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THE METABOLISM OF AMERICIUM IN THE RAT*

By

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Crocker Radiation Laboratory
University of California
Berkeley, California

December 15, 1947

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ABSTRACT

An investigation of the metabolism of americium (element 95) in the rat has been conducted following its administration by intramuscular injection and by stomach tube.. Each animal received .3 micrograms of $AmCl_3$ in 1 cc of isotonic saline at pH 5. No appreciable absorption took place from the digestive tract. Within 24 hours after parenteral administration, 55% of the total amount of americium absorbed from the site of injection was deposited in the liver and 20% was accumulated by the skeleton. Excretion occurred primarily by way of the digestive tract and most of the americium eliminated appeared to come from the liver, which released this element at an almost exponential rate for 30 days with a half-time of 10 days. Thereafter, excretion became progressively slower. There was no significant change in the content of americium in the skeleton throughout the entire interval of the experiments, which extended for 256 days. The only other tissues studied that demonstrated any striking degree of accumulation of americium were the kidneys and spleen. The distribution of americium in the bone was studied by means of the radicautographic technique and it was observed that deposition of this element occurred in the region of the osteoid matrix and about the small blood vessels of the cortex.

University of California
Radiation Laboratory
Berkeley, California

Contract No. W-7405-eng-48A

THE METABOLISM OF AMERICIUM IN THE RAT

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For research, development, or manufacturing work.

December 15, 1947

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The announcement of the discovery of element 95 was made in 1946 by Seaborg and his associates (1). This new element, named americium by its discoverer, is preceded in the periodic table by plutonium. Like neptunium (element 93), plutonium and curium (1), (element 96), americium is radioactive and has not been observed in nature in appreciable amounts. During the war an extensive study was made of the metabolic properties of plutonium (2) and several other members of the heavy elements, notably, thorium, protoactinium (3), uranium (4) (5), and neptunium (3). Similar studies were also made with a large number of the radioactive elements which arise from fission (6). This work was done at laboratories of the Manhattan project located in different parts of the country. Particular attention was directed towards a very detailed investigation of the metabolism of plutonium in rats and other laboratory animals. These studies, initiated at the University of California at Berkeley, were done also at the University of Chicago, Oak Ridge, and Los Alamos.

The outstanding characteristic of the metabolism of plutonium is the high degree of localization and prolonged retention of this element in the skeleton following parenteral administration. Unlike calcium, strontium, barium, and radium, plutonium is not deposited primarily in the mineral structure of the skeleton, but rather is laid down in the region of the osteoid matrix of the bone (7). At the present time, it cannot be definitely stated whether or not all of the plutonium so accumulated is in the organic components of the osteoid matrix. A significant fraction of plutonium may be laid down on the adjacent surfaces of the mineral structure of the bone. If this be so, the amount of penetration in the normal adult rat has been demonstrated by the radioautographic techniques to be less than 25 microns. The accumulation of plutonium in the other tissues of the body following parenteral administration is quite small as compared to the deposition in the skeleton, and moreover, its elimination from the soft tissues is relatively rapid as compared to its extraordinary high degree of retention by the skeleton. Plutonium is not absorbed to any appreciable extent from the digestive tract. In view of these interesting and obviously dangerous metabolic characteristics of plutonium it was felt that a study of the behavior in the body of its recently discovered and immediate chemical neighbor might prove to be of interest.

EXPERIMENTAL PROCEDURES

The isotope of americium employed, Am^{241}, has a half-life of five hundred years and emits alpha particles to form Np^{237} which in turn decays by alpha particle emission with a half-life of 2.25 million years to produce Pa^{233}. The presence of Np^{237} was of no practical concern in the experiments to be described since its half-life is more than four thousand times longer than that of the americium isotope employed, and thus the existence of the neptunium and its radioactive descendents was not detectable by the procedures employed in these studies.

Ten micrograms of americium, which was made available to us by Professor G. T. Seaborg and his associates, was obtained as a solution of $AmCl_3$ in one cc of one normal hydrochloric acid. This original solution was diluted with normal saline and carefully brought to pH 9 by the addition of dilute sodium hydroxide. Any further neutralization would have resulted in the precipitation of the highly soluble americium hydroxide. The final solution contained 0.3 micrograms of americium per cc with a radioactivity of 1 microcurie. One cc of this solution was administered by intramuscular injection into the left hind leg of each of 15 adult white rats and the same amount by stomach tube to three more animals. In addition, two rats received 5 micrograms each, by intramuscular injection, for the purpose of preparing radioautographs to study the distribution of this element in bone. The fifteen animals that received the 0.3 micrograms by intramuscular injection were divided into groups of three and were placed in metabolism cages which made it possible to collect daily specimens of both the urine and feces. The five groups of rats were sacrificed at 1, 4, 16, 64, and 256 days. The three rats which were given americium by stomach tube were placed in one metabolism cage and daily collections of urine and feces made. They were sacrificed after an interval of sixteen days. The rats for bone radioautographic study were sacrificed at 16 days. The rats were sacrificed by means of chloroform and the thorax was immediately opened to permit the withdrawal of from 1 cc to 5 cc of blood from the heart. The left leg, which was the site of the injection, was removed at the pelvic girdle for separate assay. The remainder of the animal was skinned, and the following organs and tissues were removed and weighed for separate assay: liver, kidney, spleen, heart, lungs, muscle, bones of the right leg, and gastrointestinal tract. These tissues, the left hind leg, excreta, skin, and remaining carcass, were dried for two days at 100° C. and then ashed at from 500 to 600° C. for 24 hours. Preliminary tests demonstrated that no measurable amount of the americium was lost by volatilzation at the temperature employed for ashing the tissues. The carcass, which was dried and ashed in one piece, was carefully sifted through a fine mesh screen so that the skeleton was separated almost completely from the ash of the other remaining tissues in the carcass, which were chiefly muscle, blood and fat. The procedure employed for the assay of americium in the tissues and excreta is described in the appendix.

