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ABSORPTION OF PLUTONIUM FED CHRONICALLY TO RATS I FRACTION DEPOSITED IN SKELETON AND SOFT TISSUES FOLLOWING ORAL ADMINISTRATION OF SOLUTIONS OF VERY LOW MASS CONCENTRATION

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August 10 1953

HANFORD ATOMIC PRODUCTS OPERATION
RICHLAND WASHINGTON

Operated for the Atomic Energy Commission by the General Electric Company under Contract # W-31-109-Eng-52

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ABSTRACT

1. The gastro-intestinal absorption and deposition of plutonium in bone and soft tissue of 180 rats after 5'7 low-level feedings of Pu^{238} were determined at doses that varied from 0.04 to 1.2 times the currently accepted maximum permissible concentration of plutonium in drinking water. Within the 95 per cent limits of confidence, the mean per cent total, soft tissue, and skeletal deposition was 0.00261 ± 0.00038, 0.00021 ± 0.00010, and 0.00234 ± 0.00028 respectively for animals receiving plutonium levels equivalent to 1.2 times the maximum permissible concentration.

2. No significant decrease in skeletal plutonium was observed over a period of 250 days following cessation of feedings.

3. The concentration of plutonium fed appeared to exert some effect on amount absorbed and deposited, but this is being further investigated.

ABSORPTION OF PLUTONIUM FED CHRONICALLY TO RATS. I FRACTION DEPOSITED IN SKELETON AND SOFT TISSUES FOLLOWING ORAL ADMINISTRATION OF SOLUTIONS OF VERY LOW MASS CONCENTRATION

INTRODUCTION

The principal objective of this study was to measure the fraction of plutonium administered orally to rats which is absorbed from the gastro-intestinal tract and is subsequently deposited in the skeleton and soft tissues. A survey of the literature pertaining to the metabolism of plutonium revealed that previous studies had involved the feeding of relatively high concentrations of plutonium to small numbers of animals during short periods of time[4]. The calculation of the currently accepted maximum permissible concentration (M. P. C.) for the soluble compounds of Pu^{239} in drinking water (1.5×10^{-6} μc/ml)[6] was based on gastro-intestinal absorption values obtained under conditions rather far removed from those apt to be encountered as a result of chronic contamination of food or water. The present study involved the feeding of plutonium solutions approximating the M. P. C. to 160 rats over a period constituting a significant fraction of the life span of the animal.

METHODS

One hundred and sixty young adult Sprague-Dawley rats were divided into 4 groups of 40 experimental rats each (20 males and 20 females). These animals were housed in individual cages having half-inch mesh wire floors which allowed excreta to drop through to prevent coprophagy. Dog chow pellets (Purina) and water were supplied ad libitum. In order to permit deposition of detectable amounts of plutonium in the bones and soft tissues in spite of the low mass levels of radioelement in the feeding

solutions and in spite of the low percentage absorption of plutonium from the gastro intestinal tract, Pu^{238} was used for all feedings.* By virtue of the high specific activity of Pu^{238} (about 260 times greater than that of Pu^{239}), solutions of low mass concentration could be employed.

Details of the feeding regimen are summarized in Table I. It was originally planned to administer only one dose of plutonium per day to each rat, but in order to shorten the duration of the experiment, feedings were increased first to two doses per day, then to three, and for the last month of feedings to four doses per day. Doses administered to a given rat were always separated by at least two hours.

In order to maintain control over possible sources of contamination during feedings as well as during radiochemical analyses, 40 additional rats (20 males and 20 females) were subjected to the same procedures as the experimental animals, except that plutonium was omitted from the solutions administered. The gavage apparatus used in the feedings is diagrammed in Figure 1. Separate gavage units were employed for feeding the control rats and each group of experimental animals. Adsorption of plutonium on rubber and glass was minimized by coating syringes, pipettes, catheters, and flasks with a silicone preparation (dimethyldichlorosilane, General Electric Co.).

As a control on the actual amount of plutonium administered to each group of experimental rats, one ml volumes of each feeding solution were delivered, before and after each feeding of a given rat group, directly from the gavage unit onto counting plates. Data listed in Table I for the amount of plutonium fed to each group were derived from statistical analyses of

* The Pu^{238} was obtained from Dr. G. T. Seaborg, University of California Radiation Laboratory, Berkeley, California.

