tion of bites at 2 weeks following initial term feeding as at 24 hours. 3. Virus carrying mosquitoes induced fibromas on the eyelid and close to the eye of one cottontail when given the opportunity to feed at will. 4. Possible mechanisms involved in mosquito transmission are discussed.

1. Aragao, H. B., *Mem. Inst. Oswaldo Crus,* 1943, v38, 93.

2. Fenner, F., Day, M. F., and Woodroofe, G. M., *Aust. J. Exp. Biol.,* 1952, v30, 139.

3. Shope, R. E., *J. Exp. Med.,* 1932, v56, 793.

4. Berry, G. F., and Dedrick, H. M., *J. Bact.,* 1936, v31, 50.

5. Shope, R. E., *J. Exp. Med.,* 1936, v63, 33.

6. Fenner, F., *Nature,* 1953, v171, 562.

7. Philip, C. B., *J. Parasit.,* 1942, v28, 395.

8. Kilham, L., in preparation.

9. Kilham, L., Herman, C. M., and Fisher, E. R., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 298.

10. Kligler, I. J., Muckenfuss, R. S., and Rivers, T. M., *J. Exp. Med.,* 1929, v49, 649.

11. Kligler, I. J., and Ashner, M., *Brit. J. Exp. Path.,* 1929, v10, 347.

12. Fenner, F., and Day, M. F., *Nature,* 1952, v170, 204.

13. Shope, R. E., *Cornell Vet.,* 1951, v41, 181.

Received May 22, 1953. P.S.E.B.M., 1953, v83.

## Effect of Calcium Salt of Versene upon Metabolism of Plutonium in the Rat.* (20340)

JOSEPH G. HAMILTON AND KENNETH G. SCOTT.

*From the Divisions of Medical Physics, Experimental Medicine, Radiology, Crocker Laboratory, University of California, Berkeley and San Francisco.*

Because of the potential hazard of the fission products and fissionable materials to individuals who may have been exposed to them, considerable interest has been devoted to the pharmacology of these radioactive substances as well as the development of methods for removing them from the body. Any procedure which results in a greater than normal elimination of these materials from the body has value from two points of view, first it can reduce the ultimate hazard to the contaminated individual, and second it allows a more accurate estimation of the degree of contamination which may be suspected in personnel exposed to such hazards.

One of the compounds which has received attention as a decontaminating agent has been the sodium and calcium salts of ethylene diamine tetra-acetic acid, later referred to as Versene. The pharmacology of this organic compound has been reviewed in detail by the Bersworth Chemical Co.(1). Popovici *et al.* (2) investigated the control of serum calcium levels using the sodium and calcium salts of Versene. They demonstrated that the sodium salt was many times more toxic than the calcium salt due to the fact that the former compound combined with the serum calcium and produced death by hypocalcemia. Cohn *et al.* (3) has recently investigated the effects of the calcium salt upon the deposition and retention of radio-yttrium using rats as the experimental animals. In their studies the Versene salt and radio-yttrium were administered intraperitoneally, either shortly before or after radio-yttrium was given. The animals were sacrificed at intervals up to 5 days and a very marked diminution of skeletal uptake was observed in the treated animals. The greatest effect was noted in those animals which had been pretreated and the skeletal uptake was approximately 1/10 that of the control groups and the content in the liver was decreased by a factor of approximately 20. Earlier studies at this laboratory have demonstrated that oral administration of the calcium salt of Versene increased the urinary excretion of plutonium(4).

