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Foreman and Hamilton[1,2] in this laboratory demonstrated that the administration of the chelating agent which is the calcium salt of ethylenediamine tetracetic acid increased the urinary excretion of plutonium fed to rats in their diet. This compound hereafter will be referred to as Versene. The use of the sodium salt rather than its calcium compound was felt to be unsatisfactory because of its toxicity. When administered to animals in this chemical state it combines avidly with serum calcium and produces death by hypocalcemia, with relatively small doses as compared to its calcium salt. Versene has been employed in these studies to secure additional information concerning the change in the metabolism of plutonium in rats.

METHODS

Female rats, weighing from 200 to 310 grams, were used for these experiments. Four groups of animals were employed, there being six rats in each of the first two groups and 19 in the third and fourth groups. The animals in each group were divided into approximately equal numbers to obtain a comparison between Versene-treated and control animals. Each animal received from 0.18 to 0.09 microcuries of plutonium^{238}. The plutonium was in the form of plutonyl nitrate in the presence of 0.01 M sodium

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bromate and 0.1 sodium citrate at a pH of approximately 8. The 92-year Pu^{238} was employed rather than the long-lived Pu^{239} in order to secure a high degree of specific activity and thus approach a simulation of the possible concentration of plutonium that might be present in human beings who may have been exposed to this radioelement. Versene was added to the diet of the first three groups of treated animals. The amount present was 3.5 per cent of the weight of the diet. The animals were placed on this diet 24 hours before they received the plutonium.

The first group of rats received 2 milligrams of Versene by intravenous injection immediately after the plutonium was given by the same route of administration. The treated animals and control animals of this group were sacrificed at the end of 24 hours. The second group was given 2 milligrams of Versene in the same manner as that employed for the first group and the treated and control animals were sacrificed 15 days later. The third group of treated animals was given 186 milligrams of Versene by intraperitoneal injection immediately after they had received plutonium intravenously and with the control animals were sacrificed 15 days later. The fourth group was maintained on a normal diet without Versene and received plutonium by intramuscular injection. At the same time and one day later each animal received 155 milligrams of Versene by intraperitoneal injection. Because of the appearance of apparent toxicity as manifested by a severe but transient state of collapse in the treated animals, Versene was given by the subcutaneous route on the second, third, and fourth days, the amounts administered being 79, 159, and 170 milligrams respectively for each animal. No Versene was administered from the fifth to the eighth day. The following six days 155 milligrams of Versene was given daily to each animal by intraperitoneal injection. Twenty-four hours later the treated and control animals were sacrificed.

Both treated and control animals were maintained in standard metabolism cages, each cage containing from three to five rats. The urine and feces were collected. In addition to the collection of excreta, the following organs and tissues were removed at the time of sacrifice for assay of their plutonium content: spleen, blood, liver,

kidney, gastro-intestinal tract, muscle, skeleton, skin, injected leg of the fourth group, and finally, the tissue described as "balance". After the animals had been skinned the carcass was ashed and the skeleton removed from the remaining ash. There was deducted from the measured values of this ash the calculated amount of activity present in muscle and blood. In the case of the animals which received plutonium by intramuscular injection, an additional correction was made, since the injected leg was removed in toto. Thus, the balance may be presumed to represent structures such as glandular, lymphoid, and connective tissue.

Some of the tissues were assayed for their plutonium content by the technique which has been described in detail earlier by Scott et al.,[3] part of which is outlined in the first section of the Appendix. The specimens containing relatively large amounts of calcium such as the skeleton, balance, liver, skin, urine and feces, necessitated the use of a second assay technique which is also given in the Appendix. The purpose of the second technique was to avoid the interference from the presence of calcium in these tissues and excreta. Following these assay techniques, the plutonium content of the samples was determined by the use of the argon gas counter. A sufficient number of counts was taken to reduce the statistical fluctuations to approximately ± 7 per cent. The average recovery of the plutonium administered to the animals ranged from 82 to 90 per cent. With the use of spiked samples the recovery ranged from 95 to 99 per cent with both assay techniques. The data were computed in such a manner as to correct the values for tissues and excreta to 100 per cent. In the case of the fourth experiment this value of 100 per cent was calculated on the basis of the fraction absorbed from the site of injection. A more detailed account of these procedures has been described by Scott et al.[3]

