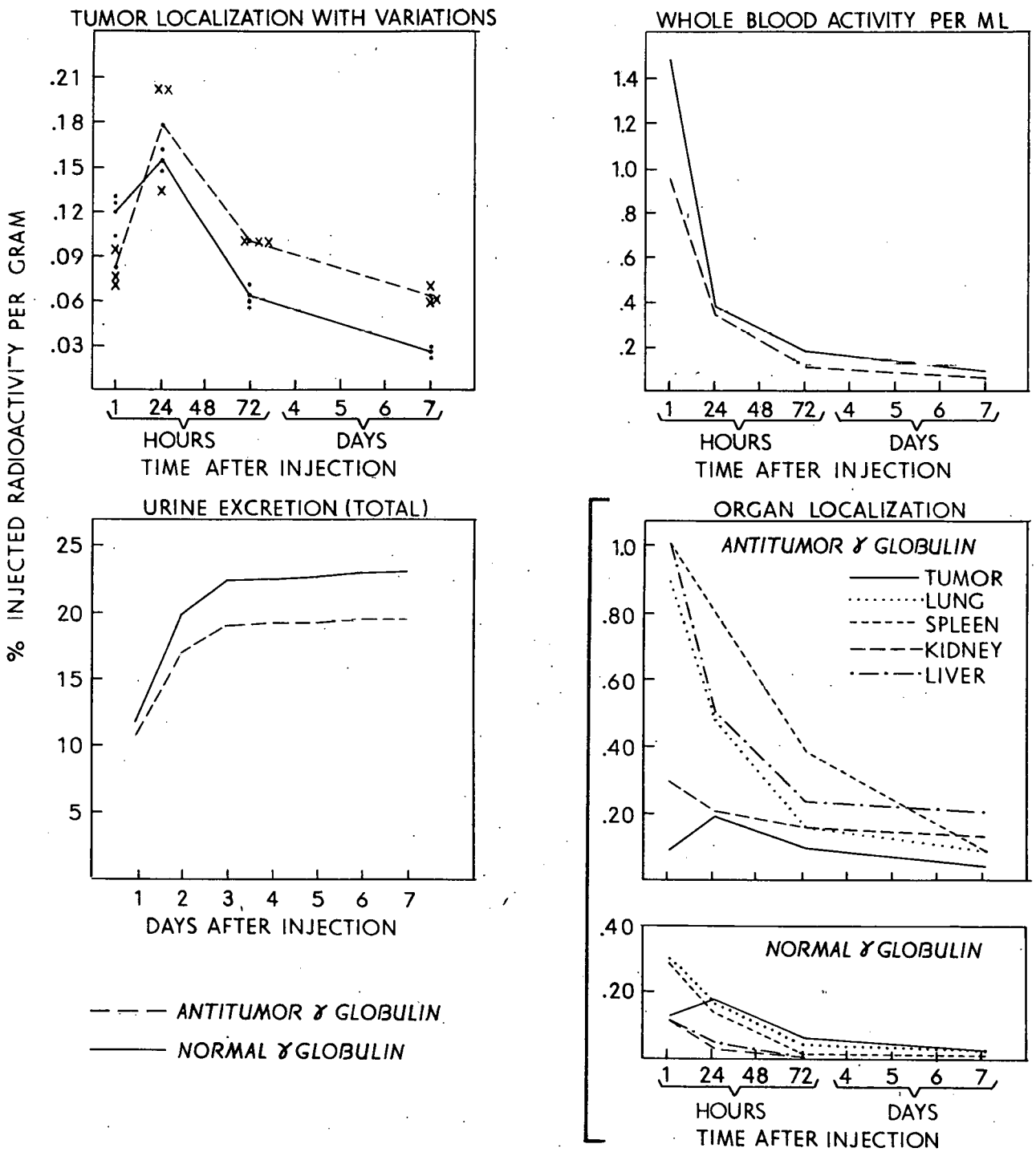

Figure 7. The localization and fate of I^{131} labeled eluted anti-Flexner-Jobling and normal γ globulin injected by the intravenous route into rats bearing the Flexner-Jobling tumor.

Apparently the capillary membranes, tenuous as they are in these transplantable tumors, will not allow the specific globulin to pass into the tumor in sufficient quantity to permit specific localization.

In vivo experiments with the Ehrlich's ascites tumor of mice: With the Ehrlich's ascites tumor, the problem of capillary permeability was circumvented since the radioactive labeled immune sera could be injected intraperitoneally to achieve close contact with the growing tumor. It was thought that if one could establish the principle that such labeled immune serum was an effective carcinocidal substance, the problem of getting the material across the capillary membrane might then be attacked profitably.

Several experiments were performed using the ascites tumor, but only the two most recent ones (AEA-5 and AEA-6) are described in this report. Since the results had varied somewhat in previous experiments, the following possible variables were investigated.

1. Variation in the amount of initial tumor inoculum: Two groups of 10 mice each (groups I and III) were inoculated intraperitoneally, respectively, with 0.02 and 0.5 ml of ascitic fluid, and 20 mice in another group (group II) were inoculated with 0.1 ml of ascitic fluid from a 7-day growth. Five days later, 6 animals were chosen from each of the above groups of 10 and 12 animals from the group of 20. The mice selected within each group apparently had equivalent amounts of tumor as determined by the increment in weight following inoculation.
2. Variation in type of globulin: Group II was further subdivided into 2 subgroups. The animals in one received intraperitoneally 1 cc of iodinated anti-Ehrlich ascites tumor γ globulin (I^{131} AEA) (3.5 million counts per cc), and those in the other, 1 cc of iodinated normal rabbit γ globulin (I^{131} NR) (3.0 million counts per cc) with the same protein content per cc. The other 2 groups received only the iodinated AEA.
3. Effect of fasting on tumor: Since earlier experiments suggested that the uptake of AEA I^{131} was increased when the tumor-bearing animals were deprived of food following the injection of the AEA, the effect of fasting was investigated: half of the animals in each of the above groups were fasted after the injection of the γ globulin while the others were fed ad libitum.
4. Distribution of I^{131} associated with tumor: The tumor and the globulin were combined in vitro in the following manner: ascitic fluid was removed from 6 mice that had been given 0.1 ml of Ehrlich's ascites fluid 5 days prior and was pooled. One-half of the pool was mixed with 3 cc of AEA I^{131}, and the other, with 3 cc of NR I^{131}. Both were shaken in the cold for 1 hour. They were then centrifuged in the cold, and the precipitates con-

taining the tumor cells were washed twice with chilled saline. Each portion was then diluted to 3 cc with saline and injected intraperitoneally in 1-cc aliquots into normal animals (group IV).

5. The significance of the I^{131} distribution in normal mouse organs: The fate of AEA I^{131} and NR I^{131} in normal mice was studied to determine the distribution of I^{131} in the normal mouse. One cc of each of the above preparations of globulin was injected intraperitoneally into each of 3 normal mice (group V).

All animals were sacrificed about 20 hours following the injection of the iodinated γglobulin. Ascitic fluid and blood were removed, and the mice were perfused with saline before the liver, spleen, kidneys, and thyroid were removed. The ascitic fluid was centrifuged (about 10 minutes at 3000 rpm), and the supernatant fluid was poured off and counted separately. The results are summarized in Table 4.

The data from the above investigation indicate that the AEA tumor cells localized 2 to 5 times the per cent of injected dose per cc (per cent ID/cc) found in the supernatant ascitic fluid and blood, and from 5 to 10 times that localized in other tissues counted (except the thyroid).

The I^{131} localizing capacity of the tumor was more marked in fasted animals than in those that were fed -- the tumor in the former groups localizing 2 to 3 times more radioactivity than in the animals which were fed ad libitum.

In those animals of group II receiving NR I^{131} (groups II-C and II-D), there was about 3 times as much radioactivity in the supernatant fluid as in the tumor cells. The organs localized less I^{131} than the tumor in these cases, while the blood had about twice the radioactivity of the tumor. It is of interest that there was little difference between the starved and fed groups receiving the NR I^{131}.

A comparison of the localization of I^{131} in animals containing different amounts of tumor suggests that there was an excess of antibody with respect to tumor antigen, since there was a progressive increase in the total counts bound by the tumor as the amount of tumor increased.

The distribution of AEA I^{131} that was injected bound to tumor cells (group IV) suggested that an appreciable part of the radioactivity, which was found in the organs in the previous groups, might have been at some time associated with tumor.

The data from group V-A and V-B showing the distribution of AEA I^{131} and NR I^{131} in normal mice indicated a greater I^{131} uptake in organs with the AEA than with the normal rabbit γglobulin, and reciprocally, a lower amount remaining in the blood. This localization cannot be accounted for by cross reactions between stroma of tumor and organs, since the ascites tumor has no stroma at all; it must be due to common or related cellular antigens (as was also suggested by the results of the in vitro experiments).

Table 4

SUMMARY OF RESULTS OF EXPERIMENT AEA-5

Group	Variables			Tumor			Supernatant		Blood	Spleen	Kidney	Liver	Thyroid
	Initial inoculum (ml)	Injected γ globulin	Nutrition	Ave. final wt. (g)	Per cent inj. dose/total	Per cent inj. dose/g	Per cent inj. dose/total	Per cent inj. dose/g	Per cent inj. dose/cc	Per cent inj. dose/g	Per cent inj. dose/g	Per cent inj. dose/g	Per cent inj. dose/total
I A	.02	AEA	fasted	.290	5.76	27.57	8.73	10.76	5.37	2.55	4.17	2.69	7.59
I B	.02	AEA	fed	.255	2.69	9.77	4.81	3.86	4.25	1.77	1.46	1.46	7.04
II A	.1	AEA	fasted	.888	16.62	18.36	12.51	6.17	4.03	1.85	2.46	1.99	5.83
II B	.1	AEA	fed	.946	12.49	11.08	11.19	4.97	3.51	1.95	1.55	1.26	3.25
II C	.1	N	fasted	.801	1.68	2.13	9.36	6.54	4.72	.83	.79	.74	.09
II D	.1	N	fed	.796	1.56	2.16	7.77	6.29	4.04	.86	1.22	.76	.12
III A	.5	AEA	fasted	1.417	24.90	17.72	17.82	4.53	3.17	1.08	.97	1.25	1.41
III B	.5	AEA	fed	1.464	14.30	9.79	16.71	4.25	3.08	1.14	1.14	.82	2.01
IV A*	.1	AEA	fasted	.963	1.36	3.68	.41	1.27	.60	.41	.42	.32	.13
IV B*	.1	N	fasted	.387	.03	.07	.07	.17	.05	.02	.03	.02	.11
V A	--	AEA	fasted	--	--	--	--	--	6.07	3.43	2.24	2.13	7.55
V B	--	N	fasted	--	--	--	--	--	7.95	2.09	1.66	1.59	5.42

* γ globulin + tumor reacted in vitro before injection.

