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THE METABOLISM OF PLUTONIUM IN RATS FOLLOWING INTRAMUSCULAR INJECTION*

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PLATES 1 AND 2

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The metabolism of plutonium in man has become a matter of concern with the development of the chain-reacting pile. For this reason a portion of the first plutonium produced by the Clinton pile was set aside for animal studies in order to evaluate the hazards which plutonium might present to man. In March, 1944, 11 mg. of plutonium were made available to this laboratory for animal studies from the first production of the Clinton pile. Later studies were undertaken at the Metallurgical Laboratory, University of Chicago (1), and at Los Alamos (2).

Plutonium is produced by the neutron irradiation of U^{238}; the U^{239} initially formed decays by the emission of a negative β-particle. The Ne^{239} created in this process in turn decays by negative β-particle emission to [illegible]. This isotope of plutonium, having a half life of 24,000 years, decays by the release of α-particles to U^{235}, which occurs in nature and has a half life of [illegible] years.

Methods

Preparation of Material—The plutonium was prepared in the three valence states for injection by Professor R. Connick and his colleagues working under the direction of Dean W. M. Latimer in the College of Chemistry.

The +3 valence state was prepared by the reduction of $PuCl_4$ by SO_2 in 0.47 N HCl. The +4 plutonium was prepared prior to the intramuscular injection by first precipitating the +4 hydroxide from the original $PuCl_4$ solution and dissolving the precipitate in 0.47 N HNO_3. The +6 plutonium was prepared by oxidation of the $PuO_2(NO_3)_2$ with dichromate. The plutonium was precipitated as sodium plutonyl acetate, the preparation dissolved in HCl, and then precipitated as $NaPuO_2Ac_3$. The precipitate was again dissolved in HCl and the plutonium reprecipitated with NaOH. Finally, the precipitate was dissolved in 0.45 N HCl.

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The solutions containing the three separate valence states of plutonium were tested qualitatively by a spectrophotometric method and each was found to contain less than 20 per cent of plutonium in the form of valence states other than the one specifically desired. These stock solutions contained approximately 2 mg. of plutonium per cc. Immediately before injection the solutions were diluted with sufficient isotonic saline to bring the pH to 2.5, and they contained approximately 15 γ of plutonium per cc.

Administration to Rats—Owing to nutritional and metabolic similarities to man, the animal chosen for this work was the rat. The three plutonium [illegible], as described above, were immediately administered to rats. In order to avoid undue plating of the plutonium on the syringes and needles [illegible] injection, they were washed with 0.01 N HCl before use. In spite of this precaution, considerable difficulty was experienced with plating, especially with respect to Pu in the +4 state. However, the equipment [illegible] injection was assayed for plutonium in order that the actual dose of Pu administered to the rats could be determined.

Twelve groups of three animals each were injected with approximately 15 γ of Pu intramuscularly in the left leg, the Pu solutions being employed in the three valence states described above. Following this, groups of rats were sacrificed at 4, 16, 64, and 256 days after administration of plutonium in the +3, +4, and +6 valence states.

Three groups of rats were given plutonium in its three valence states orally and sacrificed 4 days later.

The excreta were collected from all groups at daily intervals. In order to reduce the rather laborious plutonium assays as much as possible, the urine and feces were pooled for the following intervals, 0 to 2 days, 2 to 4 days, 4 to 8 days, 8 to 16 days, and every 8 days thereafter. When the animals were sacrificed, the organs removed included the liver, kidney, testes, spleen, muscle, skin, stomach, large and small intestine together with the cecum, bone, lung, brain, and blood. The left hind leg, which was the site of [illegible] administration, was removed and assayed separately in order to determine the percentage of plutonium remaining unabsorbed. Testes, spleen, muscle removed from the uninjected right leg, stomach, small and large [illegible], lungs, brain, and 3 to 5 cc. of blood obtained by heart [illegible] were pooled for each group. In the case of bone, the uninjected right leg bones were assayed separately from the rest of the skeleton. The skinned [illegible], which consisted of the rest of the skeleton, muscle, fat, and blood, was dried and ashed as a unit. The skeleton was separated from this by sifting the ash through a fine screen. The plutonium content of both of these portions was determined. The plutonium present in this ash, not included in the skeleton, is listed under "balance" in Tables I, III, and V after the calculated plutonium content of the muscle system of the animal had been subtracted from it.