TABLE I

Dosage Schedule

(Each group represents 40 rats)

Group	Pu^{238} per Average Dose		Fraction of M.P.C. (weight basis)*	Total Amount of Pu^{238} Fed**	
	d/m	µµg		d/m	µµg
I	36	1	0.04	18,821 ± 210	523
II	110	3	0.12	56,698 ± 770	1,575
III	365	10	0.40	188,707 ± 752	5,242
IV	1121	31	1.2	579,873 ± 2256	16,108

Chemical form of administered Pu:	$Pu(NO_3)_4$
pH of administered solution:	2.0
Mode of administration:	stomach tube
Volume administered per dose:	1.0 ml
Frequency of administration:	1-4 doses per day, 5 days per week
Duration of experimental feeding period:	$9\frac{1}{2}$ months
Total doses administered:	517

* Since the specific activity of Pu^{238} is about 280 times greater than that of Pu^{239}, a mass concentration of Pu^{238} equal to the M.P.C. for Pu^{239} (1.5×10^{-6} µc/ml, or 3.4 d/m/ml) would have an activity of about 900 d/m.

** The Pu^{238} employed was chemically and radiochemically pure. The presence of a slight isotopic impurity of Pu^{239} below radiochemically detectable limits may have been present. Such impurities may have increased the weights fed over those listed above by an amount not exceeding 25 per cent.

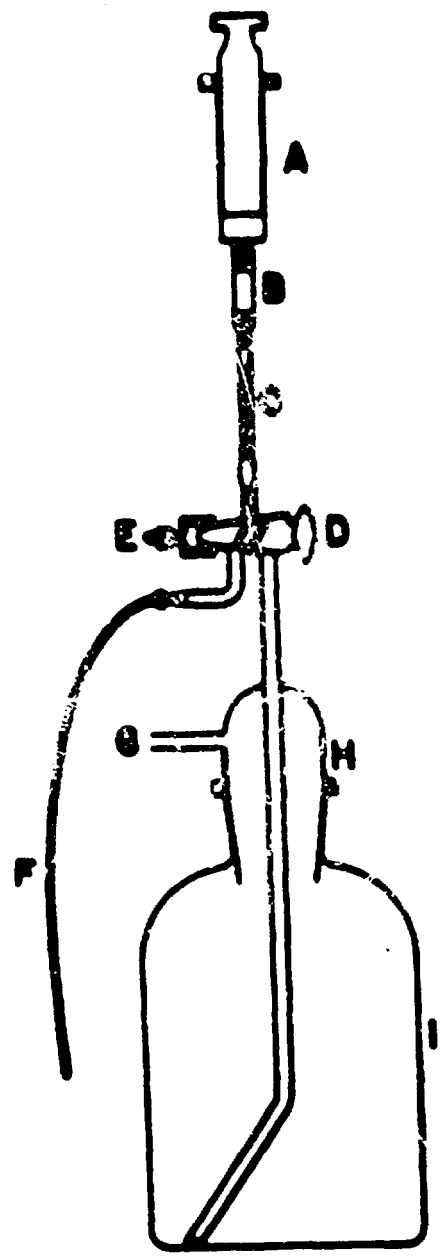

FIGURE 1

GAVAGE APPARATUS USED FOR FEEDING PLUTONIUM TO RATS

A - 3 ml syringe
B - Rubber sleeve
C - 1 ml pipette
D - 3-way stopcock
E - Stopcock adapter
F - Rubber catheter (French 8)
G - Air vent
H - Ground glass connection
I - Plutonium solution flask

TABLE 2

Comparison of Total Amounts of Pu Found in Fresh and Autoclaved Bone ($d/m \times 10^{-3}$)

Rat No.	Fresh Bone Sample	Autoclaved Bone Sample
1	2.64	2.69
2	3.09	2.76
3	2.39	2.16
4	2.82	2.76
5	3.22	3.01
6	2.96	3.14
7	3.16	2.76
8	2.76	2.69
9	3.11	2.81
10	2.36	2.54
Total	28.51	27.32

In view of the extended duration of the feeding period, it was necessary to obtain knowledge of the biological turnover of deposited plutonium. Ten of the forty rats receiving the highest plutonium concentration (designated as Group IVa) were sacrificed at the same time as the other experimental rats. The remaining 30 rats in Group IV were divided into 3 subgroups of 10 rats each (designated as Groups IVb, IVc, and IVd) and these groups were sacrificed 90, 180 and 250 days after feedings had been discontinued.

