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45-8-429 Issued to: R. S. Stone, M.D.

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PROGRESS REPORT FOR THE MONTH OF AUGUST 1945

CONTRACT # W-7405-eng-48-A

Joseph G. Hamilton, M.D.

August 17, 1945

CLASSIFICATION CANCELLED
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For The Atomic Energy Commission
Chief, Declassification Branch

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PART I

SUMMARY

B. Metabolic Studies of Plutonium

Additional radioautographic studies substantiate further the view that plutonium is accumulated principally in the osteoid matrix. The human excretory studies are now completed through the 87th day, and the rate of elimination from the body since the 20th day is relatively constant and averages approximately .005% per day. Parallel excretory studies in rats done at the same time from the same Pu^{238} solution used in the patient indicated the same rate of urinary elimination but fecal excretion in the rat was much greater. The decontamination studies with plutonium have shown that a decrease of surface alpha activity over the endosteal region of the femur in rats of up to 30 fold has been achieved by the decalcification - re Calcification Cycle in rats. The uptake of plutonium by the callus following fracture of the tibia in rats has been found to be at a maximum before calcification and decreases as the new bone is laid down. This substantiates further the opinion that plutonium is not incorporated to a significant degree in the apatite structure of bone. The blocking effect of carrier-free radio-iodine by the thyroid glands of rats is not as great as had been anticipated. The metabolism of plutonium in plants is described. The isolation of Pa^{233} for limited toxicity studies is underway. The radioautographic, tracer and human studies are to be continued.

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PART II

SECTION A.

TECHNICAL PROGRESS REPORT ON THE METABOLIC STUDIES OF PRODUCT

1. Radioautographic Studies

Additional bone radioautographs from the normal calcium and phosphorous deficient rats substantiated further the conclusion that Plutonium is not deposited within the apatite structure of the bone but is tightly held in the osteoid matrix. A group of representative sections and radioautographs was forwarded to Doctor Bloom for his interpretation. He concluded that, from the specimens, Plutonium did not precipitate in the cartilage in regions where bone salts would be deposited, that osteoid matrix accumulated more Plutonium than any other forms of collagen, and that growing bone did not take up much Plutonium. It is my impression that our findings agree in principle rather conclusively with the results at Chicago.

2. Tracer Studies

The following table (Table I) presents the results obtained for the urinary and fecal excretion of Plutonium in the human subject who received Pu^{238} over three months ago. The figures express excretion in terms of the per-cent per day elimination of the administered dose. The fecal specimens were collected for one day out of every ten due to certain practical considerations, primarily those of the patient. For the purpose of comparison, the data for the entire period is presented below.

TABLE I

RE 16 H Table for Urine Samples
The Daily Rates of Excretion of Pu in a Human Subject Following the Administration of 5 gms. by Intravenous Injection

Sample	Days	Activity in % of Dose	Weight in Grams	Sample Vol. in Ml.	Sample	Days	Activity in %Dose	Weight Gms.	Sampl Vol. l
	0-1	0.181	3.0	1500		15-16	0.0160	3.2	1500
	1-2	0.147	3.0	1500		16-17	0.00558	1.2	600
	2-3	0.123	--	1500		17-18	0.0102	2.8	1800
	3-4	0.0314	--	1500		18-19	0.00674	2.4	--
	4-5	0.0365	--	--	#1	19-20	0.0048	--	1500
	5-7	0.0296	--	--	#2	20-21	0.0077	--	1500
	7-8	0.0155	--	400	#3	21-22	0.005	--	1800
	8-9	0.0687	--	1800	#4	22-23	0.00905	--	1900
	9-10	0.0262	--	2000	#5	23-24	0.0076	--	1200
	10-11	0.0360	--	2000	#6	24-25	0.0105	--	1200
	11-12	0.0290	--	2400	#7	25-26	0.0028	--	800
	12-14	0.0343	--	4000	#8	26-27	0.00436	--	800
	14-15	0.0130	--	500	#9	27-28	0.00735	--	1200

Note: Sample number is for Laboratory use.