The data presented in the four Tables gives the plutonium content of the tissues, organs, and excreta; this is expressed as the per cent content of plutonium and to this information is added the standard deviation. Also included are the values

for the injected leg in the fourth experiment, the measured recovery of the administered plutonium, and finally, the number of rats for each experiment. In addition to the four Tables the determinations of the urinary content of plutonium have been plotted for the last three groups of treated animals and their controls. This information appears in Figure 1.

RESULTS

The data obtained from the first group of animals are summarized in Table I. The distribution of plutonium in the tissues and its content in the feces was not significantly different in the treated animals and the normal controls. There would appear to be a slight enhancement of urinary excretion. Thus it would seem that the small amount of Versene given immediately following the injection of plutonium was not sufficient to chelate a significant fraction of the plutonium and cause its subsequent excretion. Likewise it appeared that the oral ingestion of Versene from the preceding 48 hours had little or no effect.

The results from the second group of animals are presented in Table II. It appears that there was a significant increase of the urinary excretion in the treated animals which was approximately five times greater than that observed in the untreated controls. The values for balance and muscle also appear to be lower in the treated animals of this group. These effects would seem to have been produced by the Versene added to the diet. These results are similar to those observed by Foreman.[2] None of the other tissues or organs of the treated animals appear to be significantly different from the corresponding structures in the normal controls.

Table III presents the results of the intraperitoneal injection of a large dose of Versene upon the distribution and excretion of plutonium. There was noted nearly an eightfold increase of urinary excretion and the decrease in plutonium content of liver, muscle, skeleton, skin, and balance appearsto be definite. The amount of plutonium retained in the skeleton was nearly one-half that of the untreated

control animals. The degree of depletion was apparent but possibly not as striking in the case of spleen, blood, kidney, and muscle. A comparison of the data in Table III with those in Table II indicates that the much greater effect was due to the administration of a single large dose of Versene rather than the action of the diet, since the treated animals of both groups had Versene added to their diet.

The results obtained by the multiple injections of large amounts of Versene in animals which had not received this substance in their diet are summarized in Table IV. In general it may be said that the relative degree of depletion of plutonium in the tissues and the increased excretion is quite comparable to that observed in Table III which was the experiment in which the treated animals received but one injection of Versene. A noteworthy observation in the fourth experiment is the apparent mobilization of plutonium at the site of injection which in this case was by the intramuscular route.

Figure 1 presents a chart indicating the effect of Versene upon the urinary excretion of plutonium. The data for the treated and control animals were obtained obtained from the last three experiments. In the half-shaded symbols the squares indicate the data from those animals which received 2 milligrams of Versene immediately after the administration of plutonium, the triangles represent data from the animals which received a large single dose of Versene following plutonium administration, and the circles indicate the effects of multiple injections of Versene. The corresponding open symbols are the data from the control animals. The last two experiments indicate the effect of a single large dose of Versene in enhancing the urinary excretion. The secondary rise of plutonium excretion in the treated animals following the four-day interval during which they received no Versene and were subsequently given this substance is quite evident.