The purpose of the next experiment (AEA-6) was to test the effect of multiple injections of iodinated γglobulin on its net localization in tumor-bearing and normal mice; varying the initial inoculum size, type of γglobulin (AEA or NR γglobulin), and state of nutrition, as in the previous experiment. About 1.2 million counts of either I^{131}, AEA or NR γglobulin, were injected on each of 3 consecutive days into mice bearing either 4-day tumors (groups I, II, III) or normal mice (group IV). The mice were all sacrificed on the 4th day. The results, summarized in Table 5, were, in general, comparable to those of the previous experiment but showed an additive effect of the multiple injections. The tumor localized 5 to 10 times the AEA I^{131} (in per cent injected dose/g) found in the supernatant fluid and blood and showed considerably greater localization with respect to the other organs. The NR I^{131} was again localized to a greater extent in the supernatant fluid and blood than in the tumor cells, but greater in the latter than in the other organs with the exception of the thyroid.

An additive effect of the multiple injections can be seen by comparing the per cent injected dose per g in the two mouse experiments; in AEA-6 the tumor localized from 40 to 100 per cent of that found in AEA-5. (If there was no additive effect, one would expect to find only about 33 per cent of the total injected dose when 3 injections were given.)

The difference in localization of AEA I^{131} in tumors of fasted and fed animals was still marked but somewhat less than that following a single injection of the γ globulin. This difference was present in other tissues as well, with those of fasted animals showing greater localization. In addition, there was a similar differential localization in the fasted animals given NR I^{131}, suggesting that the increase involved factors other than increased specific antigen-antibody combination.

As in the previous experiment, the per cent injected dose per g was greatest in animals bearing the smallest amount of tumor and decreased with increasing tumor, while the per cent injected dose per total varied in an inverse manner, indicating the presence of excess antibody. The localization of AEA I^{131} and NR I^{131} in nontumor-bearing animals was also similar to that found in the previous experiment.

The main difference between the two mouse experiments was the considerable mortality in AEA-6; a number of animals died between the 2nd and 4th day after γ-globulin injections were begun. The mortality was greatest in groups receiving AEA I^{131} which had either the smallest amounts of tumor or none at all.

DISCUSSION

These experiments may help to explain the failure of most previous attempts to influence actively growing neoplasms in man and experimental animals by intra-

Table 5

SUMMARY OF RESULTS OF EXPERIMENT AEA-6

Group	Variables			Tumor			Supernatant		Blood	Spleen	Kidney	Liver	Thyroid
	Initial inoculum (ml)	Injected γ globulin	Nutrition	Ave. final wt. (g)	Per cent inj. dose/total	Per cent inj. dose/g	Per cent inj. dose/total	Per cent inj. dose/g	Per cent inj. dose/ml	Per cent inj. dose/g	Per cent inj. dose/g	Per cent inj. dose/g	Per cent inj. dose/total
I A*	.02	AEA	fasted	.514	5.70	13.26	.58	1.20	--	1.22	.88	1.28	1.52
I B*	.02	AEA	fed	.639	5.45	9.64	1.62	1.52	1.08	.92	.59	.88	2.45
II A†	.1	AEA	fasted	.959	7.20	7.29	2.29	1.29	1.24	.63	.48	.62	2.62
II B	.1	AEA	fed	1.585	8.39	5.32	2.84	.75	.72	.23	.25	.27	1.46
II C	.1	N	fasted	1.119	1.78	1.52	6.00	3.83	2.91	.49	.85	.45	1.37
II D	.1	N	fed	1.688	1.07	.64	6.38	1.95	1.61	.26	.29	.17	.99
III A†	.5	AEA	fasted	1.892	13.36	7.43	3.23	1.43	.99	.52	.31	.55	.61
III B	.5	AEA	fed	2.090	11.51	5.51	4.63	1.06	.89	.34	.36	.34	1.17
IV A‡	--	AEA	fasted	--	--	--	--	--	--	3.14	1.55	2.63	.49
IV B	--	N	fasted	--	--	--	--	--	4.57	.83	.45	.35	2.23

* Two of the three animals included in this ave. died before autopsy.

† One of the three animals included in this ave. died before autopsy.

‡ All three of the animals in this group died before autopsy.

venous injection of antitumor-cell antibodies.[2] It seems likely that the major obstacle is not the potency or specificity of the antisera, since it has been repeatedly demonstrated that antitumor antibodies will combine with tumor cells in vitro. In vivo, the main obstacle appears to be the difficulty of passage of the macromolecular γ globulin across the capillary membrane. It is of interest that the organs showing the greatest nonspecific localization of the antitumor antibodies in vivo have been those organs with a sinusoidal circulatory system which presumably have a more permeable vascular wall. It is also noteworthy that a recent rather successful attempt to alter the growth of an established neoplasm with specific heterologous antiserum utilized a tumor that probably has a sinusoidal circulation.[22] Whether the specific in vivo localization of antibodies to tumor can be facilitated by the simultaneous administration of substances that increase capillary permeability without producing severe toxicity remains to be investigated. Furthermore, study of the injection of the labeled antitumor γ globulin directly into the tumor may be profitable although this procedure would seem to have little promise of practical therapeutic value in the treatment of inoperable malignancies.

The results reported in this paper are not in disagreement with the frequent observation that immunological mechanisms may prevent the growth of a transplantable tumor if the antibodies are present at the time of inoculation and before the tumor has established a blood supply. However, the failure of multiple injections of the anti-Ehrlich's ascites tumor γ globulin to cause a decrease in the growth of this tumor during a 4-day period of therapy indicates that, for this tumor at least, the mere presence of an excess of the specific γ globulin combined with the tumor cells is not sufficient to retard tumor growth (AEA-6). Recent work in Japan suggests that with relatively more antitumor antibodies, marked diminution in the quantity of a rat ascites tumor can be achieved in vivo, and cures have been reported.[23]

Whether growth retardation of the Ehrlich's ascites tumor can be achieved when either more antibody is injected or when more I^{131} is attached to the specific γ globulin is now being studied. The results of this study should help indicate whether or not immunological means would be effective in localizing therapeutic quantities of radioactive substances in tumor tissue.

The in vivo experiments with the Ehrlich's ascites tumor that have been performed to date raise at least two questions. The increased avidity of both normal tissues and the tumor cells for the antitumor antibodies in fasted mice, even though the period of fasting is quite brief, may indicate that the tissues of the fasted animals metabolize the intraperitoneally injected foreign serum protein. Such an interpretation seems unlikely, however, since the fasted animals given normal serum fractions showed either no such difference as compared to comparable nonfasted

animals (AEA-5) or only a moderately increased combination of the labeled normal γ globulin with the tumor cell (AEA-6).

The apparent increased toxicity of the I^{131}-labeled anti-AEA γ globulin for animals bearing no tumors or for those receiving smaller inoculums, as judged by the survival of animals in AEA-6, is also puzzling. Does this indicate that the binding of this eluted antiserum by tumor cells decreases its general toxicity for the animal as a whole? If so, the greater toxicity of the antiserum as compared to normal serum may result from a cross reaction of the antiserum with vital organs and tissues.

One of the most interesting aspects of these experimental results has been the in vitro demonstration that there is a more marked cross reaction of a given antitumor serum with a second unrelated tumor tissue than there is between the antiserum and certain normal tissues. Similar results have been reported by others.[9,11] This broad specificity of an antitumor serum for neoplastic tissue in general suggests an immunological similarity between neoplasms and may indicate a unique biochemical structure of some part of the neoplastic cell. These data may indicate that immunological methods may be applicable to further investigation of the essential difference between neoplastic cells and normal cells. The recent demonstration by Kidd[24] of a serologically distinct constituent in the Brown-Pearce carcinoma supports the idea that this may be an additional profitable approach to the cancer problem.

SUMMARY

This study indicates that multiple injections consisting of lyophilized rat tumor tissue given to rabbits by multiple portals and preceded by a single intramuscular injection of the same material in Freund's adjuvant resulted in potent and fairly specific antisera. These antisera have been studied in vitro by means of complement fixation or by labeling the specific γ globulin with I^{131} and determining the amount that combines with the tumor. The specific antitumor antibodies became more apparent following purification of the labeled globulin by means of elution with additional unlabeled antitumor sera. There was a complete in vitro cross reaction between antiserum produced to Flexner-Jobling tumor and Jensen sarcoma.

Intravenous injection of the eluted I^{131} antitumor γ globulin into rats bearing the homologous tumor resulted in only slight selective localization of the I^{131} γ globulin in the tumor as compared with the localization of similarly labeled normal γ globulin. This failure of localization is probably due to the low permeability of the tumor blood vessels to the large antibody globulin molecules. When labeled antibodies to Ehrlich's ascites tumor were injected intraperitoneally into mice bear-

ing proliferating ascites tumors, the labeled antibody globulin was clearly differentially localized on the tumor cells.

Thus far no significant retardation of growth of the Ehrlich's ascites tumor following in vivo combination with the specific labeled antibodies has been observed.

The toxicity of the intraperitoneally injected labeled antiserum preparation for the tumor-bearing mouse varies inversely with amount of tumor growing in the mouse.

Fasting of the mice during the period following injection of the labeled antiserum increases the localization of the specific antibodies on the tumor cells.

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FURTHER STUDIES ON THE RADIOSENSITIVE STAGES IN HEMOLYSIN FORMATION*†

By

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SUMMARY

Large doses of X radiation have been found to affect antibody formation by causing 1) a marked to complete suppression of antibody formation when animals are irradiated before the antigen has been injected[1-5]; 2) a general retardation of the immune process with no lessening of the amount of antibody formed when the radiation is delivered before the animals have received antigen or afterwards when antibody formation is active[3,5]; and 3) an enhancement of antibody formation superimposed upon the general immune process.[5]

This research was aimed toward a more complete elucidation of the development and early recovery from the X-ray-induced maximum suppression of antibody formation and to further delineate the various phases of the radiation response. In addition, the radiosensitivity of the anamnestic response was re-investigated.

The materials and methods that have been described for other related investigations were used.[5-7] Only a few of the salient features are given in this report.

Sheep red cells, which were standardized photometrically, were used for all immunizations and titrations. The amount of injection is expressed for convenience in terms of a 1 per cent suspension. In the first series of experiments (Table 1), 0.125 ml of a 1 per cent suspension (i.e., 2×10^7) of red cells per kg were injected intravenously into the rabbits. In the series devoted to the study of the radiosensitivity of the anamnestic reaction, the injection unit was 1 ml of a 10 per cent suspension.

All titers were based on photometric determinations of hemolysis and are expressed in 50 per cent hemolytic units either as 1) the amount of serum necessary to produce 50 per cent hemolysis in 2 ml of 1 per cent sheep red cells in the presence of four 50 per cent units of complement or 2) as the number of such units per ml of serum.