The various tissue segments, bone, soft tissue, pelt and gastrointestinal tract were digested with concentrated nitric acid, evaporated to dryness, and muffled to a white ash at 600°C. The ash was dissolved

in 2 N nitric acid and analyzed according to a standard procedure for biological samples of low activity density now in use in our laboratory[2].

Tissues from experimental rats were analyzed simultaneously with similar tissues from rats in the control group and with a tissue to which a known amount of plutonium was added. Results on experimental tissues were corrected for the activity found in the control tissues and for the per cent recovery obtained from the spiked tissue. In order to minimize the possibilities of cross contamination, all tissue segments from rats in a lower plutonium feeding level were analyzed before commencing analyses on tissues from the next higher level.

Samples were counted on proportional low background alpha counters (manufactured by the Nuclear Instruments Corp.) having a 52 per cent geometry and operating at the 95 per cent confidence level. Background was less than 0.5 counts per minute.

RESULTS

A mortality of about 8 per cent occurred over the entire feeding period of 9 1/2 months. Deaths usually could be attributed to respiratory ailments. The average plutonium contents in the body fractions of the experimental rats are given in Table 3. These values have been corrected for the activity detected in the simultaneously analyzed control rats as well as by appropriate factors based on average spike recovery data obtained for each group and for each type of tissue. Values shown in parentheses were not statistically different at the 95 per cent confidence level from values obtained on similar tissues from control animals.

In the case of animals on the lowest plutonium feeding level (Group I) only the gastro-intestinal tract showed significant plutonium retention, while the rats in Group II which received solutions of 0.12 of the M.P.C.

TABLE 3

Average Plutonium Deposition in Body Fractions

(d/m ± 95% Confidence Limits)

(Values in parenthesis are not significantly different from controls)

Group	G.I. Tract	Pelt	Skeleton	Soft Tissue
I	2.94 ± 0.55	(0.09 ± 0.36)	(0 ± 0.36)	(0.50 ± 0.52)
II	4.65 ± 1.36	0.21 ± 0.17	1.10 ± 0.29	(0.04 ± 0.18)
III	14.80 ± 5.05	0.69 ± 0.33	4.64 ± 0.59	0.84 ± 0.76
IVa	104.48 ± 80.96	2.88 ± 2.20	14.68 ± 3.79	1.28 ± 0.93
IVb	(0.14 ± 0.75)	(0.87 ± 0.87)	13.57 ± 2.59	1.16 ± 0.76
IVc	10.42 ± 6.63	(0 ± 0.53)	2.10 ± 1.65	4.62 ± 2.53
IVd	(0.82 ± 1.44)	(0.23 ± 1.82)	13.03 ± 2.48	2.88 ± 2.37

showed significant retention in all body fractions except the soft tissues. The skeleton and soft tissues of the animals which received solutions of 0.4 of the M.P.C. and 1.2 times the M.P.C. were found to contain significant amounts of plutonium. The same was true of the gastro-intestinal tracts and pelts of the rats in these last two groups which were sacrificed immediately after cessation of plutonium feedings. The plutonium content in the pelts and gastro-intestinal tracts of the rats on the highest level of plutonium administration (Group IV) which were sacrificed at various time intervals following cessation of feedings showed a decreasing contamination with time and reached non-significant levels at the end of 90 days.

Table 4 shows the per cent of fed plutonium which was absorbed from the gastro-intestinal tract and deposited in the skeleton and soft tissues of the experimental rats. In calculating the total per cent absorbed

TABLE 4

Average Per Cent of Gastro-Intestinal Absorption
and Deposition of Plutonium Fed to Rats
(95% Confidence Limits)

Group	Fraction of M.P.C. in Solutions Fed	Skeleton	Soft Tissues	Total
I	0.04	-	-	-
II	0.12	.00194 ± .00051	-	.00194 ± .00051
III	0.4	.00246 ± .00031	.00044 ± .00040	.00290 ± .00051
IVa	1.2	.00253 ± .00065	.00022 ± .00016	.00275 ± .00067
IVb	1.2	.00234 ± .00045	.00020 ± .00013	.00254 ± .00047
IVc	1.2	.00208 ± .00028	.00080 ± .00044	.00288 ± .00051
IVd	1.2	.00226 ± .00043	.00050 ± .00041	.00276 ± .00058
IV Combined	1.2	* .00234 ± .00028	** .00021 ± .00010	** .00261 ± .00033

* Combined values for Group IV were obtained by pooling weighted components of sub-groups IVa, IVb, and IVd (See text under Discussion).