The results obtained from the assaying of the content of americium in the different organs and tissues are expressed in terms of the percent uptake of this element on a per organ and per gram

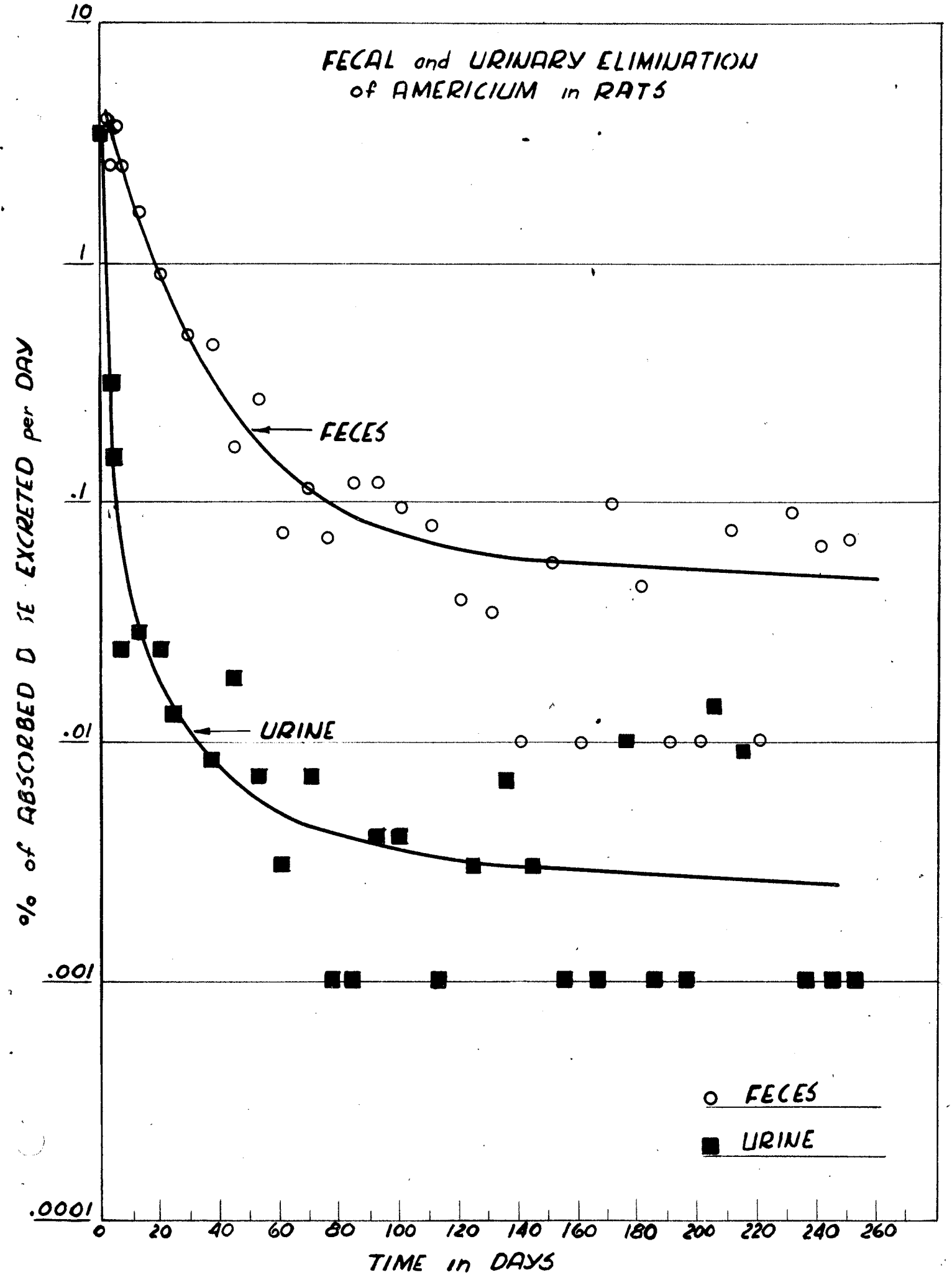
wet weight basis. The left hind leg, which was the site of injection, was assayed as a unit. The carcass ash, after separation of the skeleton by the sifting procedure mentioned earlier, was assayed as well as the skeleton itself. The urine and feces from each group of three animals were likewise ashed and assayed and the values obtained divided by three, which thus gave the average value of excreta for each of the three animals. The total content of americium in all of these organs, tissues and excreta, was obtained by adding up the individual values and this was compared against the amount given. This computation is, of course, a quantitative indication of the recovery of the administered americium and represents the fraction of americium given which could be determined quantitatively by the analytical procedures employed in these studies. The results were fairly satisfactory because the average recovery value observed in the series of 18 rats was 87% and the variations in the different groups ranged from 75% to 100%.