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The tissues were assayed in the following manner: Immediately after the animals were sacrificed, the organs and tissues were removed and weighed while wet. During the first part of the experimental program with plutonium, all of the tissues were ashed in an electric muffle following a preliminary drying at 150°. Several determinations were done in which a known amount of plutonium in its three valence states was added to inactive animal ash, which was brought up to a temperature of 500° and maintained at this level for 24 hours. No measurable amount of plutonium was lost from this ashing method by volatilization.

In later studies the tissues were wet ashed with concentrated HNO_3 and 30 per cent H_2O_2. When the ashing was completed, all of the acid was boiled off, leaving a white ash. Following either wet or dry ashing, the ash was dissolved in a solution which was 2 N HNO_3 and 0.2 M hydroxylamine. The final concentration of animal ash in the nitric acid hydroxylamine solution was 20 mg. per cc. 0.5 cc. of the solution was used for the assay of plutonium. Duplicate assays were made for every tissue and the details of the analytical procedure were as follows:

500 γ of $La(NO_3)_3$ in 25 microliters were added to 0.5 cc. of a solution of tissue ash in a 2 cc. centrifuge cone. (Because of the presence of α-emitting contaminants in some of the $La(NO_3)_3$ available, the latter must be checked for α emitters by running blanks on each batch received. The radioactive contamination present is believed to be actinium, whose chemical properties resemble closely those of lanthanum, as well as plutonium in the +4 state.)

0.2 cc. of 6 N HF was added and the mixture stirred with a platinum rod. The cone was then centrifuged at 6000 R.P.M. in a small centrifuge for 30 minutes. The cone was removed and the walls washed down with the supernatant solution. The mixture was centrifuged for 3 minutes and the supernatant solution discarded. The precipitate of LaF_3 containing the plutonium was then dissolved in concentrated nitric acid and transferred to a platinum dish 1 inch in diameter. The cone was washed with a small amount of water, and this was also added to the dish. 2 drops of 6 N HF were added to the solution in the platinum dish in order to reprecipitate the LaF_3 and the plutonium.

The dish containing the precipitate was gently warmed on a hot-plate until dry and then flamed to a dull red heat for a few seconds.

The precipitate was found to form a very thin even film which was quite adherent to the platinum. The α-particles from these samples were counted, each sample being counted twice. It was found that the LaF_3 precipitated from the diluted ash samples of bone, feces, and urine was somewhat more bulky than would be expected from the amount of lanthanum used as a carrier. A large series of α-particle measurements was made from the ash of these three types of material, and a relatively constant

to 22 per cent for the feces and bone [illegible] to the fact that the remainder of this extraneous material in the [illegible] cipitate would have necessitated a slow and laborious purification [illegible] cedure, the appropriate correction factor was applied to these sample[illegible]

TABLE I

Deposition of +3 Plutonium in Tissues of Rat after Intra[illegible] Left Leg

The values given are in per cent of the dose. Average value [illegible] time interval.