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Table 1

EXPERIMENT 1: PRIMARY HEMOLYSIN RESPONSE* FOLLOWING ONE INTRAVENOUS INJECTION OF 0.125 ML OF 1% SHEEP RED CELLS PER KG RABBIT FROM 4 DAYS BEFORE TO 28 DAYS AFTER 500 R TOTAL/BODY IRRADIATION ALONG WITH CONTROLS

Series	Time of red cell injection	Mean peak titer			Rate of accumulation k		Length in days of:		Number of rabbits
		Log	Geometric x 10^3	Arithmetic x 10^3	First phase	Derived when > one phase	Induction period	Antibody rise to peak	
	Red cells before 500 r								
1	4 days	2.76 ± 0.35	1.75	1.31 ± 0.48	0.7 ± 0.16*	0.4 ± 0.4***	4.1 ± 0.6	9.3 ± 2.6***	6
2	2 days	3.07 ± 0.31	1.17	2.60 ± 1.26	0.7 ± 0.22*	0.4 ± 0.11**	7.2 ± 1.6***	9.5 ± 2.3**	5
3	1 day	3.23 ± 0.50	1.70	1.72 ± 0.25	0.9 ± 0.16	0.7 ± 0.22	6.0 ± 0.2***	9.3 ± 3.5*	4
4	2 hours	3.74 ± 0.14**	5.50	8.08 ± 1.87***	0.9 ± 0.08	0.6 ± 0.05**	7.5 ± 0.7***	10.5 ± 0.8***	10
5	0.2 hours	3.09 ± 0.23	1.23	2.76 ± 1.24	1.3 ± 0.16	0.6 ± 0.11*	9.7 ± 0.6***	8.8 ± 0.8***	8(1)
	Red cells after 500 r								
6	0.5 hours	3.29 ± 0.16	1.95	2.94 ± 0.97	1.1 ± 0.08	0.8 ± 0.14	7.7 ± 0.5***	7.2 ± 1.4	6
7	1 hour	2.96 ± 0.12	0.91	1.44 ± 0.50	0.9 ± 0.12	0.8 ± 0.11	9.5 ± 0.6***	5.3 ± 0.5	11
8	4 hours	2.63 ± 0.14**	0.43	0.67 ± 0.24**	0.9 ± 0.11	0.7 ± 0.11	9.3 ± 0.3***	7.8 ± 1.5	8
9	1 day	1.52 ± 0.06***	0.03	0.04 ± 0.004**	0.3 ±	8.4 ±	7.6 ±	±	1(9)
10	2 days	1.88 ± 0.12***	0.09	0.09 ± 0.02**	0.3 ± 0.03***	0.2 ± 0.02***	18.8 ± 2.5***	10.4 ± 2.0	6(1)
11	1 week	2.54 ± 0.26**	0.35	0.59 ± 0.38	0.8 ± 0.12	0.4 ± 0.09*	8.8 ± 2.1***	11.4 ± 2.5***	4
12	2 weeks	2.33 ± 0.28**	0.21	0.38 ± 0.14*	0.6 ± 0.22*	±	12.0 ± 2.8***	5.7 ± 1.5	4
13	3 weeks	2.75 ± 0.42	0.56	1.57 ± 0.87	1.1 ± 0.29	0.9 ± 0.38	5.7 ± 0.4	6.0 ± 1.3	5
14	4 weeks	2.72 ± 0.21	0.22	1.01 ± 0.26	0.9 ± 0.09	0.9 ± 0.14	5.4 ± 0.7**	6.3 ± 2.4	7
15	8 weeks	3.41 ± 0.26	2.57	7.10 ± 2.53	1.1 ± 0.19	1.0 ± 0.20	3.7 ± 0.6	4.9 ± 0.7	12
	Red cells alone: no X rays								
16	Controls	3.25 ± 0.09	1.78	2.82 ± 0.39	1.1 ± 0.08	1.0 ± 0.08	3.6 ± 0.19	5.2 ± 0.47	31

* All values are means ± standard errors. Data from individual rabbits were obtained from fitted curves. Numbers in parentheses give additional rabbits which elicited no antibody response. Mean peak titers were calculated from the total sample; other values only from those rabbits which elicited an antibody response. The significance of the difference between each irradiated group and the control group are indicated by the following:

*** P = 0.001

** P = 0.002 to 0.01

* P = 0.02 to 0.05

Log peak titer was used as a measure of the amount of antibody formed. The induction period, the rate (k) of antibody rise in the first order equation describing the initial rise of serum antibody or the derived rate for the entire initial rise when the latter consisted of two rates, and the length of antibody rise to peak titer were the measures of the rate of the immune process.

Fifteen groups of from 4 to 12 rabbits were injected with 0.125 ml of 1 per cent red cells per kg at 4, 2, or 1 days and 2 or 0.2 hours before 500 r X irradiation; or after the same amount of irradiation at 1/2, 1, 4, or 48 hours; or 1, 2, 3, 4, or 8 weeks. Data on the hemolysin response in the experimental animals (108 rabbits) and the control group (31 rabbits) are given in Table 1.

The groups that received antigen before irradiation had mean peak titers that were statistically similar to that of the control group except for the group that was injected 2 hours prior to exposure. This group had a statistically higher mean peak titer. In these irradiated groups, the rate of the immune process was markedly retarded (if the statistical significance of the differences from the control group of the length of the induction period, the derived k, and the length of the antibody rise to peak titer are considered).

Animals that were injected with antigen at 10 minutes before or 1/2 hour after irradiation had mean peak titers that were almost identical with those of the controls. However, beginning with injection at 1 hour after exposure, the log peak titer decreased rapidly for 1 day (Figure 1). During this time, the induction period was significantly longer and the derived k was significantly lower for individual series. At 1 day after irradiation, 1 of 9 rabbits and at 2 days, 5 of 6 had a slight rise in titer. Recovery at one week was marked and was complete at 8 weeks.

In the second part of this investigation the radiosensitivity of the anamnestic response was studied in 36 rabbits. The animals were given a re-injection of 1 ml of 10 per cent red cells per kg at 1 to 2 hours before or at 2 days after 500 r. The controls were given identical injections but were not irradiated. Re-injections were made from 28 to 40 days after the first injection of sheep red cells.

The typical anamnestic response that we described previously[5] is illustrated by the data for series 19 in Table 2. The susceptibility of the response to X radiation is shown by the findings for series 17 and 18. All means in series 17 through 19 are statistically similar to a corresponding series that we discussed in 1952.[5]

The sharp drop in the curve of Figure 1 shows the development of X-ray injury, which prevents antibody formation. These studies indicate that the drop in the log peak titer is almost linear with arithmetic time from 1/2 hour to 1 day after irradiation. The linearity extends to the enhanced log peak titer at the 2-hour pre-irradiation time.

Recovery of the capacity to form antibodies was rapid. A plot of mean log peak antibody against the log of the postirradiation time of antigen injection indicates

an approximately linear relationship (Figure 2). Recovery was more or less complete at 8 weeks (Table 1). The median time for 50 per cent recovery was found to be 4.05 ± 1.6 days.

The radiosensitive period in the marked suppression of antibody must involve an early stage in antibody formation, though not necessarily the first, because antibody synthesis can be stimulated by injections of antigen if these are given immediately after irradiation. The length of the radiosensitive period cannot be sharply delimited from our data.

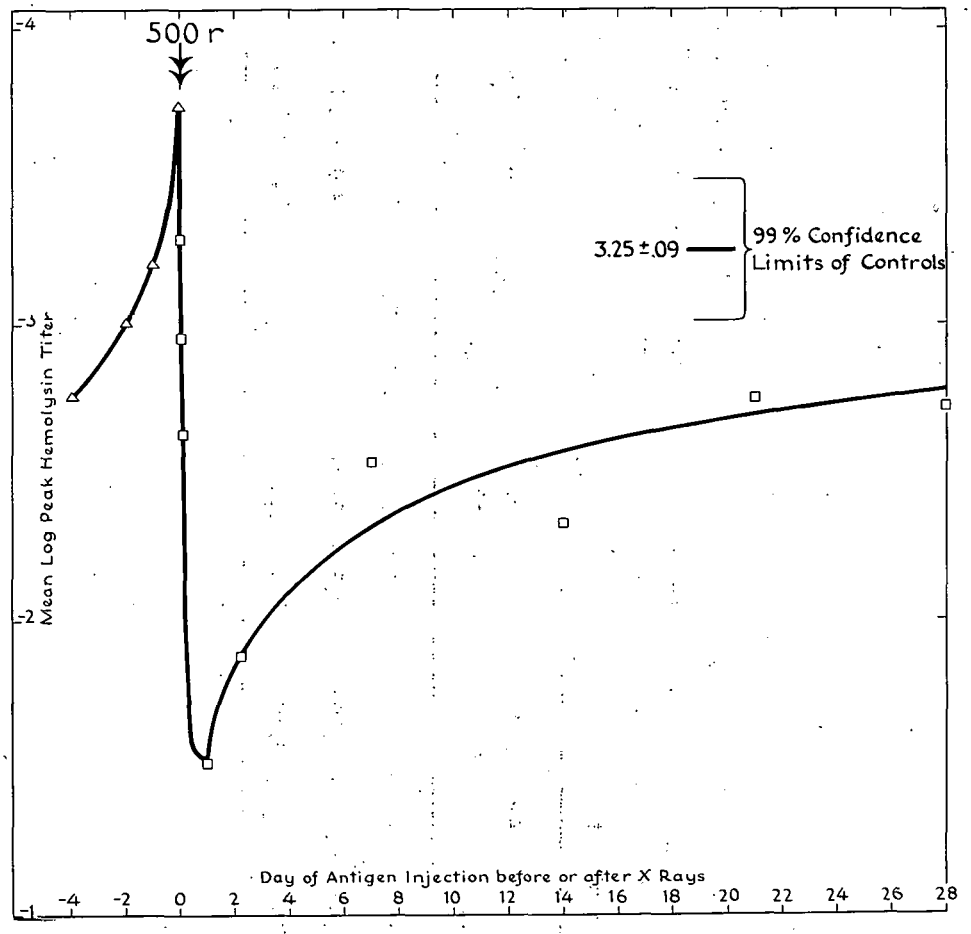

Figure 1. Log mean peak hemolysin titer as related to the time of administering 0.125 ml of 1 per cent sheep red cells per kg rabbit before and after irradiation.

It has been suggested that the X-radiation injury may be related to the radiosensitivity of the initial events involved in the fixation of the antigen by the cells.[3] We have suggested that it is probably due to the localization of antigen in the antibody-forming sites and/or the early metabolism of the antigen. Possible support for these ideas is given by Harris[8] who found that normal lymph node cells, incubated in vitro at 37°C with organisms that produce dysentery or with a soluble

Table 2

EXPERIMENT 2: SECONDARY HEMOLYSIN RESPONSE† FOLLOWING ONE INTRAVENOUS INJECTION OF 1 ML OF 10% SHEEP RED CELLS PER KG RABBIT (17) 1 TO 2 HOURS BEFORE OR (18) 2 DAYS AFTER 500 R TOTAL/BODY IRRADIATION ALONG WITH (19) CONTROLS.

Series	Mean peak titer			Adjusted rate of accumulation (k)		Length in days of:		Number of Rabbits
	Log	Geometric x 10^3	Arithmetic x 10^3	First phase	Derived when > one phase	Induction period	Antibody rise to peak	
17	3.39 ± .41	2.46	8.53 ± 2.73	1.4 ± .21*	1.3 ± 22*	3.4 ± 0.2***	5.8 ± 0.8***	20(1)
18	1.76 ± .17***	.057	0.34 ± 0.20***	0.5 ± .20***		9.0 ± 3.2***	6.2 ± 1.8***	5(10)
19	3.43 ± .10	2.70	3.87 ± 0.59	2.0 ± .20		2.0 ± 0.13	2.7 ± 0.2	18

† See footnote in table 1.

antigen from them, then washed, and injected into X-irradiated rabbits, cause antibodies to appear in the recipients within 4 days.