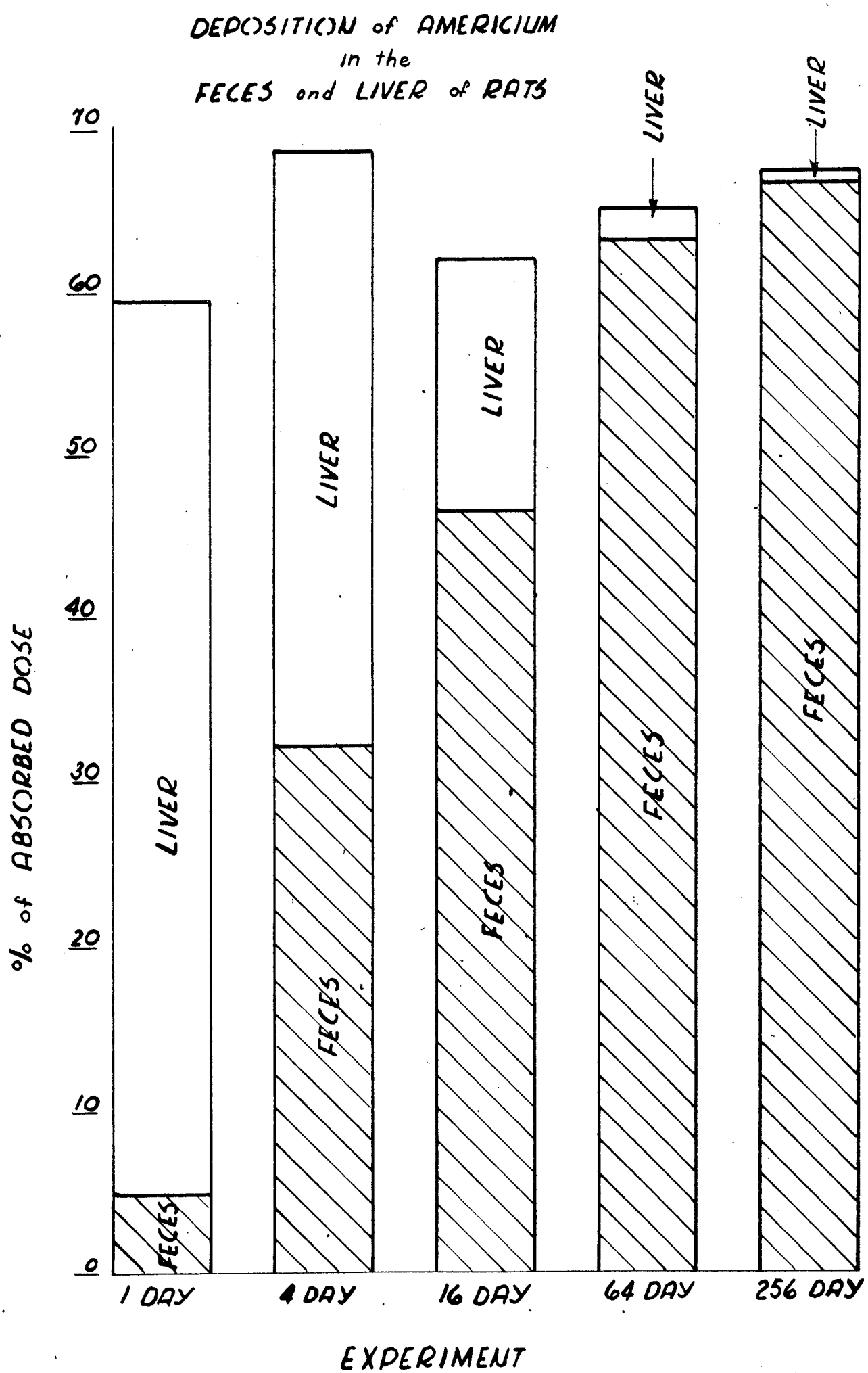
The average measured content of americium in different organs, tissues, and the excreta for the intramuscular experiments are shown in Table I. The amount in the skeleton was obtained by adding twice the measured americium content of the bones of the right hind leg to the value secured from the assay of the major portion of the skeleton that was separated from the carcass ash. This was done to correct for the americium content of the bone of the injected left hind leg, which was not included in the rest of the skeleton. The necessity for doing this arose from the fact that from 20 to 60 percent of the injected americium was retained at the site of administration. Presumably this was due to the precipitation and adsorption of insoluble compounds of americium at the injection site. It is of interest to note that most of the americium remaining behind in the injected leg was found to be in the soft tissue, although the content in the bones of that leg was of the order of twice that of the opposite injected leg. It was not determined whether this was the result of extravasation of the injected solution onto the bone surfaces followed by adsorption or occurred during the ashing procedure. The relatively small excess of americium in the carcass ash over the estimated value derived from the measured samples of blood and muscle was probably due to the presence of small fragments of carcass skeleton which passed through the screen employed for sifting out the bones. The estimated americium content of muscle and blood were made on the basis of the per gram values noted for these two structures and assuming that their total weights in the intact animal were 45% and 6% respectively of the total body weight. In Table II the data is presented after correcting for the unabsorbed fraction in the injected leg and for the deviation of the measured recovery value from 100%. The rate of excretion of americium is shown in Figure I for both urinary and fecal elimination. The relative proportion of americium appearing in the liver and feces is graphically indicated in Figure 2 and the change of total americium content in the liver and skeleton appears in Figure 3.