DISCUSSION

These studies indicate that the oral administration of Versene did not produce a pronounced depletion of plutonium in the soft tissues and apparently had very little effect upon the skeletal content of plutonium. The parenteral administration of a small amount of Versene likewise appears to be productive of little effect. An increase of urinary excretion was noted after the prolonged feeding; this effect, however, is not of sufficient magnitude to influence significantly the content of either the skeleton or soft tissues. The parenteral administration of large amounts of Versene has been shown to decrease the skeletal content of plutonium and to increase the absorption from the intramuscular injection site. The latter observation is of interest, since one probable route of exposure of an individual to plutonium might be its subcutaneous or intramuscular introduction into the body. It is unrealistic to consider that plutonium might enter the body by the intravenous route. Earlier studies at Crocker Laboratory[3] and other work[4] have shown that only minute amounts of plutonium are absorbed from the digestive tract. It will be of interest to determine if the parenteral administration of Versene is capable of reducing the plutonium content of the lungs following inhalation of the plutonium aerosol.

With the current information available it would appear that Versene is of very limited practical value for the treatment of chronic plutonium poisoning. The relatively small increase of urinary excretion after this substance has been deposited in the skeleton, together with the large amounts of Versene which appear necessary, would seem valid reasons for not giving this substance as a practical therapeutic agent. It must be kept in mind, moreover, that in the rat the amounts of Versene necessary to secure an appreciable increase of urinary excretion are in the toxic range. The immediate use of large amounts of Versene following the accidental entry of plutonium into the body through cuts or abrasions would appear

to offer some promise. Before such attempts are made, however, a thorough knowledge of both the acute and chronic toxicity of Versene at the dose levels required should be made.

REFERENCES

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APPENDIX

(a) Lanthanum Fluoride Method for Separation of Plutonium from Tissue Ash

1. Dissolve ash in 2 N HNO_3 plus 0.1 M hydroxylamine hydrochloride for 20 minutes. Use 5 to 25 cc. of solution, depending upon amount of ash.
2. Divide 1 cc. sample in half and put each sample in 2 ml centrifuge cone.
3. Add .025 ml $La(NO_3)_3$ and .2 ml 6 N HF to each tube.
4. Centrifuge for 30 seconds.
5. Stir and centrifuge for 3 minutes. Draw off supernatant and discard.
6. Dissolve precipitate in .2 ml of 12 N HNO_3.
7. Draw off sample with pasteur pipette and place in porcelain dish.
8. Rinse centrifuge tube with distilled water.
9. Add 2 drops of 6 N HF to precipitate plutonium.
10. Evaporate and count.

(b) TTA Method for Separation of Plutonium from Tissue Ash

1. Add 50 ml of 2 N HNO_3 plus 0.1 M hydroxylamine hydrochloride solution to ashed sample and let stand for at least 2 hours.
2. Put 10 cc sample in centrifuge cone.
3. Add 3 cc of $La(NO_3)_3$ without stirring.
4. Add 10 ml of 12 M HF and stir.
5. Centrifuge for 5 minutes at 2000 rpm.
6. Pour off supernatant when precipitate is well packed down.
7. Add 10 ml of 1.5 M HF to cone and stir up precipitate.
8. Centrifuge again for 5 minutes at 2000 rpm and pour off supernatant when precipitate is well packed down.
9. Stir precipitate to break it up. Add 25 ml $Al(NO_3)_3$ and stir until precipitate dissolves.

10. Add .25 ml $NaNO_2$.
11. Stir and let stand at least 15 minutes before pouring into a separatory funnel.
12. Add 10 ml of TTA solution and shake for 10 minutes.
13. Let stand one hour and drain off bottom layer of liquid.
14. Wash twice with distilled water.
15. Add 10 ml of 8 M HNO_3 and shake for 15 minutes.
16. Let stand for 5 minutes.
17. Drain product into porcelain dish.
18. Rinse through funnel tube once with 8 M HNO_3.
19. Evaporate and count.

TABLE I

THE EFFECT OF VERSENE ON THE DISTRIBUTION OF Pu^{238} IN THE RAT ONE DAY AFTER ITS INTRAVENOUS ADMINISTRATION. TREATED ANIMALS WERE GIVEN 3.5 PER CENT OF VERSENE IN THEIR DIET STARTING 24 HOURS PRIOR TO ADMINISTRATION OF Pu^{238} AND 2 MILLIGRAMS OF VERSENE INTRAVENOUSLY AT THE TIME OF Pu^{238} INJECTION. THE DATA ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED AS THE PER CENT OF THE ADMINISTERED DOSE. THERE WERE THREE ANIMALS IN EACH GROUP.