	4 days		16 days		63 days		[illegible]	
	per cent per organ	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*
Lungs	0.05	0.04	0.06	0.05	[illegible]	[illegible]	[illegible]	[illegible]
Spleen	0.08	0.13	[illegible]	0.15	0.22	[illegible]	[illegible]	[illegible]
Blood	0.45	0.04	0.20	0.02	[illegible]	[illegible]		
Liver	1.12	0.14	0.87	0.14	[illegible]	[illegible]	[illegible]	[illegible]
Kidney	0.30	0.18	0.10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Brain	<0.01*	<0.01	<0.01	<0.01	[illegible]	[illegible]	[illegible]	[illegible]
Stomach	0.06	0.02	0.03	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Intestines	0.25	<0.01	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Bone	10.8	0.64	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Muscle	[illegible]	<0.01	[illegible]	[illegible]	[illegible]			
Balance	[illegible]		[illegible]		[illegible]		[illegible]	
Left leg	77.1		[illegible]		[illegible]		[illegible]	
Skin	0.61	0.03	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Gonads			0.06	0.02				
Tail			[illegible]		[illegible]	[illegible]		
Urine	0.11		0.19		0.87		[illegible]	
Feces	[illegible]		[illegible]		[illegible]		[illegible]	
Actual recovery	[illegible]		[illegible]		[illegible]		[illegible]	

* Although samples in this [illegible] measurable amounts of activity, the [illegible] not significant to more than two decimal [illegible]

Bone radioautographs were prepared from [illegible] by techniques described elsewhere [illegible] sacrificed at intervals ranging from 8 to 250 days after intramus[illegible] administration. Doses ranging from 15 to 25 γ were used.

Results

The average value of absorption of plutonium in each of its three valen[illegible] states from the gastrointestinal tract was found to be 0.007 per cent. This

value may be even lower, since it is predicated primarily upon the limits of the sensitivity of the counting apparatus used and the number of micrograms of plutonium available for these studies.

Data are presented in Tables I and II which give the relative deposition of plutonium in the tissues of the rat when administered in the +3 state. These studies were carried out for 256 days after administration of the plutonium, as were those involving the +4 and +6 valence states. It can be seen from an examination of Table I that +3 plutonium is only partly absorbed from the injection site, 77 per cent remaining at 4 days and 23 per

TABLE II

Deposition of +3 Plutonium in Tissues of Rat Corrected for Recovery and for Unabsorbed Balance at Injection Site

Average values for three rats at each time interval.

	4 days		16 days		64 days		256 days	
	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.
Lungs	0.32	0.25	0.26	0.22	0.15	0.09	0.11	0.08
Spleen	0.50	0.82	0.40	0.66	0.38	0.69	0.32	0.64
Blood	2.84	0.25	0.88	0.09	0.17	0.02		
Liver	7.06	0.88	3.84	0.62	2.94	0.52	1.41	0.18
Kidney	1.89	1.13	0.44	0.31	0.46	0.33	0.47	0.26
Brain	0.03	0.02	0.03	0.02	0.02	<0.01	<0.01	<0.01
Stomach	0.38	0.23	0.13	0.09	0.01	0.01	0.14	0.05
Intestines	1.58	0.06	1.02	0.13	0.14	<0.01	0.18	<0.01
Bone	68.1	4.04	82.6	4.99	52.1	3.59	46.3	2.98
Muscle	3.15	0.04	3.36	0.04	4.71	0.05	2.51	0.03
Skin	3.85	0.19	2.74	0.13	0.84	0.03	0.30	0.01
Gonads			0.26	0.09				
Tail					2.89	0.41		
Urine	0.69		0.84		1.67		1.69	
Feces	9.65		3.27		33.5		46.6	

cent at 256 days. The largest portion of the material absorbed is deposited in the skeleton. Liver, kidney, and spleen were the only other tissues examined which contained relatively large concentrations of plutonium. The excretion of plutonium occurred primarily in the feces. In Table II the same data are presented with corrections[1] for recovery and absorption from the injection site.

[1] These corrected values were obtained by calculating the relative amounts of plutonium present in all of the tissues and excreta, exclusive of the plutonium remaining unabsorbed in the left hind leg, and the amount of plutonium in the balance less the calculated quantity present in the blood, muscle, and carcass. This type of extrapolation, which attempts to correct for the amount of unabsorbed plutonium

Tables III and V demonstrate the deposition of plutonium in the tissues of the rat when administered as the +4 and +6 valence states. The corrected values are given in Tables IV and VI. These data are similar to those obtained for plutonium in the +3 valence state, demonstrating major deposition in the skeleton. Of all of the soft tissues, the liver, kidney, and spleen show the highest affinity for plutonium per gm. How-

TABLE III

Deposition of +4 Plutonium in Tissues of Rat after Intravenous Administration into Left Leg

The values given are in per cent of the dose. Average values for three rats at each time interval.