For the purpose of securing bone radioautographs, thin histological sections, which ranged from 5 to 8 microns in thickness, were prepared from the undecalcified femur by the techniques developed by McLean and Bloom (8) and by Axelrod (9). The relatively large dose of americium employed was necessary in order to secure enough activity in the sections to obtain satisfactory radioautographs. It is believed to be obligatory to avoid decalcification procedures in the preparation of the sections for taking the radioautographs so that the possibility of either leaching or migration of the deposited material be avoided. Plate I is a photomicrograph of a section of undecalcified femur and its corresponding radioautograph showing the distribution of americium in this bone. In Plate II, a small region of the bone section and its corresponding radioautograph shown in Plate I are pictured at a much higher magnification.

The observed and the corrected data for the intramuscular series shown in Tables I and II presents an interesting finding, namely, the high degree of accumulation in the liver and bone. In the case of the liver, most of the accumulated americium is removed at a fairly rapid rate, presumably by way of the bile, since the quantity of americium in the feces paralleled that lost by the liver and this reciprocal relationship is indicated in Figure 2. After most of the americium is eliminated from the liver, it can be seen in Figure 1 that the excretion is very slow but still the digestive tract acts as the principal channel of elimination. During the entire period of these experiments, including the 256 day interval, there was no significant decrease in the content of americium in the skeleton. A comparison of the uptake and retention of americium by the liver and bone is given in Figure 3. The only other soft tissues that show significant accumulation are the spleen and kidneys, whose initial content on a per gram basis at the earlier time intervals, is considerably lower than that of the liver. The fraction of americium absorbed from the digestive tract following oral administration was observed to be less than .01% of the 0.3 microgram dose given to each rat.

The radioautographic studies of the deposition of americium in the femur of the rat suggests that this substance is deposited in the region of the osteoid matrix, Plate I. In addition to the fixation of this element in this structure, it will be noted that there is a very spotty localization in the cortical bone. When this was first observed, it was felt that the americium present in the mineral portion of the bone had been fixed in the region of the small blood vessels. In Plate II, which is at a higher magnification, it will be seen that this surmise would appear to be correct in that there was definite evidence of accumulation of americium in the region of the small blood vessels. The resolution of radioautographs was not sufficient to determine whether this accumulation was limited to the blood vessel walls or actually extended out into the surrounding mineral structure of the bone for a distance of from 10 to 20 microns. Further technical developments in the field of radioautography must be successfully applied to this problem before this issue can be settled.


FECAL and URINARY ELIMINATION
of AMERICIUM in RATS
% of ABSORBED DOSE EXCRETED per DAY
10
1
.1
.01
.001
.0001
FECES
URINE
FECES
URINE
0
20
40
60
80
100
120
140
160
180
200
220
240
260
TIME in DAYS