	Treated		Control	
	% per organ	% standard deviation	% per organ	% standard deviation
Spleen	0.65	48.5	0.43	40.0
Blood	13.2	14.9	15.7	12.3
Liver	14.9	21.4	11.05	12.6
Kidney	1.04	16.1	1.20	24.3
G.I.	4.21	12.4	3.56	8.66
Muscle	5.70	9.70	7.23	12.3
Skeleton	47.1	7.09	47.8	4.66
Skin	7.37	40.8	9.88	27.2
Urine	3.37	-	0.89	-
Feces	2.36	-	2.23	-
Balance	-	-	-	-

TABLE II

THE EFFECT OF VERSENE ON THE DISTRIBUTION OF Pu^{238} IN THE RAT 15 DAYS AFTER Pu INTRAVENOUS ADMINISTRATION. THE ANIMALS WERE MAINTAINED ON A VERSENE DIET AS NOTED IN TABLE I, AND RECEIVED 2 MILLIGRAMS OF VERSENE INTRAVENOUSLY AT THE TIME OF Pu^{238} ADMINISTRATION. DATA ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED AS THE PER CENT OF THE ADMINISTERED DOSE. THERE WERE THREE ANIMALS IN EACH GROUP.

	Treated		Control	
	% per organ	% standard deviation	% per organ	% standard deviation
Spleen	0.73	46.5	0.55	60.0
Blood	0.45	11.8	0.45	9.46
Liver	5.72	30.0	7.73	12.0
Kidney	0.56	27.7	0.57	50.2
G.I.	0.79	35.3	0.76	1.61
Muscle	1.12	23.2	2.48	31.0
Skeleton	59.1	4.94	64.6	5.28
Skin	2.11	49.3	1.09	22.3
Urine	10.5	-	1.90	-
Feces	17.6	-	16.0	-
Balance	1.05	-	3.13	-

TABLE III

THE EFFECT OF VERSENE ON THE DISTRIBUTION OF Pu^{238} IN THE RAT 15 DAYS AFTER Pu INTRAVENOUS ADMINISTRATION. THE ANIMALS WERE MAINTAINED ON A VERSENE DIET AND RECEIVED 186 MILLIGRAMS OF VERSENE BY INTRAPERITONEAL INJECTION AT THE TIME OF Pu^{238} ADMINISTRATION. DATA ARE CORRECTED FOR RECOVERY AND ARE EXPRESSED AS PER CENT OF THE ADMINISTERED DOSE. THERE WERE NINE TREATED ANIMALS AND TEN CONTROLS.

	Treated		Control	
	% per organ	% standard deviation	% per organ	% standard deviation
Spleen	0.36	72.2	0.61	33.3
Blood	0.28	58.4	0.41	50.0
Liver	3.60	33.4	8.75	20.2
Kidney	0.49	51.8	1.36	30.6
G.I.	0.31	46.4	0.88	26.0
Muscle	0.70	41.4	1.41	38.4
Skeleton	30.6	8.58	55.6	17.9
Skin	0.51	38.7	1.47	22.3
Urine	50.6	-	6.10	-
Feces	11.6	-	20.7	-
Balance	0.90	-	2.69	-

TABLE IV

THE EFFECT OF LARGE DOSES OF VERSENE ON THE DISTRIBUTION OF Pu^{238} IN THE RAT 15 DAYS AFTER Pu INTRAMUSCULAR ADMINISTRATION. THE VERSENE WAS ADMINISTERED BY MULTIPLE INTRAPERITONEAL INJECTIONS. THE DATA ARE CORRECTED FOR RECOVERY AND ARE EXPRESSSED AS PER CENT OF THE ADMINISTERED DOSE. THERE WERE TEN TREATED ANIMALS AND NINE CONTROLS.