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TABLE I
(Cont'd.)

Days	Activity in % of Dose	Weight in Grams	Sample Vol. in Ml.
29	0.0043	--	1200
30	0.0069	--	600
31	0.0077	--	1000
32	0.0063	--	800
33	0.0073	--	700
34	0.0084	--	500
35	0.0065	--	1000
36	0.00786	--	1200
37	0.0063	--	1300
38	0.0085	7.0	1900
39	0.0064	5.8	1400
40	0.00719	5.4	1200
41	0.00795	4.5	1400
42	0.00805	9.5	600
43	0.00764	12.0	1300
44	0.00550	7.8	1200
45	0.0063	8.5	500
46	0.0073	16.0	700
47	0.00586	9.0	1100
48	0.00586	6.0	500
49	0.0063	10.05	900
50	0.0078	11.45	800
51	0.00815	10.8	1500
52	0.0098	14.2	1400
53	0.0074	6.7	800
54	0.0077	9.5	1100
55	.0096	11.8	1400
56	.0064	5.1	1500
57	.00495	10.2	1200
58	.00589	4.1	1800
59	.0098	6.1	1000
60	.00674	6.2	400
61	.00655	4.1	800
62	.00584	1.2	800
63	.0077	7.7	1000
64	.0042	11.5	1000
65	.0042	5.2	1000
66	.0047	3.7	400
67	.0064	7.2	700
68	.0068	3.7	600
69	.0070	12.3	500
70	.00999	13.42	600
71	.0072	8.5	300
72	.0092	13.3	700
73	.00688	11.9	400
74	.0079	7.72	800
75	.00512	4.8	800
76	.00414	9.8	800
77	.0065	11.1	800
78	.0074	11.9	700
79	.0066	4.5	700
80	0.0048	5.9	300
81	0.0055	10.9	1100
82	0.00795	6.92	1200
83	0.00675	8.44	1200
84	0.00222	5.8	1500
85	0.00926	9.92	900
86	0.0101	10.36	1000
87	0.0079	10.68	1000

TABLE II
RE 16 H Table for Feces

Days	Activity in % of Dose	Weight in Grams
0-1	--	--
1-2	--	--
2-3	0.306	2.0
8-9	0.0092	--
17-18	0.0165	2.0
18-19	0.0105	2.4
29-30	0.00218	--
39-40	0.00326	--
49-50	0.00143	--
59-60	0.0013	--

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As can be seen from Table I, the urinary excretion rate leveled off about the 20th day and showed very little change thereafter. It ranged from .004 to .01 percent per day of the administered dose. The rate of fecal elimination averaged from one-third to one-eighth the urinary excretion.

Table II presents the rates of urinary and fecal excretion of Plutonium in three rats which were given Pu^{238} at the same time this material was administered to the patient.

TABLE II

The Daily Rates of Urinary and Fecal Excretion of Pu^{238}
in the Rat Following I.V. Administration
RE 16g Parallel Experiment to RE 16H

Days (average per day)	% Urinary Excretion	% Fecal Excretion
0-1	0.323	1.39
1-2	0.152	2.83
2-3	0.067	1.54
3-4	0.0325	1.92
4-8	0.0230	1.35
lost	0.0307	0.74
16-22	0.023	0.94
22-32	0.052	0.46
32-40	0.018	0.04
40-50	0.0085	—
50-58	0.0039	—
58-68	0.0096	—

It will be noted that the urinary excretion paralleled quite closely that of the human subject. However, fecal excretion in the rats was very much greater.

3. Decontamination Studies

Plutonium and certain other radioactive elements present a hazard from chronic radiation toxicity analagous to that from radium poisoning. This is due to localization in the skeleton as a very stable deposit which is eliminated extremely slowly.

The following is a survey of work carried out during the past year to further elucidate this problem. A more detailed report will follow.

a. Effect on elimination of Plutonium of the standard treatment used in radium poisoning.