	4 days		16 days		64 days		256 days	
	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.	per cent per organ	per cent per gm.
Lungs	<0.01	<0.01	0.01	0.01	0.03	0.02	0.02	0.01
Spleen	<0.01	<0.01	0.01	0.02	0.03	0.07	0.05	0.12
Blood	0.05	<0.01	0.01	0.01	0.04	0.01		
Liver	0.08	<0.01	0.19	0.03	0.56	0.08	0.41	0.04
Kidney	0.03	0.02	0.08	0.04	0.20	0.05	0.07	0.03
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Intestines	0.05	<0.01	0.05	<0.01	0.11	<0.01	0.07	<0.01
Bone	1.44	0.08	2.18	0.14	12.9	0.84	11.5	0.60
Muscle	0.03	<0.01	0.08	<0.01	0.63	<0.01	0.59	<0.01
Balance	2.35		1.05		1.68		0.28	
Left leg	95.8		87.6		68.1		65.8	
Skin	<0.01	<0.01	0.06	<0.01	0.17	<0.01	0.21	<0.01
Gonads			<0.01	<0.01	0.05	0.02	0.06	0.02
Tail					0.84	0.13		
Urine	0.01		0.04		0.39		0.43	
Feces	0.11		0.43		4.70		9.42	
Actual recovery	100.0		91.8		90.3		89.9	

ever, their concentration of plutonium per gm. averaged from one-fifth to one-tenth that of bone. The corresponding concentration of plutonium in the other soft tissues was very much less.

present, appears to be the best method of indicating the true metabolic behavior of this substance. The observed quantity of plutonium in the balance, less that estimated to be present in the blood and muscle of the carcass, is thought to represent the amount of the injected solution which extravasated beyond the point of amputation and remained unabsorbed.

For all groups it will be noted that the corrected data show that there is no apparent and significant difference in either the distribution or excretion of plutonium in its three valence states. This is demonstrated in Text-fig. 1 which shows the relative proportions of the absorbed plutonium in the three valence states in the excreta and in the skeleton. Their similarity suggests that plutonium exists in the body in the same valence state, regardless of the valence state in which it was administered.

TABLE IV

Deposition of +4 Plutonium in Tissues of Rat Corrected for Recovery and for Unabsorbed Balance at Injection Site

Average values for three rats at each time interval.

	4 days		16 days		64 days		256 days	
	per cent per organ	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*
Lungs	0.22	0.17	0.19	0.16	0.15	0.10	0.09	0.04
Spleen	0.33	0.30	0.32	0.95	0.15	0.34	0.22	0.53
Blood	2.76	0.22	0.19	0.01	0.19	0.01		
Liver	4.40	0.39	6.03	0.95	2.72	0.39	1.79	0.18
Kidney	1.66	1.10	2.54	1.27	0.49	0.24	0.31	0.13
Brain	0.04	0.03	0.02	0.02	<0.01	<0.01	0.04	0.03
Stomach	0.11	0.05	0.13	0.06	0.05	0.03	0.04	0.02
Intestines	2.76	0.28	1.59	0.16	0.15	0.04	0.31	0.02
Bone	79.3	4.40	69.3	4.44	62.7	4.09	50.3	2.63
Muscle	1.66	0.02	2.54	0.03	3.06	0.03	2.58	0.02
Skin	0.22	0.03	1.91	0.06	0.83	0.03	0.92	0.02
Gonads			0.32	0.10	0.24	0.10	0.26	0.09
Tail					4.09	0.63		
Urine	0.56		1.27		1.90		1.88	
Feces	6.06		13.6		22.9		41.2	

However, a very great difference in the amounts of plutonium absorbed from the site of injection was noted for the three valence states. The greatest degree of absorption was observed for +6 plutonium, the least for +4 plutonium, and +3 was intermediate in character. These dissimilar rates of uptake from the injection site are shown in Text-fig. 2.