	Treated		Control	
	% per organ	% standard deviation	% per organ	% standard deviation
Spleen	0.21	77.3	0.49	21.8
Blood	0.17	32.8	0.52	23.2
Liver	2.22	28.2	7.36	31.6
Kidney	0.40	33.2	1.06	24.7
G.I.	0.33	24.0	0.87	42.3
Muscle	0.68	30.5	1.36	36.9
Skeleton	32.9	20.9	64.1	10.2
Skin	0.38	23.8	1.15	24.4
Urine	49.0	-	4.60	-
Feces	13.7	-	18.5	-
Injection site	6.50	-	28.7	-
Balance	0.85	-	2.82	-

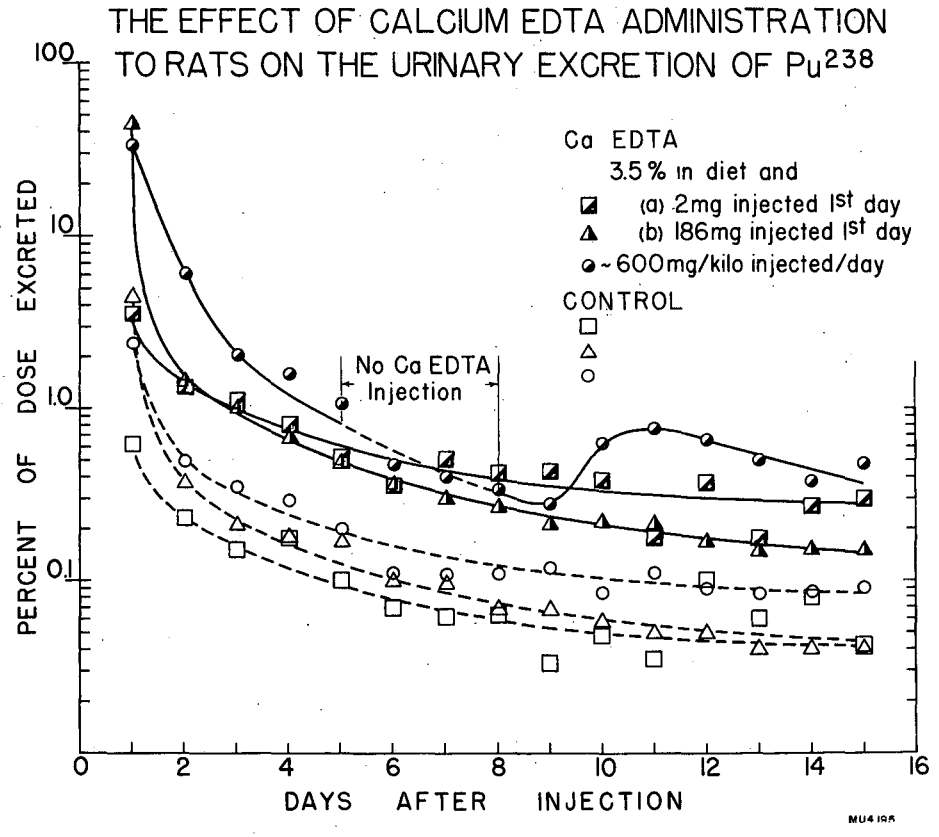
THE EFFECT OF CALCIUM EDTA ADMINISTRATION
TO RATS ON THE URINARY EXCRETION OF Pu238
PERCENT OF DOSE EXCRETED
100
10
1.0
0.1
Ca EDTA
3.5% in diet and
(a) 2mg injected 1st day
(b) 186mg injected 1st day
~600mg/kilo injected/day
CONTROL
No Ca EDTA Injection
0
2
4
6
8
10
12
14
16
DAYS AFTER INJECTION

Fig. 1