DEPOSITION of AMERICIUM
in the
FECES and LIVER of RATS
% of ABSORBED DOSE
0
10
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30
40
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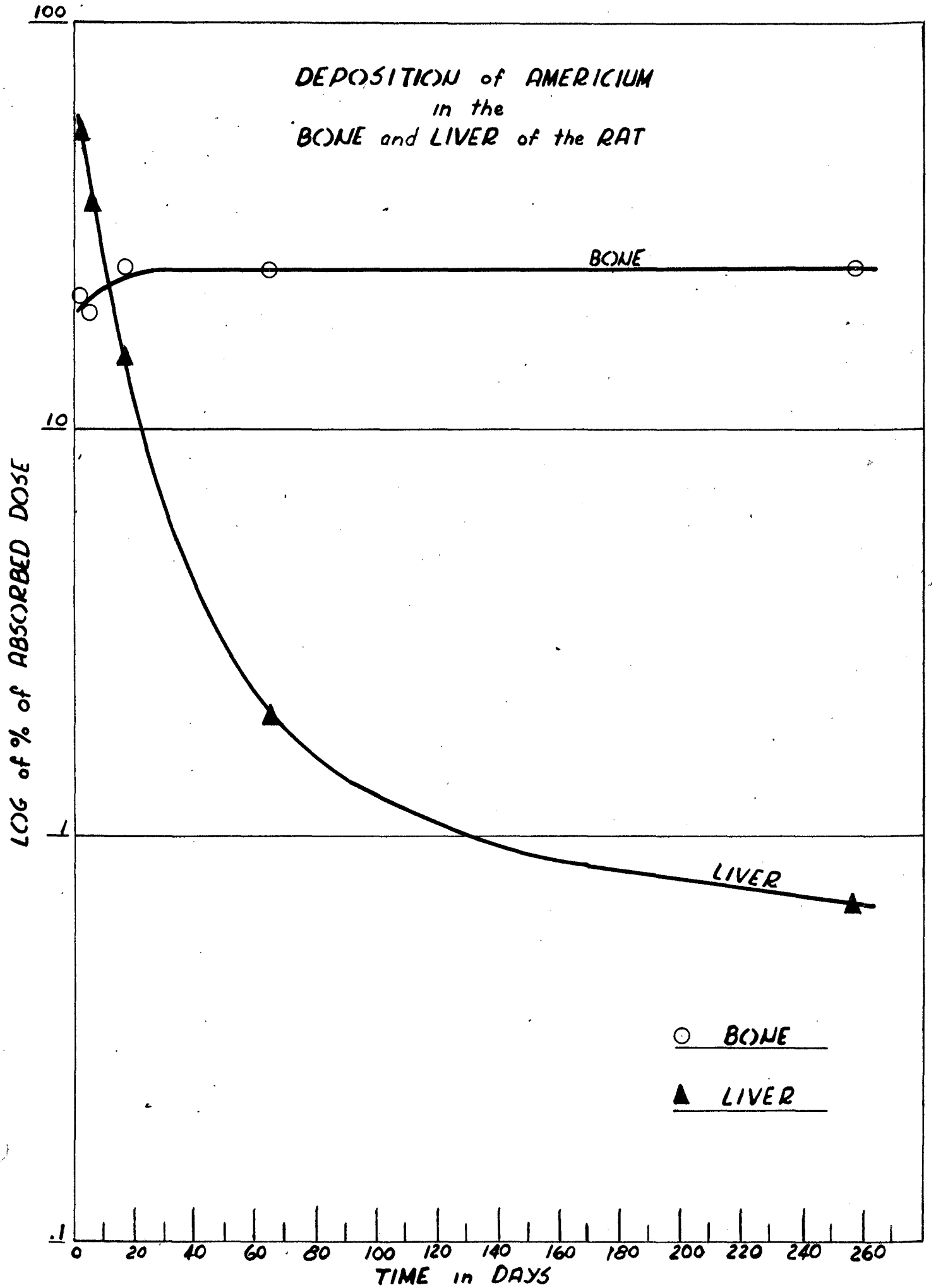
60
70
LIVER
FECES
1 DAY
4 DAY
16 DAY
64 DAY
256 DAY
EXPERIMENT


DEPOSITION of AMERICIUM
in the
BONE and LIVER of the RAT
BONE
LIVER
BONE
LIVER
100
10
1
.1
LOG of % of ABSORBED DOSE
0
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80
100
120
140
160
180
200
220
240
260
TIME in DAYS