Radioautographs

Radioautographs of adult rat femurs were made at periods ranging from 8 to 256 days after plutonium administration. All of these showed the deposition of plutonium on bone surfaces; *i.e.*, in the region of the periosteal and endosteal bone surfaces, and on the endosteal covering of the trabecular

Deposition of +6 Plutonium in Tissues of Rat after Intramuscular Administration into Left Leg

The values given are in per cent of the dose; average values for three rats at each time interval.

	4 days		16 days		64 days		256 days	
	per cent per organ	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*
Lungs	0.08	0.06	0.10	0.08	0.09	0.06	0.05	0.03
Spleen	0.10	0.16	0.22	0.40	0.28	0.47	0.20	0.34
Blood	0.86	0.07	0.21	0.02	0.11	<0.01		
Liver	3.58	0.47	1.89	0.34	2.24	0.20	1.09	0.13
Kidney	0.50	0.28	0.47	0.28	0.91	0.48	0.20	0.12
Brain	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	0.17	0.05	0.06	0.03	0.08	0.04	0.03	0.01
Intestines	0.59	0.03	0.50	0.05	0.26	0.02	0.09	<0.01
Bone	17.2	0.83	43.7	1.78	28.7	1.96	24.6	2.31
Muscle	0.65	0.01	1.56	0.02	0.83	0.01	0.73	0.01
Balance	4.73		1.50		3.57		2.71	
Left leg	70.4		30.0		23.6		12.6	
Skin	0.56	0.02	0.60	0.02	0.60	0.02	0.25	0.01
Gonads			0.10	0.04				
Tail					1.72	0.28		
Urine	0.10		5.51		2.30		3.28	
Feces	1.95		13.4		14.1		32.4	
Actual recovery	101.5		99.8		90.4		88.3	

TABLE VI

Deposition of +6 Plutonium in Tissues of Rat Corrected for Recovery and for Unabsorbed Balance at Injection Site

Average values for three rats at each time interval.

	4 days		16 days		64 days		256 days	
	per cent per organ	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*	*per cent per organ*	*per cent per gm.*
Lungs	0.30	0.23	0.15	0.12	0.17	0.11	0.07	0.04
Spleen	0.38	0.61	0.32	0.59	0.53	0.88	0.27	0.47
Blood	3.27	0.27	0.31	0.03	0.21	0.02		
Liver	13.6	1.78	2.77	0.50	4.21	0.56	1.49	0.18
Kidney	1.90	1.06	0.69	0.41	1.71	0.90	0.27	0.16
Brain	0.03	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Stomach	0.65	0.19	0.09	0.04	0.15	0.08	0.04	0.01
Intestines	2.24	0.11	0.73	0.07	0.49	0.04	0.12	<0.01
Bone	65.3	3.15	64.0	2.61	55.8	3.69	47.4	3.17
Muscle	2.47	0.03	2.28	0.03	1.56	0.02	1.00	0.01
Skin	2.13	0.08	0.88	0.03	1.13	0.04	0.36	0.01
Gonads			0.15	0.06				
Tail					3.23	0.53		
Urine	0.38		8.07		4.32		4.51	
Feces	7.40		19.6		26.5		44.4	