Fitch and his coworkers[9] and Ingraham[10] have found no marked difference in the gross distribution of antigen following heavy total-body X irradiation. This is also true of intracellular distribution.[10] As yet, it is not known whether these investigations relate to the localization of the functional antigen in antibody-forming sites.

The retardation of the immune process without a significant reduction in peak titer has been studied by Kohn[3] and by the authors and Janssen.[11] This slowing-down probably occurs whenever the antigen is given before irradiation so that antibody is being formed at a rate that is high enough to permit the detection of injury.

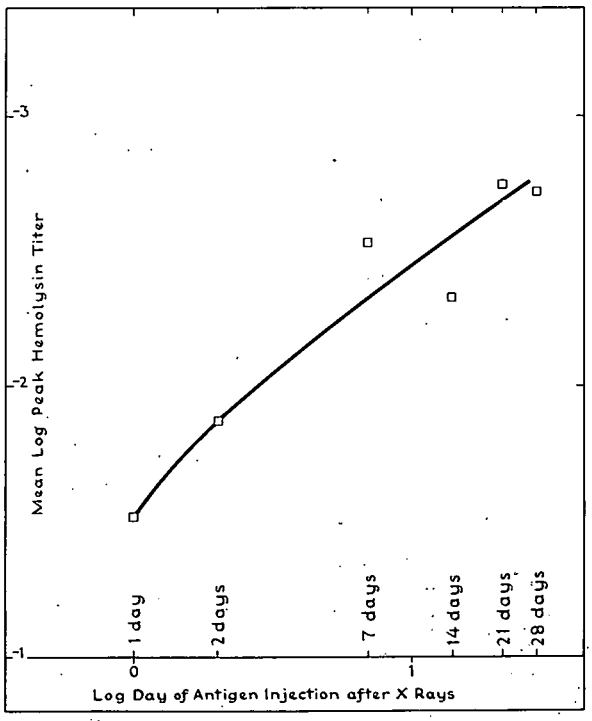

Figure 2. Recovery of antibody-forming power as indicated by the log mean peak hemolysin titer plotted against the log time of injecting 0.125 ml of 1 per cent sheep red cells per kg after irradiation.

It is quite likely that the period between the onslaught of radiation and an observable effect is short although it may be as long as the period of suppression of the antibody. When irradiation was administered at the end of the induction period, the rate of antibody rise to peak titer was shortened.

The enhancement of the peak antibody that follows the injection of antigen, shortly before irradiation, probably involves a mechanism that is superimposed on the general retardation of the immune response because the two are always associated. Enhancement was found to occur when antigen was injected at 2 hours before

irradiation but not at either 1 day or 12 minutes before. The increased antibody formation might result from the superimposition of stimulation on the injury that is associated with a slowing of the general immune response, and it might represent the removal of some limiting factor thereby permitting an injured antibody-forming mechanism to continue until it produces more than normal amounts.

Re-examination of the anamnestic reaction revealed that it is as radiosensitive as the primary response to X irradiation.

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EFFECT OF X RAYS ON HEMOLYSIN FORMATION FOLLOWING VARIOUS IMMUNIZATION AND IRRADIATION PROCEDURES*†

By

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SUMMARY

The purposes of this investigation were to 1) obtain data that would give base line information for further research on the mechanism of the effects of X radiation on antibody formation and 2) to restudy some of our earlier work on antibody enhancement.(1) The calculation of approximate dosage-response curves for both antigen and X-ray exposure is an integral part of our first purpose. The corroborative aspect of the second is highly desirable because there are so many unknown variables in this type of radiobiological research.

Since the materials and methods have been described previously(1-3), only a few pertinent details are outlined below. The antigen that was used for injection and for in vitro titrations consisted solely of sheep red cells. The suspensions of red cells were standardized in terms of hemoglobin liberated by distilled water hemolysis so that 1 ml of suspension in 4 ml of distilled water (designated a 1 per cent suspension, i.e., 1.6×10^8 cells per ml) gave a reading of 150 on the Klett Summerson photoelectric colorimeter with a #54 filter. The 1 per cent suspension was used as the injection unit per kg rabbit although the actual fluid given never exceeded 30 ml. The cells from several sheep were used for all injections and titrations. The cells that were used for all injections were washed and suspended in 0.85 per cent NaCl, and were always injected into the vein of the right ear. Hemolysin titers were obtained in 50 per cent units. This unit is the amount of serum that induces 50 per cent hemolysis of 2 ml of 1 per cent sheep cells in the presence of four 50 per cent units of complement.

The equation that was used to describe the rise and fall of antibody is

$$A_t = A_0 e^{k_0 t}$$

in which A_t and A_0 represent antibody titers at times t and 0, respectively, and k_0, the rate of rise of serum antibody. The peak titer determined from fitted curves of

* This work was done under contract AT(11-1) 175 between the U.S. Atomic Energy Commission and the University of Chicago. It was also supported by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

† A summary of a paper that appears in the Journal of Infectious Diseases, 95:117-133, 1954.

‡ The Department of Microbiology, formerly the Department of Bacteriology and Parasitology, The University of Chicago.

log serum antibody in time was used as a measure of the amount of antibody that was formed. The length of the induction period, the rate of increase of (k) of the initial rise, and a derived average, k, when the initial rise consisted of more than one phase were used as measures of the rate of the immune process.

Total-body X irradiation was delivered to the rabbits at rates ranging from 15 to 35 r per minute, and total exposure ranged from 10 to 700 r. The target to rabbit distance varied from 75 to 92 cm. Two rabbits, each weighing from 2 to 2.5 kg, were irradiated at a time in aluminum boxes, which were turned at the midpoint of the exposure.

Log peak titers were used for statistical analyses of the experimental result, while rates and times were treated arithmetically.

Each of the 17 experiments that comprised this investigations, with two exceptions, consisted of 3 series: 1) irradiated rabbits that were given a single injection of antigen or the first of a series of injections 1 to 4 hours before X irradiation; 2) X-irradiated animals that were given antigen 2 days after exposure; and 3) nonirradiated controls that were injected with antigen.

The effect of varying the amount of antigen while the X-ray exposure is held constant at 500 or 600 r is shown by 4 experiments in Table 1 (249 rabbits). From the data it appears that although enhancement occurred with all doses of antigen given 1 to 4 hours prior to 500 or 600 r, an injection of 10 ml or more caused the greatest increase in the peak titer. The smaller injections gave the most significant evidence of slowing the rate of the immune process.

When antigen was injected 2 days after 500 or 600 r, suppression of antibody formation occurred throughout the 2000-fold range of antigen dosage. Thus, there was a highly significant decrease in the amount of antibody formed as indicated by the log peak titers. At the same time, there was marked retardation of the immune process in the animals that formed measurable amounts of antibody. This was shown by the lengthening of the induction period and a decrease in the initial and derived k values.

The amount of antigen that was injected was found to modify the degree of X-ray-induced suppression of antibody formation. For example, the mean log peak titer was significantly higher in the series that received 250 ml than in those receiving 0.125 ml of 1 per cent cells per kg.

The changes in the irradiated rabbits as compared with the control are represented in Figure 1.

Data on the effect of repeated injections of antigen upon X-ray-induced antibody suppression are shown by 3 experiments in Table 2 (76 rabbits). In the first group, one injection was given before irradiation and the rest afterwards, and in the second, all injections were given after exposure. The third group consisted of nonirradiated controls. Nine or 10 injections of 0.125 ml or 10 ml of 1 per cent cells per kg

Table 1

PRIMARY HEMOLYSIN RESPONSE* FOLLOWING ONE INTRAVENOUS INJECTION WITH SHEEP RED CELLS (A) 1 TO 4 HOURS BEFORE OR (B) 2 DAYS AFTER 500 OR 600 R TOTAL-BODY IRRADIATION ALONG WITH (C) CONTROLS.

Experiment & series		1% red cell per kg	Mean peak titer			Rate of accumulation k		Length in days of:		Number of rabbits
			Log	Geometric x 10^3	Arithmetic x 10^3	First phase	Derived when > one phase	Induction period	Antibody rise to peak	
			A. One injection 1 to 4 hours before X rays							
1	1	0.125 ml	3.74 ± .14**	5.50	8.08 ± 1.87***	0.9 ± .08	0.6 ± .05**	7.5 ± 0.7***	10.5 ± 0.8***	10
2	4	1.25 ml	3.86 ± .08***	7.25	9.69 ± 2.12***	0.9 ± .07***	0.7 ± .04***	5.7 ± 0.4***	0.5 ± 0.5***	20
3	7	10 ml	4.05 ± .10***	11.22	14.19 ± 3.11**	1.3 ± .10	0.9 ± .17	7.0 ± 0.5***	7.9 ± 1.4*	10
4	10	250 ml	4.10 ± .08***	12.59	14.24 ± 1.66**	1.2 ± .09**	1.0 ± .09	5.4 ± 0.3***	7.4 ± 0.6	7
			B. One injection 2 days after X rays†							
1	2	0.125 ml	1.68 ± .08***	0.04	0.06 ± 0.01***	0.3 ± .04***	0.2 ± .02***	17.3 ± 2.5***	10.5 ± 1.9***	6(8)
2	5	1.25 ml	1.81 ± .11***	0.06	0.11 ± 0.02***	0.6 ± .20***	0.3 ± .04***	12.6 ± 1.0***	8.3 ± 1.7***	12(6)
3	8	10 ml	2.09 ± .14***	0.12	0.37 ± 0.08***	0.6 ± .11***	0.4 ± .07***	10.8 ± 1.7***	0.1 ± 2.0*	9(3)
4	11	250 ml	2.61 ± .18***	0.41	0.78 ± 0.31*	0.8 ± .19***	0.5 ± .11**	7.9 ± 0.2***	7.9 ± 1.2*	9
			C. Red cells alone: no X rays							
1	3	0.125 ml	3.25 ± .09	1.78	2.82 ± .39	1.1 ± .08	1.0 ± .08	3.7 ± 0.19	5.2 ± 0.47	31
2	6	1.25 ml	3.40 ± .04	2.52	3.60 ± .37	1.3 ± .05	1.2 ± .05	3.2 ± 0.11	4.3 ± 0.16	71
3	9	10 ml	3.43 ± .06	2.70	3.21 ± .42	1.4 ± .10	1.1 ± .20	2.7 ± 0.14	5.0 ± 0.44	18
4	12	250 ml	3.61 ± .08	4.08	5.54 ± .93	1.9 ± .08	1.2 ± .12	2.6 ± 0.19	5.4 ± 0.57	26

* All values are means ± standard errors. Data from individual rabbits were obtained from fitted curves. Numbers in parentheses give additional rabbits which elicited no antibody response. Mean peak titers were calculated from the total sample: other values only from those rabbits which elicited an antibody response. The significance of the differences between each irradiated group and the corresponding control group are indicated by the following:

*** P = 0.001

** P = 0.002 to 0.01

* P = 0.02 to 0.05

† Antigen given either one or two days after X rays in series 2 and 5.

were given over a 15- to 17-day period. The effect of the series of injections given prior to X irradiation could not be studied because by the 15th day, serum antibody is rising very slowly or falling. However, injection of the first dose 1 to 4 hours before exposure to 500 or 600 r caused a significant lengthening of the induction period, but other than that there was little indication of any effect on antibody response.