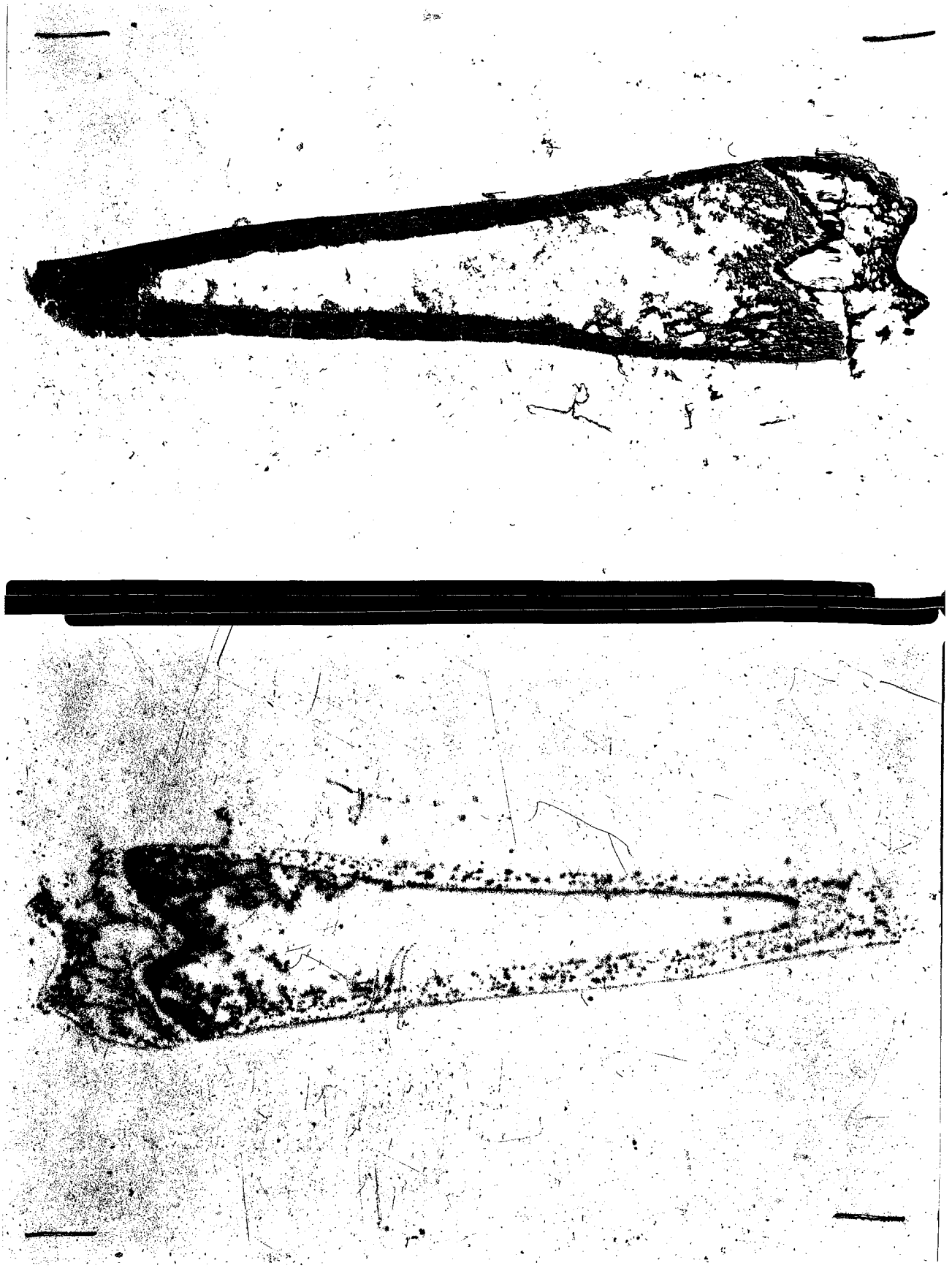

PLATE I

Undecalcified section (upper) of rat femur and corresponding radioautograph (lower) showing the deposition of americium in bone. The element is deposited on the bone surfaces, in the region of the osteoid matrix, and in or around blood vessels of the shaft. Hematoxylin and eosin. (x 7)

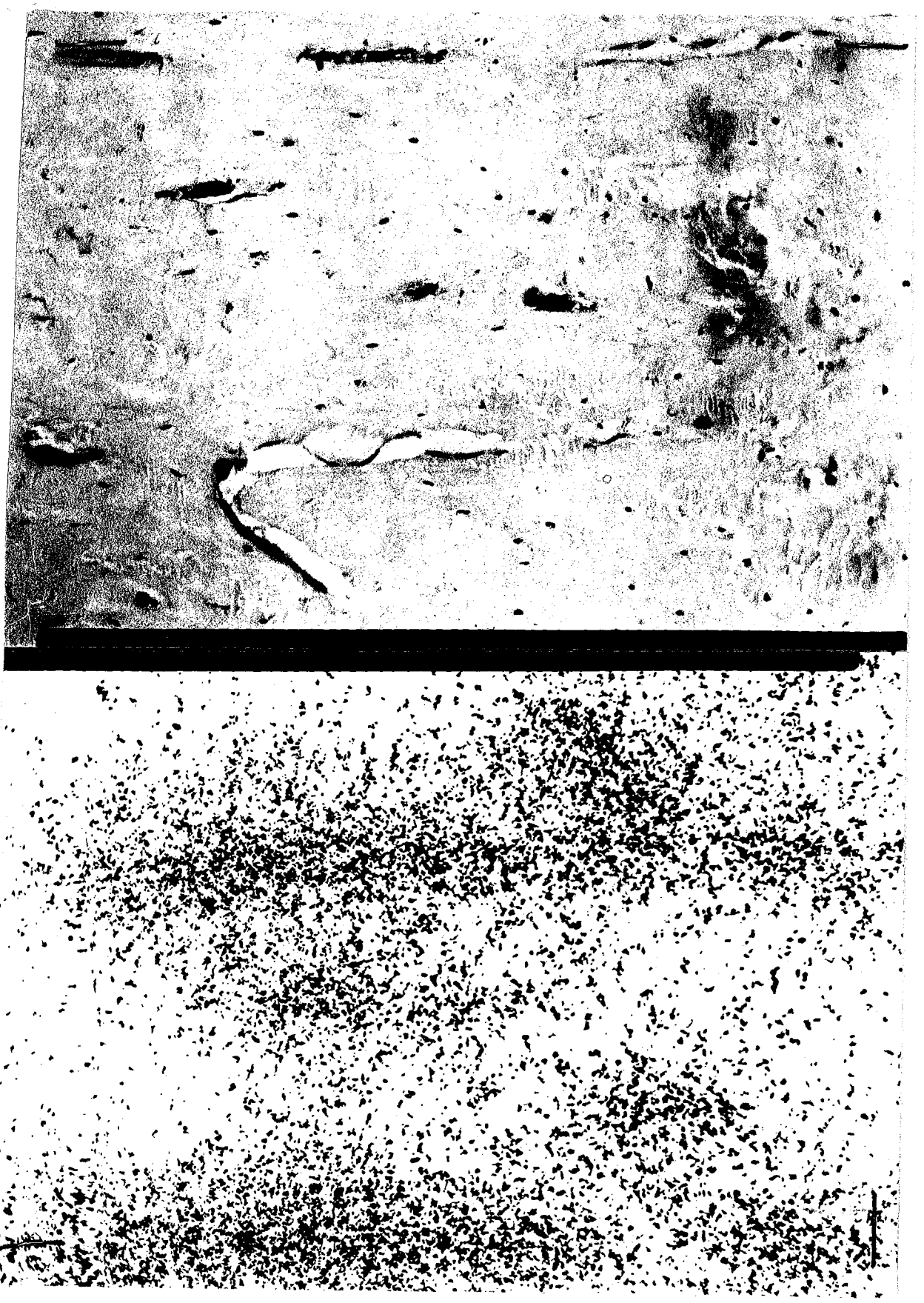

PLATE II

Higher magnification of bone section and radioautograph shown in Plate I demonstrating the deposition of americium in the region of the blood vessels in the cortical bone. Hematoxylin and eosin. (x 290)