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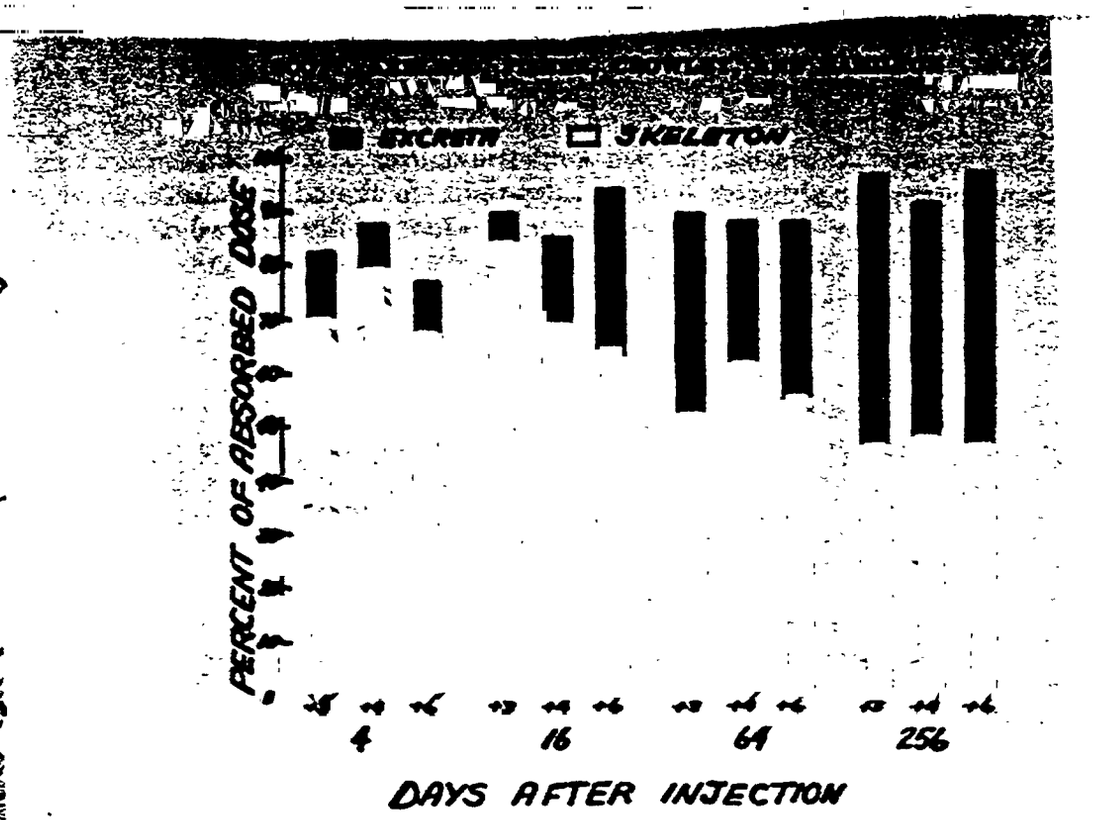
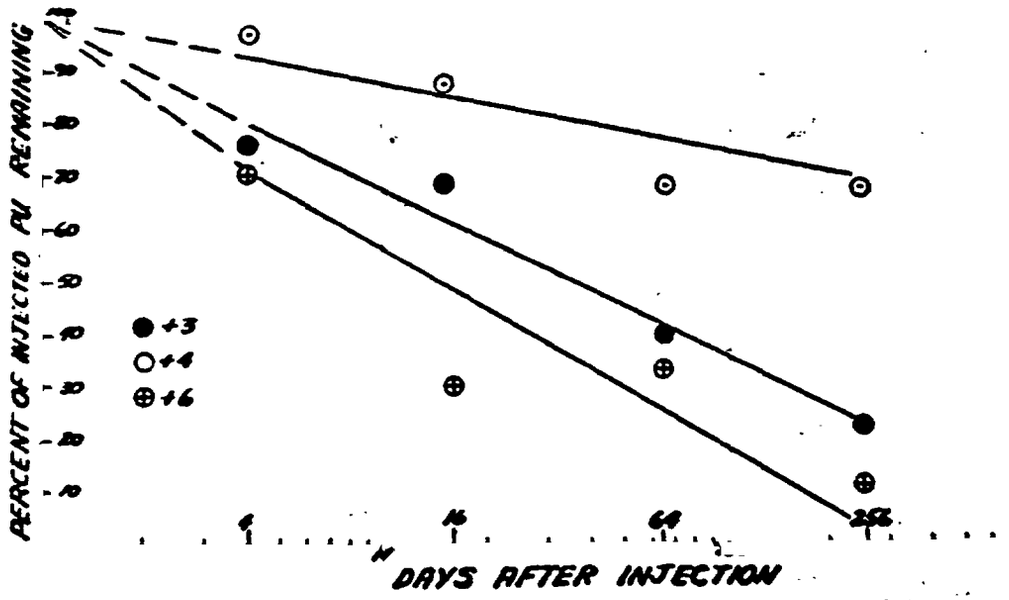