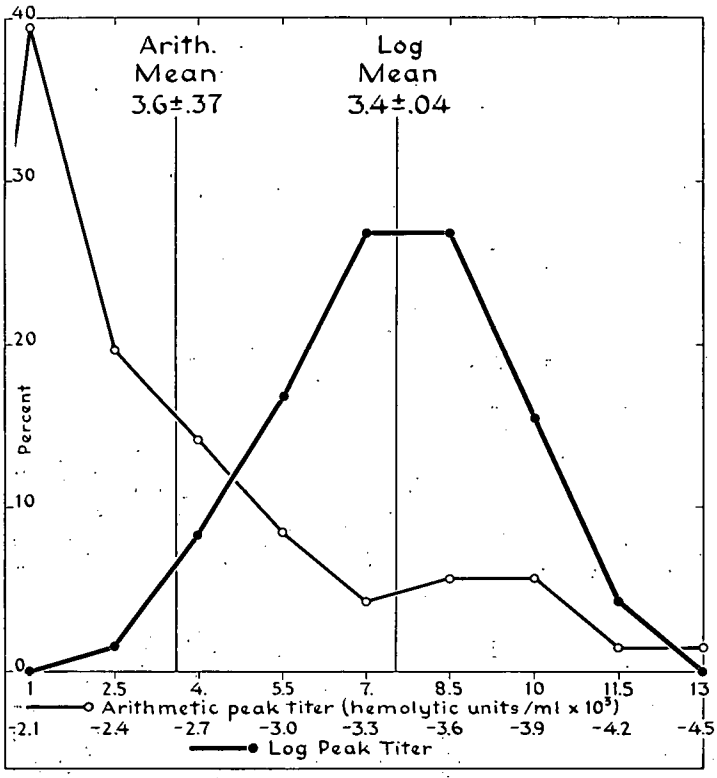

Figure 1. Representative examples of series 10, 11, and 12 in experiment 4 showing the hemolysin curve of 3 rabbits which received 1 injection of 25 ml of 10 per cent red cells per kg 4 hours before and 2 days after 500 r, and of an unirradiated control.

When the animals were given the entire course of injections after exposure to 500 or 600 r, there was a marked inhibition of antibody formation as compared with the values for their controls. Nevertheless, the antibody formed was similar in amount to that formed in nonirradiated controls that received a single injection of antigen. When, however, the same number of injections was given over a 5 1/3-day postirradiation period, the inhibition was no greater than that observed after a single injection of antigen that was given 2 days after exposure, the time of maximum suppression. Thus, antibody formation after exposure to X rays is due chiefly to the recovery of the animal from irradiation and not due to the summation of small antibody responses that occur in animals that received a single injection.

Table 2

HEMOLYSIN RESPONSE* FOLLOWING 9 TO 10 INTRAVENOUS INJECTIONS WITH SHEEP RED CELLS DURING 15 TO 17 DAYS.

In section A, the first injection was 1 to 4 hours before and in section B, 2 days after 500 or 600 r total-body irradiation. Controls in section C were not irradiated.

Experiment & series		Red cell per kg (ml) (%)	Mean peak titer			Rate of accumulation k		Length in days of:		Number of rabbits
			Log	Geometric x 10^3	Arithmetic x 10^3	First phase	Derived when > one phase	Induction period	Antibody rise to peak	
A. First injection before irradiation and others after irradiation										
5	13	0.125 ml	4.07 ± .08	11.75	13.91 ± 3.06	1.3 ± .07	0.9 ± .07	8.6 ± 0.6***	7.8 ± 0.5	9
6		0.125 ml	Not tested							
7	18	10 ml	4.30 ± .12	19.93	26.75 ± 5.63	1.3 ± .10	1.1 ± .11	5.7 ± 0.4***	7.0 ± 0.5*	10
B. All injections after irradiation										
5	14	0.125 ml	3.11 ± .24**	1.29	2.98 ± 0.96**	0.9 ± .23*	0.6 ± .08*	11.3 ± 1.1***	7.5 ± 0.9	8
6	16	0.125 ml	1.57 ± .21***	0.04	0.06 ± 0.02***	0.6	0.3	14.4	5.6	2(4)
7	19	10 ml	3.67 ± .14***	4.68	6.14 ± 1.88**	1.0 ± .17**	0.8 ± .09	7.6 ± 0.1***	8.8 ± 1.1	7
C. Red cells alone: no X rays										
5	15	0.125 ml	3.96 ± .10	9.13	11.13 ± 2.54	1.4 ± .12	1.1 ± .20	3.4 ± 0.36	8.3 ± 2.10	8
6	17	0.125 ml	3.74 ± .15	5.50	6.23 ± 1.32	2.1 ± .37	1.5 ± .47	3.6 ± 0.31	5.9 ± 1.33	6
7	20	10 ml	4.13 ± .05	13.49	15.51 ± 1.68	1.6 ± .21	0.8 ± .13	2.5 ± 0.13	10.5 ± 1.11	22

* See first footnote in Table 1.

Antibody formation was studied in 187 rabbits in experiments 8 through 17 that were given 1.25 ml of 1 per cent red sheep cells per kg before or after total-body irradiation ranging from 10 to 700 r. When the antigen was given 2 days after exposures between 200 and 700 r, antibody formation was totally suppressed in some rabbits and markedly so in others. In those forming measurable amounts of antibody, the rate of the process of immunity was decreased markedly. A statistically valid suppression of antibody was induced by 200 but not by 100 r. The median suppression was calculated to be induced by 240 r (see Figure 2B).

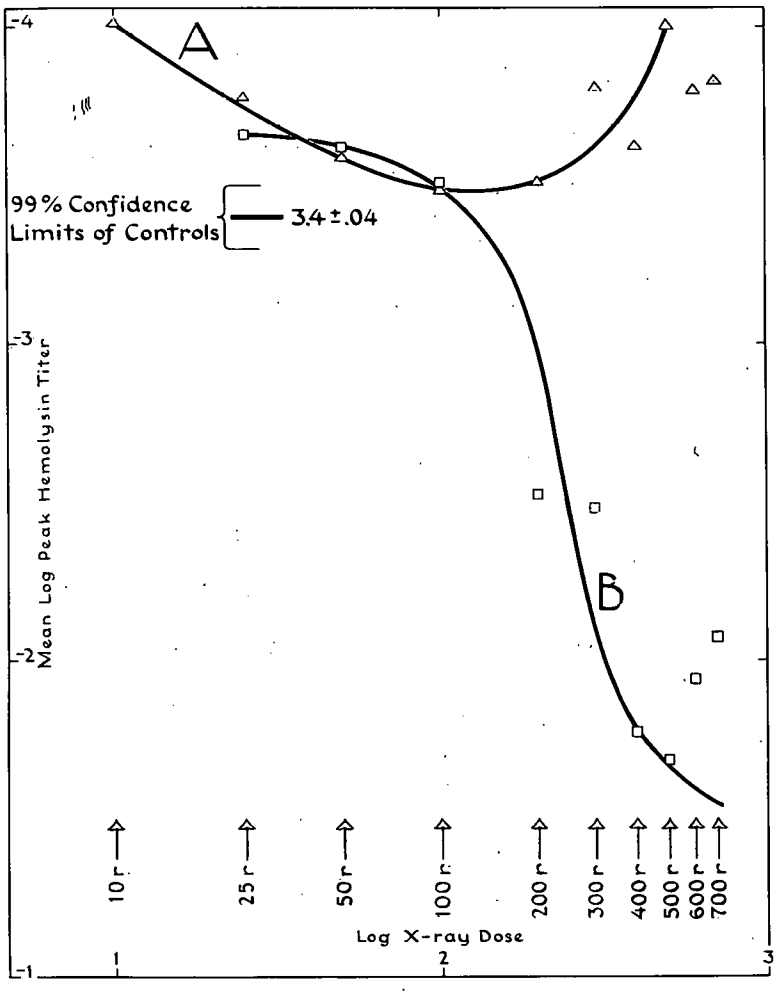

Figure 2. Dosage-response curve relating log peak hemolysin titer to log dose of X rays in experiments 8 through 17 (controls = series 40): 1.25 ml of 1 per cent red cells given (A) 1 to 4 hours before irradiation, and (B) 2 days after irradiation.

When the antigen was given 1 to 4 hours before irradiation at doses of 300 or 700 r, the enhancement of the mean peak titer was found to be associated with a marked retardation in the process of immunity. When 10 and 25 r were given, enhancement occurred with no inhibition of the immune process. These data are shown

in the dose-response curve in Figure 2A. Neither this nor the increased enhancement associated with exposures ranging between 300 and 700 r can shed any light on the reported curative action of X rays upon infections because any enhancing action absorbed before irradiation would be more than offset by the suppression of antibody from antigen that was liberated after irradiation.

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PRELIMINARY STUDIES ON THE EFFECT OF INTRAVENOUS INJECTION OF PLASMA FROM X-IRRADIATED, SPLEEN-SHIELDED MALE RATS ON ERYTHROPOIESIS IN NORMAL MALE RATS*

By

T. F. Dutcher and W. F. Bethard

A DISSERTATION

INTRODUCTION

In 1949, Jacobson and his associates[1] showed that spleen-shielding effectively increases the survival of animals subjected to total-body X irradiation. Since that time, a great amount of work has been done in the attempt to find the factor(s) released by the spleen and responsible for this increased survival. In 1952, the literature concerning the effect of spleen-shielding and allied techniques on the survival of mice, rats, rabbits, and dogs following total-body irradiation was reviewed by Jacobson.[2] He concluded from the available evidence that the factor(s) responsible for recovery from radiation injury is probably a humoral substance (or substances) produced by the living cells of the shielded or injected hematopoietic tissues and is capable of instituting the recovery of certain body tissues vital to survival.

Erslev[3] reported increased erythropoietic activity, as measured by increased erythrocyte counts, hematocrits, reticulocyte counts, and hemoglobin levels, in normal rabbits that were injected with plasma from rabbits made anemic by repeated bleedings. The amount of plasma given to each test animal approximated the plasma volume of the recipient rabbit and was given over a period of 4 to 8 days. He postulated that the increased erythropoietic activity was due to a humoral factor in the plasma of the anemic rabbits. The authors of this report thought that by using a technique similar to that of Erslev it might be possible to obtain more positive

* Based upon a dissertation submitted to the faculty of the School of Medicine of the Division of Biological Sciences, The University of Chicago, for a degree of M.D. with honors.