*Methods.* The dosage of Versene and mode of administration is summarized in each table. Wherever administered, Versene was given as

* This document is based on work performed under the auspices of the Atomic Energy Commission.

the calcium salt at a pH of 7. Female rats weighing from 200 to 310 g were used. The treated animals and controls received $Pu^{238}$. The dosage varied in the several experiments from 0.18 to 0.09 $\mu$c. The plutonium was administered at plutonyl nitrate in the presence of 0.01 M bromate which kept it in +6 valance state and 0.1 M citrate which forms a complex with plutonium thereby preventing the formation of a plutonium colloid. The 92-year $Pu^{238}$ was used rather than the longer lived $Pu^{239}$ in order to secure a higher degree of specific activity and thus approach a plutonium concentration as to the number of atoms that might be present in human beings who may have been exposed to this radioelement and who might have of the order of 1 $\mu$g of the longer lived isotope $Pu^{239}$ stored in their body. In all 5 experiments the treated and control animals were maintained in metabolism cages, each cage containing from 3 to 5 rats. The urine and feces were collected separately. The organs and tissues removed at the time of sacrifice were assayed for plutonium content and these included: spleen, blood, kidney, gastrointestinal tract, muscle, skin, injected leg of the fourth group as well as the "balance" of the rat. The balance represented the ashed remainder of the rat after it had been skinned, the skeleton removed from the ash and the calculated amount of plutonium in blood, muscle and skeleton subtracted from the total plutonium content of the balance. In the case of the animals which received plutonium by intramuscular injection an additional correction was made since the injected leg was removed at the proximal end of the femur. Thus the balance may be presumed to represent structures such as glandular, lymphoid, connective tissue and cartilage. Most of the tissues were assayed for their plutonium content by the technic which has been described in detail earlier by Scott *et al.*(5). In other cases specimens containing relatively large amounts of calcium such as the skeleton, balance, liver, skin, urine and feces necessitated the use of a second assay technic in which the chelating agent Thienyl Trifloroacetone was used to separate the plutonium from the tissue ash. This chemical, commonly called TTA, will effect a quantitative removal of plutonium from animal residues when the following method is employed. *TTA method for separation of plutonium from tissue ash.* 1. Add 50 cc of 2 *N* $HNO_3$ plus 0.1 M hydroxylamine hydrochloride solution to ashed sample and 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 (20 mg $La(NO_3)_3$ per cc). 4. Add 10 cc 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 cc of 1.5 M HF to cone and stir precipitate. 8. Centrifuge again 5 minutes at 2000 rpm and pour off supernatant when precipitate is well packed down. 9. Stir precipitate to break it up. Add 25 cc of 3.5 M $Al(NO_3)_3$ and stir until precipitate dissolves. 10. Add .25 cc of 0.1 M $NaNO_2$. 11. Stir and let stand at least 15 minutes before pouring into a separatory funnel. 12. Add 10 cc of TTA solution containing 25 g of TTA in 500 cc of benzene and shake 10 minutes. 13. Let stand one hour and drain off bottom layer of liquid. 14. Wash twice with distilled water. 15. Add 10 cc of 8 M $HNO_3$ and shake 15 minutes. 16. Let stand for 5 minutes. 17. Drain product and 8 M $HNO_3$ rinse from separatory funnel into porcelain, gold at platinum dish. 18. Evaporate to dryness and count.

TABLE I. Distribution of 0.18 $\mu$c of $Pu^{238}$ at 1 and 15 Days after I.V. Inj. to Control and Versene Treated Rats. The former given 2 mg Versene I.V. at time of inj. plus 3.5% in diet 24 hr prior to $Pu^{238}$ inj. 3 animals in each group. Values expressed are % of the dose per g wet wt and % excreted.

| | 1 day | | 15 days | |
|---|---|---|---|---|
| | Control | Versene | Control | Versene |
| Muscle | .06 | .05 | .01 | .01 |
| Skeleton | 2.33 | 2.38 | 2.98 | 3.07 |
| Urine | .89 | 3.37 | 1.9 | 10.5 |
| Feces | 2.23 | 2.36 | 16.0 | 17.6 |

*Results.* The results obtained from the first 2 experiments are summarized in Table I, and show that the oral administration of Versene is not an effective way of removing plutonium from the rat. The simultaneous injection of Versene intravenously at a dosage of 8 mg per kilo of body weight had little effect on altering the eventual distribution of plutonium in the