TEXT-FIG. 1. Proportion of plutonium found in excreta and skeleton of the ra after intramuscular administration of plutonium in +3, +4, and +6 valence state Values corrected for absorption from the left leg.



TEXT-FIG. 2. Loss of plutonium from the hind left leg of the rat after intramuscular injection of the +3, +4, and +6 valence states. The ordinate scale gives the per cent of plutonium remaining at the injection site.

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bone (Fig. 1). The picture of plutonium deposition at 8 days was the same as that found after 256 days. In other words, no shifting or redistribution of plutonium took place after its initial deposition in the adult animal. Fig. 2 shows a section in which the periosteum was fortuitously stripped off of the bone. The radioautograph demonstrates that a large amount of plutonium is present in this stripped layer.

Conclusions

No significant variations in the metabolic behavior of plutonium were observed in each of its three valence states. It is probable that plutonium, when absorbed and distributed throughout the body, exists in a single valence state which is independent of its valence at the time of administration.

Plutonium is not absorbed from the gastrointestinal tract to any significant degree. Following parenteral administration, the chief organ of deposition is the skeleton. The soft tissues having the greatest concentration of plutonium are liver, kidney, and spleen; their content of plutonium per gm., however, is from one-fifth to one-tenth that of bone.

The chief channel of elimination is the digestive tract. The rate of plutonium excretion is very slow and its half period of retention in the body is estimated to be greater than 2 years.

Radioautographic studies show that plutonium is deposited primarily in the region of the periosteum, endosteum, and the endosteal covering of the trabecular bone. Examination of many of these preparations suggests that the principal site of deposition is on the bone surface and that a very limited accumulation takes place in the mineral structure of the bone. No significant redistribution of plutonium took place in the bone during the period of these experiments.

The selective localization of the plutonium on the bone surfaces explains the high toxicity of plutonium compared to equivalent quantities of radium. This effect arises from the fact that the radium is distributed throughout the mineral portion of the bone, and a large proportion of the α-particles is absorbed before they can enter the marrow cavity. Plutonium α-particles can bombard bone marrow more readily, since there is relatively less self-absorption of radiation by the bone.

SUMMARY

Detailed metabolic studies of Pu^{239} administered to rats intramuscularly in the +3, +4, and +6 valence state are presented. Plutonium was not found to be absorbed from the gastrointestinal tract to any extent. The skeleton was the main organ of deposition of plutonium, and the degree of retention in this organ was very great. No significant differences were

observed in the metabolic properties of plutonium absorbed by the body for its three valence states. Radioautographs demonstrate the deposition of plutonium in the region of the endosteum, periosteum, and the endosteal covering of the trabecular bone.

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EXPLANATION OF PLATES

Plate 1

Fig. 1. Section of femur from an adult rat injected intramuscularly with 15 γ of plutonium and sacrificed after 8 weeks. The radioautograph demonstrates the superficial deposition of plutonium in the region of the periosteum, endosteum, and trabecular bone. Hematoxylin, eosin, and silver nitrate; × 8.

Plate 2

Fig. 2. Radioautograph and section of femur from a rat injected intramuscularly with 25 γ of plutonium and sacrificed after 5 weeks. During the preparation of the sections the periosteum became separated from the shaft, and the autograph shows a heavy deposit of plutonium in this stripped layer. No calcium was detected in this particular area. Hematoxylin, eosin, and silver nitrate; × 10.4.

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