Editor's note: This work was performed by a senior medical student (T. Dutcher) and represents his ideas and plans. Faculty supervision was provided by Dr. Bethard. This paper will not appear in the open literature since the research is of a pilot nature and requires extension.

evidence for a humoral factor(s) in the circulating plasma of rats following total-body irradiation with the spleen shielded.

Since the red cell uptake of radioactive iron (Fe^{59}) is a more sensitive measure of erythropoietic activity than blood and reticulocytes counts, it was used in this experiment.

METHODS AND MATERIALS

Adult male Sprague-Dawley rats of the same age and of approximately the same weight (see tables) were divided randomly into 5 groups and were treated as follows:

A. Controls

B. Normal, nonirradiated plasma donors

C. X-irradiated, spleen-shielded plasma donors (X + L donors)

D. Recipients of plasma from the 2 donor groups

E. Saline recipients.

The animals in Group C were anaesthetized with Nembutal^{R} (I.P.), and the spleens were exteriorized and placed in lead shields. Each animal then received 550 r total-body X radiation, generated by a Maxitron machine operating at 250 kvp and 17.5 ma, with 0.5-mm Cu and 1.0-mm Al filters.

Table 1

THE EFFECT OF PLASMA FROM X-IRRADIATED, SPLEEN-SHIELDED RATS ON THE UPTAKE OF Fe^{59} (EXPERIMENT 1)*

Type of plasma received	Average body weight (g)	Ave. amount of plasma received (cc)	Ave. uptake of Fe^{59} (%)	Deviation from control
Control	404	0	58.1	-----
Normal	441	12	68.8	+10.7
1 hour after X ray X + L	415	11.3	62.4	+ 4.3
3 hours after X ray X + L	380	11.9	74.8	+16.7

*Sampled 4 days after the injection of Fe^{59} or 8 days after the first plasma injection.

Table 2

COMPARISON OF THE EFFECT OF SALINE AND PLASMA FROM X-IRRADIATED AND SPLEEN-SHIELDED RATS UPON THE UPTAKE OF Fe^{59} (EXPERIMENT 2)*

Type of plasma received	Average body weight (g)	Ave. amount of plasma received (cc)	Ave. uptake of Fe^{59} (%)	Deviation from control
Control	246	0	65.4	-----
Saline	233	8	57.0	- 8.4
Normal	215	8	79.0	+13.6
3 hours after X ray X + L	241	8	79.9	+14.5
6 hours after X ray X + L	256	8	64.6	- 0.8

*Sampled 4 days after the injection of Fe^{59} or 8 days after the first plasma injection.

Table 3

COMPARISON OF THE EFFECT OF SALINE AND PLASMA FROM X-IRRADIATED AND SPLEEN-SHIELDED RATS UPON THE UPTAKE OF Fe^{59} (EXPERIMENT 3)*

Type of plasma received	Average body weight (g)	Ave. amount of plasma received (cc)	Ave. uptake of Fe^{59} (%)	Deviation from control
Control	228	0	66.7	-----
Saline	239	8.0	68.6	+ 1.9
Normal	245	7.5	75.1	+ 8.4
2 hours after X ray X + L	252	7.2	76.5	+ 9.8
4 hours after X ray X + L	241	7.0	77.5	+10.8
6 hours after X ray X + L	242	8.6	74.0	+ 7.3

*Sampled 4 days after the injection of Fe^{59} or 8 days after the first plasma injection.

Animals from the irradiated group were sacrificed after X ray at 15 minutes; 1, 3, 8, and 12 hours; and at 2, 4, and 6 days, and all obtainable heart blood was withdrawn into a heparinized syringe. The blood from several animals that had been X-irradiated simultaneously was collected at the desired interval, pooled, and chilled in an ice bath until it was centrifuged at 2500 rpm for 30 minutes in a refrigerated room. Plasma from the nonirradiated donors was withdrawn and processed in an identical manner. The plasma thus obtained was refrigerated for no longer than 4 hours and was injected into the tail veins of the recipients in amounts ranging from 7.2 to 16 cc, depending upon the group. This routine was carried out on 3 or 4 consecutive days, depending upon the experiment; on the 4th or 5th day, respectively, all recipients received intravenous injections of known amounts of radioactive iron.

The control animals (group A) received no injections prior to Fe^{59}, which was given on the same day that the animals in the recipient groups were injected.

Table 4

THE EFFECT OF PLASMA FROM X-IRRADIATED, SPLEEN-SHIELDED RATS UPON THE UPTAKE OF Fe^{59} (EXPERIMENT 4)*

Type of plasma received	Average body weight (g)	Ave. amount of plasma received (cc)	Ave. uptake of Fe^{59} (%)	Deviation from control
Control	316	0	63.4	-----
Normal	322	16	75.0	+11.6
1 hour after X ray X + L	294	16	73.8	+10.4
4 hours after X ray X + L	298	16	68.2	+ 5.2
6 hours after X ray X + L	319	16	70.1	+ 6.7
8 hours after X ray X + L	342	16	70.9	+ 7.5

*Sampled 5 days after the injection of Fe^{59} or 10 days after the first plasma injection.

Table 5

THE EFFECT OF PLASMA FROM X-IRRADIATED, SPLEEN-SHIELDED RATS UPON THE UPTAKE OF Fe^{59} (EXPERIMENT 5)*

Type of plasma received	Average body weight (g)	Ave. amount of plasma received (cc)	Ave. uptake of Fe^{59} (%)	Deviation from control
Control	231	0	69.9	-----
Normal	238	9	77.4	+ 7.5
15 minutes after X ray X + L	250	9	74.0	+ 4.1
1 hour after X ray X + L	243	9	77.5	+ 7.6
3 hours after X ray X + L	243	9	76.0	+ 6.1
8 hours after X ray X + L	237	9	77.0	+ 7.1
12 hours after X ray X + L	251	9	78.2	+ 8.3
18 hours after X ray X + L	256	9	74.2	+ 4.3
24 hours after X ray X + L	186	9	69.7	- 0.2
2 days after X ray X + L	240	9	61.7	- 8.2
4 days after X ray X + L	241	9	51.4	-18.5
6 days after X ray X + L	233	9	47.3	-22.6

*Sampled 4 days after the injection of Fe^{59} or 8 days after the first plasma injection.

Four days after injection of the radioisotope, 0.5 ml of blood was withdrawn from the tail vein into a heparinized pipette, lysed in 1 ml of distilled water, and counted in a well-type scintillation counter. Appropriately diluted standards were counted simultaneously. The blood volumes (using P^{32}) were done in the first few experiments. Thereafter, 5 per cent of the total-body weight in g was used as the basis for determining the blood volume in ml.[4]

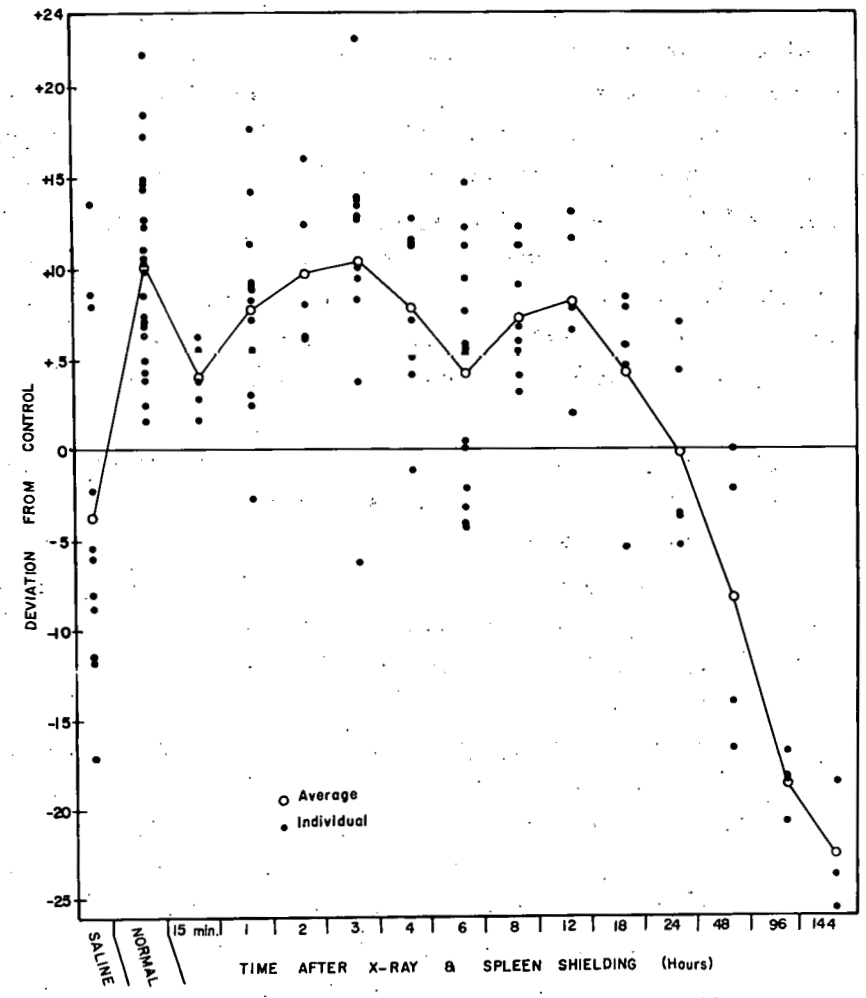

Figure 1. Percentage of Fe^{59} uptake per total blood volume (expressed as deviation from control animals) in normal adult male rats following the intravenous injection of plasma obtained from adult male spleen-shielded rats at various intervals following total-body X irradiation.

RESULTS

Five experiments were completed, and the data for each and the average of all the experiments are presented in Tables 1 to 5 and Figure 1.

In all experiments, except experiment 4, an attempt was made to inject an amount of plasma that would be approximately equivalent to the original plasma volume of the recipient animal (7 to 12 ml). In Experiment 4, the volume of the plasma injected was designed to approximate the total blood volume of the recipient (16 ml). No significant differences in the response of these animals could be detected when compared with animals in the other four experiments.

Figure 1 shows the per cent of Fe^{59} taken up by the red blood cells (expressed as deviation from the control animals) in each animal. The rather wide scatter is considered to reflect biological differences, the arbitrary use of 5 per cent of the body weight as equivalent to the total blood volume, and differences in the weights and ages of the animals. However, it is believed that the averages are an accurate representation of the effect of the injected plasma on erythropoiesis in rats.

Comparison of control and saline injected groups: Upon completion of experiment 1 (Table 1), it was thought that the observed increased uptake in the groups receiving plasma might be due to dilution only; therefore, in the next two experiments (Tables 2 and 3) a group of saline-injected recipients was included. The results indicate clearly that dilution alone plays no part in the increased erythropoiesis.

Comparison of control and other groups: In terms of statistical significance (as determined by the Fisher t test), there was a real difference between the uptake of FE^{59} by the controls and all other groups except those that received plasma from spleen-shielded donors that were sacrificed at 15 minutes and at 18 and 24 hours after X irradiation. The greatest increase in radioiron uptake was in the groups that were given normal plasma and plasma obtained from spleen-shielded rats 3 hours after X irradiation.