TABLE II. Distribution of 0.09 μc of $Pu^{238}$, 15 Days after I.V. Inj. in Tissues of Control and Versene Treated Rats. Values expressed as % of dose/organ and g wet wt. Corrected to 100% recovery. Average recoveries were 88.7% for the 10 animals in control group and 89.7% for the 9 animals in Versene group. 186 mg of Versene given I.P. at time of inj.; 3.5% in diet starting 24 hr prior to $Pu^{238}$ inj.

| | Control | | | | Versene | | | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | Mean* stand. error | % per g | Mean* stand. error | % per organ | Mean* stand. error | % per g | Mean* stand. error |
| Spleen | .60 | ± .06 | .67 | ± .08 | .17 | ± .05 | .20 | ± .09 |
| Blood | .40 | .04 | .02 | <.01 | .28 | .05 | .02 | <.01 |
| Liver | 8.77 | .52 | .89 | .04 | 3.59 | .38 | .39 | .04 |
| Kidney | 1.37 | .13 | .76 | .07 | .50 | .08 | .26 | .05 |
| G.I. tract | .88 | .07 | — | — | .32 | .04 | — | — |
| Skeleton | 55.4 | .92 | 2.62 | .12 | 30.6 | .20 | 1.35 | .09 |
| Muscle | 1.39 | .13 | .01 | <.01 | .71 | .10 | <.01 | — |
| Balance | 2.70 | .15 | — | — | .90 | .10 | — | — |
| Skin | 1.48 | .12 | .03 | <.01 | .51 | .06 | .01 | <.01 |
| Urine | 6.16 | .19 | — | — | 50.6 | 1.28 | — | — |
| Feces | 20.9 | .77 | — | — | 11.7 | .29 | — | — |

* Mean stand. error $\pm = \sqrt{\frac{\Sigma \text{ dev}^2}{n(n-1)}}$, for Tables II, III and IV.

TABLE III. Distribution of 0.11 μc of $Pu^{238}$, 15 Days after I.M. Inj. in Tissues of Control and Versene Treated Rats. Values expressed as % of dose/organ and g wet wt, corrected to 100% recovery and representing only plutonium absorbed from inj. site. Average recoveries were 90.9% for the 9 animals in control group and 86.7% for the 10 rats in treated group. 140 mg Versene given I.P. for first 5 days, none for following 3 days, 155 mg daily for next 7 days.

| | Control | | | | Versene | | | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | Mean* stand. error | % per g | Mean* stand. error | % per organ | Mean* stand. error | % per g | Mean* stand. error |
| Spleen | .44 | ± .05 | .54 | ± .03 | .20 | ± .05 | .28 | ± .08 |
| Blood | .47 | .04 | <.04 | — | .16 | .02 | .01 | <.01 |
| Liver | 6.50 | .52 | .97 | .09 | 2.12 | .16 | .27 | .01 |
| Kidney | .94 | .06 | .61 | .05 | .38 | .03 | .25 | <.01 |
| G.I. tract | .80 | .12 | — | — | .31 | .03 | — | — |
| Skeleton | 63.6 | .70 | 4.39 | .17 | 34.4 | 1.77 | 2.04 | .12 |
| Muscle | 1.21 | .13 | .01 | <.01 | .66 | .06 | <.01 | — |
| Balance | 4.29 | .57 | — | — | 1.08 | .19 | — | — |
| Skin | 1.03 | .10 | .04 | <.01 | .36 | .03 | .01 | <.01 |
| Urine | 4.10 | .15 | — | — | 47.1 | 2.18 | — | — |
| Feces | 16.6 | .71 | — | — | 13.2 | 1.03 | — | — |
| Left leg | 24.4 | — | — | — | 3.79 | — | — | — |

* See footnote, Table II.

tissues in animals which were also being fed 3.5% Versene. Although the group summarized in Table I had an increased urinary plutonium excretion no significant reduction was observed in the amount of plutonium found in the skeleton, and muscle.