** Combined values for Group IV were obtained by pooling weighted components of sub-groups IVa and IVb (See text under Discussion).

the amounts of plutonium found in the pelts and gastro-intestinal tracts were neglected, since as indicated by the sharp drop in activity following cessation of feedings, the former values represent largely contamination of pelt by excreta while the latter consists of physically adsorbed plutonium on food particles or gastro-intestinal mucosa which were not removed during the 4-day pre-sacrifice fasting period.

DISCUSSION

Statistical evaluation of data pertaining to skeletal deposition of plutonium in rats of sub-groups IVa, IVb, and IVd (see Table 4) which were sacrificed 4, 90, and 250 days, respectively, following cessation of plutonium feedings reveals that no significant differences exist among those values. Results of radiochemical analyses of skeletal plutonium deposition in rats which were sacrificed 380 days after completion of feedings (sub-group IVc) indicate a significant decrease, but this value is considered to be the result of experimental error due to the low levels of activity assayed, the length of the experiment, and the complexity of the laborious plutonium extraction procedure used. This is taken to justify the conclusion that no significant decrease in skeletal plutonium content occurred during the experimental feeding period. A pooling of values for sub-groups IVa, IVb, and IVd yields 0.00234 ± 0.00028 per cent at the 95 per cent confidence interval for the mean deposition in skeletal tissue for rats in Group IV which received 1.2 times the maximum permissible concentration of plutonium.

Similar statistical analyses of the observed plutonium concentrations in soft tissues suggested that any pooling of results in the sub-groups comprising Group IV would best be limited to those of IVa and IVb. Values from IVc and IVd were discarded on the basis that turnover of plutonium from soft tissue may be reasonably expected to be more rapid than from

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the gastro-intestinal tract on fraction absorbed. Discrepancies in coefficients of absorption calculated from the present study and from earlier work may have been due to such concentration effects.

The calculation of the maximum permissible concentration of plutonium in drinking water must not be based only on average values of plutonium deposited. It must place emphasis on individual variation in order to set up standards which will protect the exceptional as well as the average individual. With this in mind, Table 5 was prepared. Here are found statistical estimates of the maximum per cent of gastro-intestinal absorption to be expected in 99 per cent of the experimental rats, 90 per cent of the time. In spite of the pessimistic bias embodied in these values, the limiting per cent of gastro-intestinal absorption falls below the 0.01 per cent which has previously been accepted as the best experimental average value for the rat.

The presently accepted M. P. C. for plutonium in water (1.5×10^{-6} μc/ml) is based on an assumed absorption coefficient of 0.1 per cent[3]. This is a factor of approximately ten higher than the absorption coefficient determined by previous workers using small numbers of animals and high concentrations of plutonium; and a factor of 40 higher than the absorption coefficient measured in the present experiment. In view of the consistency of the data obtained in the present study, the large number of animals employed, and the low concentration levels employed, it would seem reasonable to accept a value of 0.01 per cent for the absorption coefficient. This would still leave a safety factor of four to take care of unusual individual variation and the possibility of species variation between the rat and man. Using 0.01 per cent for the absorption coefficient the M. P. C. for plutonium in water would become 1.5×10^{-5} μc/ml.

TABLE 5

Maximum Per Cent of Gastro-Intestinal Absorption and Deposition of Plutonium in 99 Per Cent of Rats 90 Per Cent of the Time.

Group	Skeleton	Soft Tissue	Total
I	-	-	-
II	.0047	.0027	.0074
III	.0050	.0038	.0088
IVa	.0062	.0009	.0071
IVb	.0043	.0008	.0051
IVc	.0033	.0027	.0060
IVd	.0039	.0019	.0058

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Patricia C. Aichele, M. P. Fujihara, and Joan O. Hess who were responsible for the feedings. The radiochemical analyses were performed by Bernice Grubb and Alene Forbes under the direction of A. C. Case. Statistical analyses of data were carried out by F. H. Tingey, Statistics Unit, and G. E. Pilcher, Control Services, Biophysics Section. Special thanks are also due M. H. Weeks for his invaluable aid and R. C. Thompson for his suggestions and advice.