Comparison of normal and other groups: There was a statistically significant difference between the group receiving normal plasma and the controls, saline recipients, and the recipients of plasma from spleen-shielded animals 15 minutes, 6 hours, and from 18 hours to 6 days following X irradiation. The differences between other groups were not statistically significant. The animals that received plasma obtained from spleen-shielded animals 3 hours after X irradiation had the same relation to other groups as did the normal plasma recipients. The recipient animals that were given plasma obtained from spleen-shielded rats later than 18 hours after irradiation had a significantly decreased radioiron uptake as compared with all other groups.

It was noted that the animals in experiment 5 that had received plasma obtained from spleen-shielded rats at 24 hours and 6 days following X irradiation appeared to be losing weight gradually. For this reason, a daily record of weight was kept for the animals in several groups, including the control. Although the weight of the animals was not recorded at the beginning of the experiment, it was

assumed that they all weighed between 200 to 220 g. This assumption was based on the knowledge that the donor rats from the same shipment weighed within this range when the Nembutal^{R} was administered.

The weight of the recipient animals that received plasma obtained from spleen-shielded rats 24 hours following X irradiation gradually decreased until an average weight loss of approximately 40 g was reached on the 7th day following the first injection of plasma. Thereafter, they regained weight, and by the 14th day following the first plasma injection, the weights were approximately at their original level. The weight of the animals that were given plasma obtained 6 days after X irradiation followed a similar though more rapid course. The maximum weight loss, however, was less (10 g) in this group and occurred on the 4th day following the first injection of plasma. On the 10th day after the first injection of plasma, the weights of these animals were the same as those of the controls. Other groups showed a continuous gain in weight that was comparable to that of the controls.

DISCUSSION

The data suggest the presence of an erythropoietic factor(s) in the plasma of normal adult male rats and in the plasma of adult male rats that have been subjected to 550 r total-body irradiation with the spleen shielded. This factor(s) is present for at least the first 18 hours and is maximally effective from 1 to 4 hours and from 8 to 12 hours following the irradiation. It cannot be stated yet that the factor(s) present in the plasma of the irradiated rats is identical with that in the plasma of normal rats, nor with the factor demonstrated by Erslev[3] in the plasma of anemic rabbits. It is of interest that Erslev did not find evidence for an erythropoietic factor in the plasma of normal rabbits.

A marked decline in erythropoiesis was observed after the administration of plasma collected from rats 2 to 6 days after X irradiation with spleen-shielding. Jacobson[5] has shown that marrow suspensions taken from mice at approximately the same intervals following total-body irradiation have an almost immediately lethal effect on about 50 per cent of the recipient mice. It is possible that the shielded spleen has the capacity to produce the factor(s) in a limited quantity, possibly in cyclic pattern, in response to total-body X irradiation. When the spleen becomes exhausted, the toxic products resulting from irradiation might reach such a high concentration that erythropoiesis is inhibited. On the other hand, it may be that substances are responsible for the cessation of splenic action insofar as the production of an erythropoietic factor(s) is concerned.

It is also possible that the spleen is the target organ of a normally circulating factor elaborated elsewhere in the body and responds to this factor by producing an erythropoietic factor(s). After total-body X irradiation, the organ that normally

produces this spleen-stimulating factor would be nonfunctional; however, the protected spleen could be expected to continue elaborating an erythropoietic factor(s) for a short time in response to the still circulating spleen-stimulating factor. The converse must also be considered; namely, that the spleen is the initiating organ and another body tissue is the target organ and producer of an erythropoietic factor(s).

If an erythropoietic factor(s) is produced by the spleen, then it is not known how this is delivered into the plasma. Cell-free extracts of the spleen have failed to enhance the survival of mice subjected to total-body X irradiation, although splenic homogenates do have a marked effect on survival and on the recovery of blood-forming tissue.[2] Thus far, it would appear that living cellular elements that are capable of elaborating some as yet unknown substance(s) may be essential for the regeneration of hematopoietic tissues following irradiation. Circulating white blood cells or platelets in the plasma of normal donors cannot be overlooked as possible sources.

It must be emphasized that there is little known direct evidence in support of any one or a combination of the possibilities mentioned above. Nor do these three hypotheses exhaust the possible explanations for the observed results. Further experiments are being planned which, it is hoped, will serve to clarify many of these problems.

More observations are necessary to confirm the observed loss of weight described above.

SUMMARY

Adult male rats were given 550 r total-body X irradiation with lead-shielding of the spleen. These rats were sacrificed at intervals of from 15 minutes to 6 days following the X irradiation. Plasma was obtained and injected intravenously into normal adult male rats. The recipient rats then received a single intravenous injection of Fe^{59}. The Fe^{59} uptake, as percentage of total blood volume, was determined on the 4th day after the injection of Fe^{59}. Groups of animals receiving plasma from normal rats, saline, and only Fe^{59} served as controls.

There was an increased uptake of iron in the animals receiving normal plasma and plasma obtained at intervals of 1, 2, 3, 4, 6, 8, and 12 hours following X irradiation of the spleen-shielded donors when compared to the control animals that received only radioiron. There was no significant difference between these control animals and those that received saline or plasma obtained at intervals of 15 minutes, 18 and 24 hours following X irradiation. There was markedly decreased uptake in the groups received plasma obtained at intervals of 2, 4, and 6 days following the X irradiation.

The significance of these findings relative to the presence of an erythropoietic factor(s) in the plasma of adult male rats is discussed.

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ON THE NATURE OF THE SPLENIC RADIATION RECOVERY FACTOR*: CELLULAR OR HUMORAL?

By

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INTRODUCTION

In the five years since Jacobson et al.[1] demonstrated the effect of the intact spleen on recovery from otherwise lethal exposures to X radiation there has been a great deal of effort expended in attempting to determine whether this effect is due to a humoral mechanism. An alternative explanation is that of transplantation and subsequent recolonization of the irradiated animal by healthy spleen cells. Until recently, the bulk of the evidence supporting the humoral hypothesis was indirect (for a summary see (2)). However, within the past year and a half, a group at the Naval Radiological Defense Laboratory in San Francisco has published results of experiments that they claim demonstrate, in a direct fashion, that the splenic radiation recovery factor is noncellular in nature.[3-5]

In those experiments, the donor spleens were homogenized in a glass Potter-Elvehjem homogenizer in an appropriate medium, then separated into fractions by centrifugal methods. The most easily sedimentable fraction, which contains nuclei, appreciably increased survival after lethal doses of X rays.[4] They found, in addition, that this nuclear fraction was inactivated by incubation at room temperature with trypsin or deoxyribonuclease (DNase). From these data, the California group concluded that the responsible factor was a deoxyribonucleoprotein, which is associated with the nucleus of the cell.[5]

The purpose of this report is to examine these conclusions in the light of some data accumulated in this laboratory. Special emphasis will be given to the necessity for as rigorous proof as possible to support the humoral hypothesis. An admirably succinct and critical review of the claims for the humoral nature of the radiation recovery factor has been published recently.[6]

MATERIALS AND METHODS

The mice used were strain LAF_1† DBA/2‡, or CF No. 1.** Irradiation was done with a G. E. Maxitron with the following factors: 250 kvp, 30 ma., 79-cm,

* While the word "factor" will be used in the singular in this report, it must be borne in mind that more than one factor may be involved in promoting recovery from radiation damage.

† Bred in this laboratory.

‡ Obtained from Jackson Memorial Laboratory, Bar Harbor, Maine.

** Obtained from Carworth Farms, New City, New York.

target distance; filtration: 0.25 mm Cu and 1 mm Al; and dose rate, 60 r per min. The DNase was obtained from the Worthington Biochemical Corporation, and three separate lots were used: D 409, D 434, and D 436. The deoxyribonucleic acid (DNA) used in some control experiments was prepared from calf thymus. The xanthine oxidase was also obtained from Worthington. Ribonuclease (RNase) was purchased from Armour and Company.

Table 1

EFFECT OF SPLEEN HOMOGENATE ON SURVIVAL

Group	30-day survival	
	Fraction	Per Cent
"Nuclei"*	10/10	100
Supernatant	4/10	40
Medium	2/10	20

* "Nuclei" were prepared according to method of Cole et al. [4] from 79 5- to 21-day LAF_1 spleens. The supernatant fraction consisted of pooled first and second supernatants.

Note: Recipient (10-week LAF_1) had received 750 r total-body X radiation; then intraperitoneal injection of 1 ml of fraction, corresponding to 7.9 spleens per mouse.

For the preparation of "nuclei" or of unfractionated homogenates, a small, motor-driven, glass Potter-Elvehjem homogenizer was used. All steps in the procedure, including centrifugations, were carried out at about 4°C. Except for the experiment recorded in Table 2, unbroken cell suspensions were made by repeated injection of the medium through a fine needle into the excised spleen, which was supported on a tilted cold Petri dish. This method leaches out a large number of intact cells that are capable of counteracting the lethal effects of X irradiation.[7]

RESULTS AND DISCUSSION

Our early attempts to prepare cell-free material from spleen or bone marrow, which would be effective against X irradiation, were uniformly unsuccessful. After the report by Cole et al.[4], we were able to confirm, under the identical conditions used, the finding that homogenates of LAF_1 baby spleen were efficacious (Table 1).

Table 2

EFFECT OF DNASE UPON WHOLE SPLEEN CELLS

Group	28-day survival	
	Fraction	Per cent
Cell suspension	6/10	60
Cell suspension + DNase*	6/10	60
Cell suspension + RNase†	6/10	60
X ray only	0/14	0

* 1 mg DNase (lot D 409) added.

† 1 mg ribonuclease (RNase) added.

Note: Spleens from 40 2- to 10-day CF No. 1 mice were broken up by ejection through #24 syringe needle. The final suspension had 13 million cells per cc. Aliquots were allowed to stand, with occasional shaking at 22° for one hour. 0.4 cc/mouse injected intravenously into mice exposed to 900 r.

However, microscopic examination of the "nuclei" preparation, which was stained with methyl-green-pyronin, revealed a large number of nuclei surrounded by a rim or crescent of cytoplasm. These could not be distinghished, visually, from some cell types seen in a smear of spleen cells prepared by a method that would not break the cellular integrity.

Table 3

EFFECT OF DNASE ON SPLEEN "NUCLEI"

Group	30-day survival	
	Fraction	Per cent
"Nuclei"*	3/8	41
DNase treated "nuclei"†	3/8	41
"Nuclei" treated with DNA hydrolyzate‡	3/8	41
X ray only	0/8	0

* "Nuclei" were prepared from 30 4- to 5-week LAF_1 mice according to method of Cole et al. [4] Approximately 57 mg of spleen equivalent was injected intraperitoneally per mouse. Recipient mice received 750 r. All suspensions of "nuclei" contained $MgCl_2$ (0.005 M) and were shaken for one hour at 24°.

† 0.7 mg DNase (lot D 434) added to "nuclei."

‡ 10 mg calf thymus DNA + 0.4 mg DNase (lot D 434) + 0.005 M $MgCl_2$ incubated 2 hours at 36° then 10 minutes at 100°, cooled and added to "nuclei."