DISCUSSION

It has been shown in studies comparable to the series of experiments described here using certain of the fission products in the carrier-free state as well as several members of the heaviest elements (6), that the high degree of accumulation and prolonged retention by the skeleton is a phenomenon common to a number of elements. This characteristic of a high degree of accumulation and retention by the skeleton has been demonstrated with the following fission products: yttrium, lanthanum, cerium, praseodymium, element 61, zirconium, columbium, strontium, and barium (6). The heaviest elements that also are deposited in the skeleton include thorium, protoactinium, uranium, neptunium, and plutonium (2) (3) (4) (5) (6). Incomplete data indicates that the same behavior is characteristic of curium (6) (10). Strontium has been observed to be distributed primarily in the mineral structure of the bone (7) and it is presumed that the same histological pattern of deposition in bone takes place with barium. Radioautographic studies of bone have been made with yttrium, cerium, element 61 (11), zirconium, strontium, thorium, and plutonium (6) (7). In each instance, with the exception of strontium, there is apparently very little accumulation in the mineralized structure of the bone. Deposition with these elements occurs in the region of the osteoid matrix. It has already been shown, in the case of americium, that in addition to this site of localization, there is apparently some accumulation about the small blood vessels of the cortical bone. This interesting characteristic has been thus far only noted with cerium and element 61. Now these two rare earth elements show the same very high degree of localization in the liver that has been deomonstrated with americium. It is predictable that this radioautographic pattern of the bone, which is characteristic for these three elements, should be observed with lanthanum and praseodymium. These two rare earths also demonstrate a similar high level of accumulation by the liver. However, their half-lives are too short to permit the preparation of satisfactory bone radioautographs. The remaining elements subjected to both tracer and radioautographic studies, notably, yttrium, zirconium, thorium, and plutonium, exhibit a relatively small uptake by the liver and do not show any significant amount of deposition in the region of the small blood vessels of the cortical bone. The correlation between the high liver uptake and deposition of material about the small blood vessels of cortical bone is a curious phenomena for which no obvious explanation is available. It is of interest to note that the rates of removal of lanthanum, cerium, praseodymium and element 61 from the liver proceed at almost the same rate that has been observed with americium.

It would appear that in the case of americium, as well as with lanthanum, cerium, praseodymium, and element 61, that there must exist a competition between the liver and the skeleton for an accumulation of these elements. Moreover, it is unlikely that any significant fraction of the very constant level of americium in the skeleton is due to transfer of this element from the liver to the bone. Evidence that may be cited for this deduction is

based on a fact that by the 64 and 256 day intervals there is no appreciable loss in the skeleton, although by 64 days, the americium content of the liver has fallen to a relatively low value. It would be of interest to explore this hypothesis further by performing tracer studies with americium upon hepatectomized animals. Under these conditions with the liver not present, one would expect from 60% to 80% of the americium absorbed from the injection site to be deposited in the skeleton. An additional item of evidence for the reasonableness of this concept is the fact that those elements of somewhat similar metabolic properties, except for their limited deposition in the liver, notably, zirconium, yttrium, thorium, neptunium, and plutonium, show an accumulation by the skeleton of the order of 65% to 70%. The possibility that americium and the other substances for which the liver shows such a high affinity are entrapped by the reticulo-endothelial system appears highly unlikely, since the per gram concentration in the spleen in the earlier phases of these experiments is roughly a fiftieth of the concentration in the liver.

The practical aspects, with respect to the dangers that might be encountered by those who may work with americium, are fairly obvious. In the first place, it is not absorbed to any significant degree from the digestive tract, which therefore places the hazard of ingesting this material by mouth at a relatively low level. The property of selective localization and prolonged retention in the region of the osteoid matrix makes it a highly dangerous agent should it gain entry into the body through cuts, abrasions, etc., due to the close proximity of the radio-sensitive bone marrow. In the light of the work with other elements showing a close metabolic similarity to americium, it seems probable that soluble compounds of this element will be absorbed through the alveolae of the lungs (12). On the basis of experience with plutonium and mixtures of the fission products, it is likely that if any soluble compound of this element should gain entry into the lungs by inhalation from 10% to 30% would be absorbed into the blood stream and roughly a fifth of this amount would eventually find its way to the skeleton where it has already been demonstrated there is an extraordinarily high degree of retention.

APPENDIX

Method of Assay of Americium in Materials of Biological Origin.