Adult rats were injected with Plutonium intramuscularly. Six weeks later, they were placed on a low calcium diet to which 2% ammonium chloride had been added. They also received 40 units of parathormone subcutaneously each day. After four weeks on this regimen, they were changed to a high calcium diet (Stock diet supplemented with 5% calcium carbonate). Four weeks later, they were sacrificed. No significant decontamination of Plutonium

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was obtained by this treatment.

b. Factors Modifying Bone Metabolism.

Differences in age, calcium dificiency, and phosphorus deficiency were all found to have a profound effect on the absorption, excretion, and deposition in bone of radio-strontium and radio-phosphorus. They had only minor effects, if any, on the deposition of Plutonium, radiocerium and radio-yttrium in bone. This suggested that the deposition of these latter elements might not be related to the laying down of bone salts.

c. Severe Phosphorus Deficiency

With the exception of an experiment reported by Day and McCollum, there has been almost no work on severe phosphorus deficiency, because of the difficulty in obtaining a source of protein free of phosphorus. By using carefully washed blood fibrin as protein source, it was possible to prepare a synthetic diet extremely low in phosphorus. This diet produced extreme deficiency symptoms in young animals within two or three weeks. In such young animals, almost no bone salt was left in the long bones, and even in adult animals, extensive decalcification took place, particularly if aluminum sulfate were added to the diet. Evidently, the phosphorus essential for the soft tissue was obtained by decalcification of the skeleton. This was confirmed by the negative calcium balance associated with phosphorus deficiency. In this way, it was possible to obtain extensively decalcified bone in young rats, and to produce decalcification in adult animals.

d. Deposition of new bone over deposits of Plutonium to shield tissues from its radiations.

Because excretion of Plutonium is very limited, experiments were carried out to determine whether, following decalcification, new non-radioactive bone might be laid down over the deposits of Plutonium, thus effectively screening the living tissues from the short range alpha particles. Rats containing Plutonium were decalcified by lactation on a calcium or phosphorus deficient diet, or by simply maintaining for six weeks on a phosphorus defident diet. They were then placed on stock diet supplemented with 2% dicalcium phosphate and 2% cod liver oil. Six weeks later, they were sacrificed. Discs were cut from the femur in a standardized procedure, with a 0.140 inch trephine. Shielding of radiation was determined by comparing the alpha radiation from the periosteal and endosteal surfaces with the total radioactive material in the disc. Measurement of disc thickness and ash weight of tibia gave an index of the extent of recalcification. The reduction in surface radiation ranged from 2 to 3 fold, to 10 to 30 fold. In the former, very little recalcification had occurred. For this reason, the results were inconclusive. This problem is being investigated further in rats, particularly to determine means of producing more effective deposition of new bone and recalcification. Experiments are also being carried out in pigeons, in which the very rapid and intense bone formation and resorption associated with egg laying should give a quick answer as to the feasibility of this method for covering the deposits of Plutonium.

e. Site of Deposition of Plutonium

The fact that the deposition of plutonium does not follow the behavior of strontium, phosphorus or calcium, suggest that it may not be deposited with the bone salts. This has been substantiated for Plutonium

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by radioautographs of bones of rachitic and phosphorus deficient animals, in which very little bone salt is present. The Plut nium was found principally in the uncalcified osteoid matrix, and was deposited most intensely in the newly formed matrix below the epiphyseal plate. It did not deposit in cartilage. This was further supported by a study of standard fractures of the fibula in the rat. Intense deposition of Plutonium in the osteoid matrix of the callus occured several days before active calcification of the callus had commenced.

f. Work projected or in progress

Further investigations of fundamentals of bone metabolism, with special attention to decalcifying and calcifying mechanisms, to the effects of severe phosphorus deficiency, and to the action of parathormone and vitamin D.

Continuation of the studies on means of shielding the tissues from radiation by deposition of new bone over the deposited Plutonium. These experiments will be carried out on rats and pigeons.