Since survival after X irradiation could not be expected to serve as a criterion of the possible effects of "naked" nuclei and whole cells, we utilized another approach to determine whether large numbers of functionally intact cells were present after homogenization; i.e., the capacity to transmit mouse leukemia. Dr. Simmons of this laboratory had already determined the relationship between time of death from leukemia and number of leukemic spleen cells administered to the experimental group of mice.[8] Using these data, we were then able to determine how many functional cells remained in a preparation after homogenization according to the method of Cole et al.[4] When homogenates of leukemic DBA/2 spleen, equivalent to one adult spleen (130 mg) per mouse, were injected into DBA/2 recipients, 10 out of 10 mice died by the 13th day. This indicated that a minimum of 3 to 5 million cells had been injected. This number of normal baby spleen cells was greater than the number required for demonstrable survival of irradiated mice.

The California group used a different method of determining whether the effect they observed with spleen homogenates was due to nuclei or to whole cells. As mentioned earlier, they had concluded from inactivation studies with DNase and trypsin that the effect was due to nuclei. This conclusion rests on the assumption that the enzymes used would have no effect on whole, viable cells. If this assumption is true such experiments would be expected to give an unequivocal answer to the question of whole cells vs. nuclei, providing the essential controls were carried out. It seemed pertinent, therefore, to investigate the possibility that DNase could act on whole cells as well as to determine whether the observed effect of DNase was due to a) a nonenzymic contaminant, b) the products of the enzymic hydrolysis of DNA, or c) contamination of the DNase with a proteolytic or some other enzyme. This last possibility assumed more importance when we determined that trypsin did cause a marked reduction in the cell count of suspensions of whole spleen cells.

The action of DNase upon whole spleen cells was tested and the results are given in Table 2. It can be seen that there was no difference between the treated and untreated groups, indicating that either the enzyme has no effect on the cells or that the number of cells present was sufficiently large so that destruction of a small number of them would not be detected. An experiment of the same sort was reported by Cole et al.[9], who used suspensions of rat marrow.

When a similar experiment was performed with a "nuclear" preparation from spleen, again no effect of DNase was seen (Table 3). In addition, this experiment was designed to test the first two possibilities described above. When the "nuclei" were incubated with the products of hydrolysis of DNA by DNase, after the enzyme was destroyed by boiling, no inactivation was seen. However, since in this experiment the DNase did not have any effect on the "nuclei," this control has no significance.

The effect of a different batch of DNase upon whole cells was tested, and the results are summarized in Figure 1. While the survival data are not unequivocal, it seems quite clear that the preparations treated with DNase had lost the capacity to extend the lifetimes of the irradiated mice. Since these data contradict those of Table 2, it was desirable to determine whether the commercially obtained DNase preparations had any other detectable enzymic activities (e.g., protease) and if so, whether these other activities were constant from batch to batch. Unfortunately, of

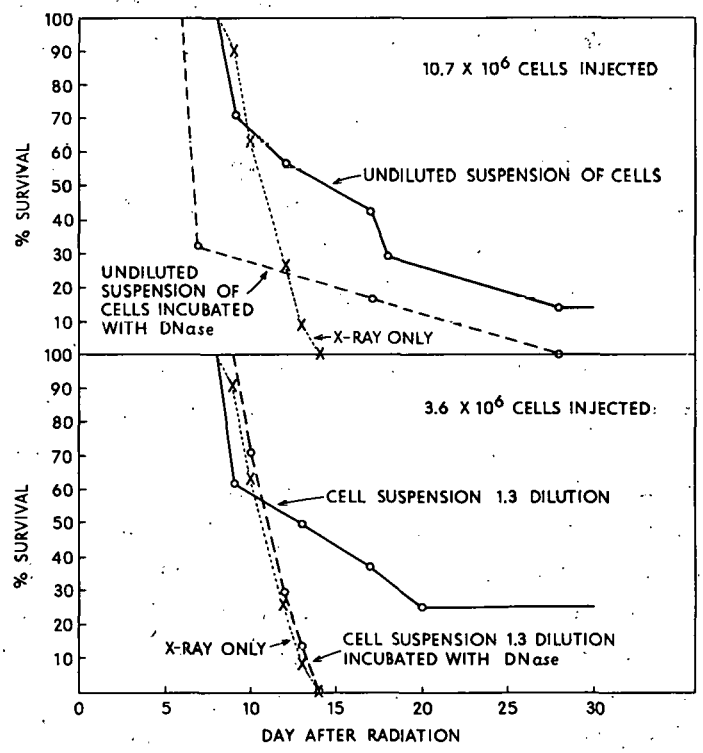

Figure 1. Cells were leached out of 95 4- to 5-week CF No. 1 spleens with medium described by Cole et al. (4), centrifuged down, then diluted to a concentration of 21.4 x 10^6 cells per ml. Appropriate sized aliquots were pipetted into 10 ml flasks, medium was added so that all flasks contained 4.0 ml, and to the treated ones were added DNase (lot D 436) in the same medium to a final concentration of 0.3 mg per ml, and $MgCl_2$ to a final concentration of 0.006 M. All flasks were allowed to stand with occasional shaking at 25° for 60 minutes, then the suspensions were injected intravenously into 10- to 12-week CF No. 1 female mice that had been given 750 r. The X-ray only curve is from the same group in both parts of the figure.

the three lots of DNase used in the experiments mentioned previously, only the last (D 436) was still available for testing. We hope to be able to test the other batches in the near future.

Since only minute amounts of protease might be expected, a highly sensitive method of detection was used. A solution of DNase in buffer was allowed to incubate for 60 minutes at room temperature with a very small amount of xanthine oxidase (lot 436), along with a control containing all constituents of the test system

except for the DNase. After the hour, an excess of hypoxanthine was added, and the extinction at 293 mμ was read as a function of time to determine the amount of enzyme present.[10] This experiment is summarized in Figure 2. It can be seen that 0.8 mg of DNase caused a fall of about 35 per cent in the activity of the xanthine oxidase preparation. The slope of the control curve is 0.002 while the slope of the inhibited curve is 0.0013. When one-half the amount of DNase was used, the observ-

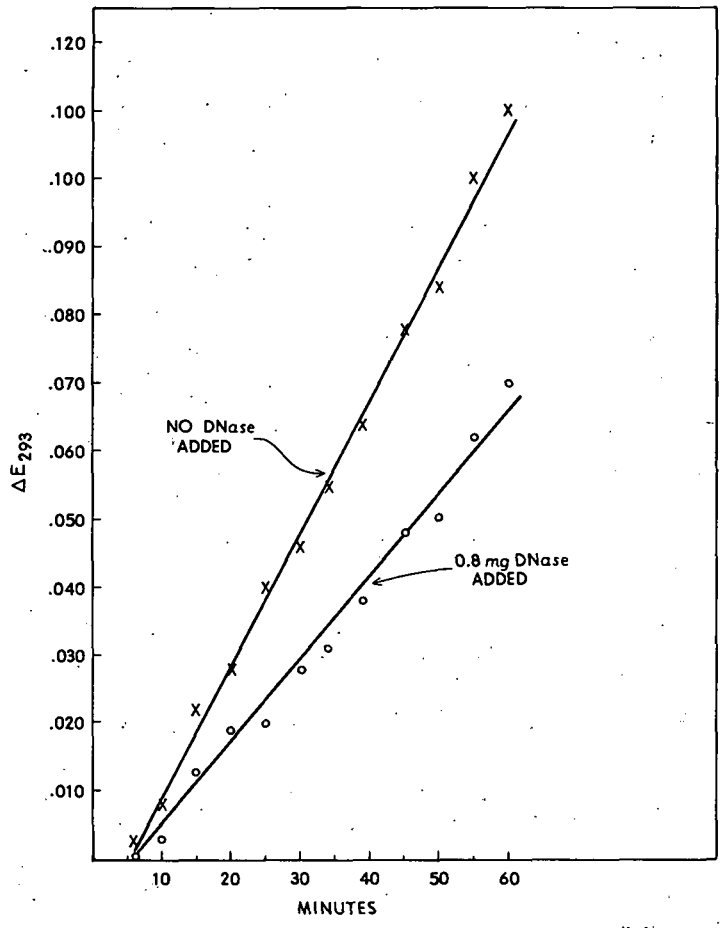

Figure 2. 0.4 ml of DNase (2 mg per ml lot D 436) in M/10 phosphate pH 7.35, 0.015 ml of xanthine oxidase (1.2 dilution of dialyzed enzyme), 0.050 ml of 0.05 M $MgCl_2$ and 0.50 ml of phosphate buffer were allowed to stand at 25° for 60 minutes. The control had 0.90 ml of buffer plus the same amounts of xanthine oxidase and $MgCl_2$. After incubation, 0.050 ml of hypoxanthine (0.001 M) were added to both, mixed, and extinctions were read.

observed inhibition was about one-half (slope = 0.0016). This type of evidence cannot be interpreted as meaning that the DNase is contaminated with protease since xanthine oxidase is a flavo-protein, and it is possible that the DNase preparation in some way acted on the flavin prosthetic group. The effect of DNase on other enzymes is being studied at present to determine whether the inhibition observed is a general one. Regardless of the interpretation of the results shown in Figure 2, it is

clear that inactivation by DNase preparations does not necessarily mean that the breakdown of DNase is the only process that occurs since it is quite a certainty that xanthine oxidase is not a deoxyribonucleoprotein.

From the data presented in this report, it seems clear that the noncellular nature of the radiation recovery factor still remains to be demonstrated.

SUMMARY

It has been determined that some preparations of commercial deoxyribonuclease (DNase) have no appreciable effect on either suspensions of spleen cells or spleen nuclear preparations on the basis of studies of survival of mice exposed to lethal doses of X rays.

A different lot of commercial DNase, however, displayed a significant effect on whole spleen cells, indicating that DNase can affect whole cells or is contaminated with something which can.

The same lot of DNase has been shown to inactivate xanthine oxidase, which indicates that the DNase is not completely free of contaminants.

These data make less certain the claim that the splenic agent responsible for survival after lethal exposures to X radiation is a deoxyribonucleoprotein.

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THE LIGHT INSENSITIVITY OF THE LIEBERMANN-BURCHARD REACTION DURING SPECTROPHOTOMETRIC DETERMINATION OF CHOLESTEROL*

By

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SUMMARY

Since Grigaut[1] first introduced a procedure for the quantitative estimation of cholesterol utilizing the Liebermann-Burchard reagent, in 1910, the question of whether light has an effect on this reaction has apparently never been adequately answered. While the majority of investigators state that color development should take place in the dark[2-9], others[10-12] have minimized the importance of light.

The discrepancies between these and other reports may be resolved by examining the whole absorption curve of the developed color in the dark and in the light. While the optical density of the two solutions measured from 580 to 740 mμ is the same, the difference in shade that is seen can be explained by the light sensitivity of the solution in the region of 540 to 340 mμ. It thus becomes apparent that the effect of light is of importance when a visual colorimeter is used, but not with a spectrophotometer since the latter can be made to measure more specifically those portions of the curve that are not affected by light.

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