The detection and quantitative measurement of alpha particles is relatively simple with the appropriate equipment. However, due to the very short range of this type of radiation, it is not possible, in most instances, to simply place samples of biological materials in the counting device and obtain an accurate determination of the number of disintegrations taking place. The sample must be spread out in a thin and even film whose mass does not exceed two to three milligrams per square centimeter. If this precaution is not observed, a fraction of the emitted alpha particles will not be able to enter the detection chamber. Most of the tissues and organs, even after ashing, contain far too much material to make it possible to spread out the sample in a sufficiently thin film to permit accurate alpha particle counting. This problem may be met by separating the radioactive material, in this case americium, from the bulk of the ash from the samples. Since the total dose of americium given was only .3 micrograms, it was necessary to add to the samples a small amount of a non radioactive element with similar chemical properties to act as a carrier for the tiny traces of americium present. The chemical properties of lanthanum and americium are very much alike in many respects. In these particular experiments, the ashed tissues were dissolved in dilute nitric acid, two milligrams of lanthanum nitrate added, and this followed by the addition of hydrofluoric acid. The americium was carried down quantitatively by the insoluble lanthanum fluoride precipitate. Thus, the americium could be quantitatively separated from several grams of tissue. The lanthanum fluoride precipitate was then spread out in a thin film on the counting dishes and the americium alpha particles measured. The specific details of this procedure appear in the following paragraph.

1. The tissue ash was dissolved in 2 normal HNO_3 with a concentration of 20 milligrams of tissue ash per cc of acid solution.

2. Two milligrams of La $(NO_3)_3$*were stirred into the ash solution used for any particular assay, this ranged from 1 to 5 cc of ash solution.

3. The solution in Part 2 is made 2.5 normal in hydrofluoric acid by the addition of an adequate amount of 8 molar HF.

4. The precipitate formed is separated by centrifugation, and the supernatant fluid is discarded.

5. The precipitate is mounted on a gold dish by redissolving it in 8 molar HNO_3, transferring the solution to the dish and adding an excess of HF to reprecipitate the lanthanum which results in a thin film upon drying on a hot plate at a temperature low enough to prevent spattering.

6. The sample is assayed for americium by determining the alpha particles emitted by the use of a parallel plate alpha counter.

* Because of the presence of alpha emitting contaminants in some of the C.P. La $(NO_3)_3$ available, the La $(NO_3)_3$ must be checked for alpha emitters by running blanks on each batch of La $(NO_3)_3$. The radioactive contamination present is believed to be actinium, whose chemical properties resemble closely those of lanthanum as well as americium.

TABLE I

THE OBSERVED DISTRIBUTION OF AMERICIUM (Am^{241}) FOLLOWING THE INTRAMUSCULAR INJECTION OF .3 MICROGRAMS OF A SOLUTION OF $AmCl_3$ INTO THE HIND LEG OF THE RAT

	1 Day		4 Days		16 Days		64 Days		256 Days	
	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Lungs	.13	.096	.18	.11	.14	.093	.11	.036	.065	.017
Heart	.11	.12	.045	.056	.083	.088	.049	.044	.012	.010
Liver	30.8	3.41	31.0	3.68	10.6	1.13	1.46	.13	.53	.063
Spleen	.28	.39	.083	.18	.079	.11	.085	.13	.045	.081
Kidney	2.49	1.29	1.97	1.13	1.02	.52	.72	.34	.27	.13
G.I. Tract	1.54	.068	4.06	.19	1.56	.075	.32	.012	.12	.005
Bone	13.1	.57	16.6	.57	17.7	.88	18.9	.73	19.2	.73
Skin	1.84	.056	1.46	.039	1.47	.037	.33	.008	.17	.005
Muscle	.83	.006	1.60	.014	2.06	.018	1.53	.012	.94	.008
Blood	.57	.053	.12	.008	.10	.007	.068	.004	.01	.001
Balance	3.68		2.51		1.61		1.59		1.13	
Unabsorbed in injected leg	43.5		12.9		5.37		5.13		1.45	
Urine	3.12		2.80		2.34		4.27		4.67	
Feces	2.66		27.3		32.1		47.6		51.6	
% Recovery of injected dose	103.2		100.9		74.1		80.6		79.3	

TABLE II

THE DISTRIBUTION OF AMERICIUM (Am^{241}) IN THE RAT FOLLOWING INTRAMUSCULAR INJECTION. THE DATA IS TAKEN FROM TABLE I AND HAS BEEN CORRECTED FOR THE FRACTION OF AMERICIUM UNABSORBED AT THE SITE OF INJECTION AND THE DEVIATION OF THE RECOVERY VALUES FROM 100%

	1 Day		4 Days		16 Days		64 Days		256 Days	
Tissue	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram	% per organ	% per gram
Lungs	.23	.17	.21	.13	.21	.13	.15	.048	.084	.022
Heart	.19	.21	.052	.065	.12	.13	.065	.058	.015	.013
Liver	54.8	6.07	35.7	4.24	15.3	1.64	1.94	.18	.68	.081
Spleen	.50	.70	.095	.21	.11	.15	.11	.17	.058	.11
Kidney	4.43	2.29	2.27	1.30	1.47	.75	.96	.44	.34	.16
G.I. Tract	2.73	.12	4.66	.22	2.25	.11	.43	.016	.15	.008
Bone	23.2	1.01	19.1	.66	25.6	1.25	25.0	.96	24.8	.94
Skin	3.26	.10	1.68	.045	2.12	.053	.44	.011	.22	.006
Muscle	1.46	.010	1.84	.017	2.97	.025	2.03	.016	1.21	.010
Blood	1.01	.095	.14	.009	.16	.010	.090	.005	<.01	<.001
Urine	5.53		3.21		3.38		5.66		6.02	
Feces	4.73		31.5		46.4		63.2		66.4	

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