Attention will be directed to the nature of osteoid matrix, the manner of combination with Plutonium and possible means of releasing it from this combination. Factors affecting the formation of osteoid matrix and the deposition of Plutonium therein will be investigated in normal and decalcified phosphorus deficient animals, and in the osteoid of the fracture callus. Two deficiencies in particular will receive attention: Vitamin C deficiency, since in scurvy formation of osteoid matrix is very defective, and deficiency of the sulfur containing amino acids, since they are precursors of certain essential components of the osteoid.

g. Deposition of Plutonium in the Callus of Healing Fractures

Previous experiments have indicated that Plutonium is probably deposited in the osteoid matrix of the bone, and not with the bone salts. In healing fractures, the osteoid matrix which appears in the fibrous callus does not show active calcification until the sixth to eight day, and the most active calcification occurs from eight to sixteen days after the fracture is produced. The following preliminary experiment was undertaken to determine whether plutonium would be deposited in the osteoid matrix of the callus before active calcification had commenced.

Standard fibular fractures were produced in rats which were then injected intramuscualrly with Plutonium. The rats were sacrificed at 4, 6, 9, and 13 days. The callus and adjacent bone were carefully removed and the radioactivity compared with that of a corresponding piece of bone from the unbroken opposited fibula of the same animal. The results are shown below:

TABLE III

Days Following Fracture	Plutonium Activity (counts/minute)				Calcification Activity % Dose Sr x 100	
	Fracture Callus		Unbroken Bone		Fracture	Control
4	500	624	72	30	22	17
6	340	360	155	105	23	10
9	200	215	80	100	35	16
13	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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There was a striking uptake of Plutonium by the callus, which was marked even at 4 days, before active calcification had begun. This lends further support to the view that plutonium is deposited in the osteoid matrix, and not with the bone salts. It is interesting that, in this small series, the amount of Plutonium in the callus appeared to decrease with time.

Further investigations are projected to determine whether Plutonium is actually removed from the callus during calcification and during the subsequent reorganization of the bone. Healing fractures also provide a means for studying factors which modify the formation of osteoid matrix and the deposition of Plutonium in it.

References: Copp, K. H., and Greenberg, D. M.
Studies on Bone Fracture Healing
Effects of Vitamins A and D. J. Nutrition 29: 261 (1945)

4. Plant Studies

The preliminary studies of the absorption of Plutonium by plants have been completed. Barley plants were placed in distilled water and in clay suspensions. In each treatment, six plants were immersed for 24 hours in 850 cc. of solution containing about 28,000 counts per second. Each treatment was carried out in triplicate. All three valence states were investigated. The results for the leaves and roots are presented in the following table.

TABLE IV

Valence States	% Plutonium Taken Up from H_2O (Average)		% Plutonium Taken Up From Clay Suspensions (average)	
	Roots	Leaves	Roots	Leaves
+3	16.1	0.0014	20.3	0.00045
+4	24.2	0.0037	24.6	0.0024
+6	29.4	0.0068	37.8	0.010

The data show that Plutonium, as with the fission elements, is fixed by the plant roots rapidly and in large percentages of the amounts offered. This is true for all three valence states of Plutonium under the conditions of the experiments. Very small amounts of the element are found in the leaves when present initially in the +3 and +4 states. Somewhat more activity is found in the case of the +6 state, especially when the Plutonium is initially adsorbed on clay.

General absorption experiments would lead one to expect a much greater translocation to the leaves of the +6 state than observed, owing to its solubility properties. It is, therefore, highly probable that the +6 state is reduced by the roots.

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In general it appears that the behavior of Plutonium is similar to that of the fission elements Zr + Cb, Y, and Ce. Plants exposed to these radio-elements would be subject to root injury. Because of the very pronounced ability of roots for the accumulation of these radio-elements in trace amounts, injury would be expected to occur at quite low levels of activity. With Sr. and presumably Cs, injury would also occur in the tops.

5. Radio-Chemical Isolation

Additional studies were made on the separation of U^{232} from Thorium and fission products. The results indicate that the substitution of Y carrier for Fe in the hydroxylamine method leads to a more convenient and satisfactory procedure.

The isolation of Pa by the di-isopropyl ketone extraction method has been investigated. It was found that minute traces of fluoride completely inhibited the extraction. This effect may be overcome by the addition of large amounts of zirconyl nitrate. A sample of Pa^{233} has been prepared in the cyclotron and is to be isolated shortly. Preparation of Pu^{238} on an internal probe in the cyclotron is continuing.

6. Radio-Iodine Tracer Studies

In table V there is summarized all the results to date obtained in a series of experiments which were designed to evaluate the possible prophylactic use of inert Iodine as a blocking agent to reduce the uptake of radio-Iodine by the thyroid gland. Inconclusive human experiments done before the war, indicated that the administration of inert Iodine prior to the giving of carrier-free radio-Iodine very significantly reduced the uptake of the latter by the thyroid. With this premise, a series of experiments on rats was undertaken in which the animals were given varying doses of Iodine at weekly intervals for periods of from four to six weeks. One week after the last dose of inert Iodine, carrier-free radio-iodine was administered and the uptake of radio-Iodine was determined in the thyroid four days later. As can be seen from the accompanying chart, the blocking action was not as great as might be anticipated. Moreover, considerable variations in the amounts taken up by the thyroids was observed in each series and in the last experiment (RE 15_4) a much small amount of active Iodine was employed and a significantly greater accumulation of the material in the thyroids of all four groups took place. This would suggest that at the ten and one microcurie dose level, the uptake of the gland had been decreased as a result of radiation effects. We are at a loss to give an adequate explanation as to why the blocking effect is as small as it appears. However, there is sufficient data accumulated to indicate that, at least in the rat, the blocking action under conditions of these experiments was surprisingly small, when one considers that the normal rat thyroid weighs from 20 to 50 milligrams and blocking doses as large as 100 milligrams per kilo, or approximately 25 milligrams per rat, were employed at weekly intervals for up to six weeks. It would appear that further studies of this character upon rats or other animals should be abandoned in the favor of direct human experimentation which can be done quite readily if it is felt that the need for such efforts still exists.

7. Projected Studies

Tracer, smoke radio-autographs and human studies will be continued for the next 2 months.

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RE 15 SERIES

% Uptake of I^{131} by the Thyroid of the Rat

	Controls No NaI				1 mg. NaI per kilo.			
Exp. No.	RE 15	RE 15_2	RE 15_3	RE 15_4	RE 15	RE 15_2	RE 15_3	RE 15_4
Dose I*	10 c	1 c	10 c	0.1 c	10 c	1 c	10 c	0.1 c
Animal A	4.5	6.84	6.75	24.4	1.81	3.65	5.74	21.0
Animal B	6.1	8.9	6.29	23.6	2.23	2.95	4.55	17.2
Animal C	3.87	6.64	—	22.8	2.13	2.95	—	23.2
Animal D	—	—	—	36.2	—	—	—	14.8
Average	4.82	7.46	6.52	26.75	2.06	3.18	5.15	19.05

(Continued)

	10 mg. NaI per Kilo				100 mg. NaI per Kilo			
Exp. No.	RE 15	RE 15_2	RE 15_3	RE 15_4	RE 15	RE 15_2	RE 15_3	RE 15_4
Dose I*	1 c	1 c	10 c	0.1 c	10 c	10 c	10 c	1 c
Animal A	0.545	0.85	5.65	15.2	.120	.26	3.18	6.03
Animal B	1.08	1.21	7.15	12.3	.33	.26	2.82	5.30
Animal C	1.24	0.914	—	13.6	.16	.432	—	6.75
Animal D	—	—	—	16.7	—	—	—	6.25
Average	0.955	0.991	6.40	14.45	.203	.317	3.00	6.08

RE 15, 15_2 and 15_3 animals were "pretreated" four weeks before administration of I*.
RE 15_4 animal was "pretreated" six weeks before administration of I*.
At the end of the last I unjection, one week was allowed to elapse before radio-Iodine injection. Animals were killed four days after radio-Iodine injection.

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