The parenteral administration of large amounts of Versene to rats following a dose of plutonium is capable of reducing the plutonium stored in most tissues of the body. When plutonium was administered intravenously and Versene given parenterally, a significant decrease of plutonium in the tissues of rats sacrificed 15 days following plutonium administration was observed. These data are summarized in Table II. There was nearly an 8-fold increase of urinary excretion with the administration of Versene. The amount of plutonium found in liver, muscle, skin and balance of these treated animals was significantly less than that in the control. The amount of plutonium retained by the skeleton was nearly one-half that of the untreated control animals. A comparison of these data with

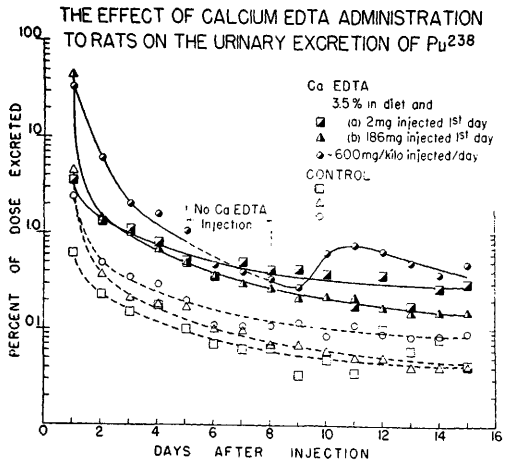


FIG. 1.

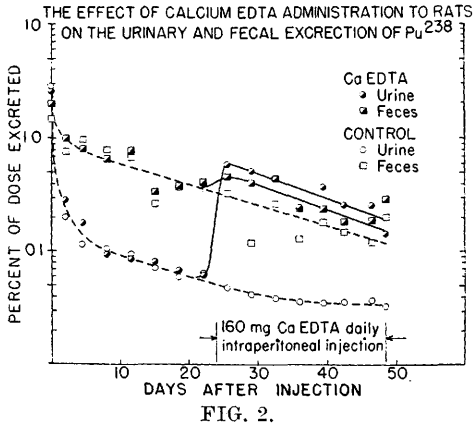


FIG. 2.

those obtained in the first 2 experiments indicates that the greater effect was apparently due to the administration of large amounts of Versene parenterally as compared to the action of this compound when present in the diet, since the treated animals in Groups 2 and 3 had the same diet.

The results obtained by the multiple injections of large amounts of Versene in animals on a normal diet are summarized in Table III. In general the results are very similar to those noted in the third experiment. An interesting observation in the fourth experiment was the effect of Versene upon the increased absorption of plutonium from the site of the injection, which was by the intramuscular route. Fig. 1 presents graphically the effect of Versene upon the urinary excretion of plutonium for the second, third, and fourth experiments. There was apparently some increase of urinary excretion in all 3 groups and it seemed greatest in the fourth group.

Fig. 2 indicates that the parenteral administration of Versene nearly a month after plutonium was given produced quite a prompt and definite increase of its excretion. The complete distribution of plutonium in the tissues in the fifth experiment is shown in Table IV. The fecal excretion of the Versene-treated and control animals was nearly the same. The depletion of plutonium in the tissues and organs was minimal. The greatest effects were seen with spleen, skin and balance. Here the plutonium content was reduced by nearly a factor of two. The skeletal content of plutonium was significantly lower in the Versene-treated animals but the reduction was not as great as that observed in the soft tissue.

*Discussion.* These studies indicate that prolonged oral administrations of Versene at levels that approached a significant degree of toxicity did not produce a pronounced depletion of plutonium in the soft tissues and had little or no effect upon the skeletal content of this element. The parenteral administration of small amounts of Versene likewise appears to be productive of little effect. The parenteral administration of large amounts of Versene at the same time plutonium was given appeared to decrease the skeletal content to a significant degree and also increase the absorption from the intramuscular site of injection. The latter observation is of interest since a very probable route of exposure of an individual to plutonium might be by subcutaneous or intramuscular introduction into the body. It would be of interest to determine if the parenteral administration of Versene is capable of reducing the plutonium content in the lungs following its inhalation as an aerosol and more particularly in a chemical state which can be absorbed and enter the blood stream, Scott *et al.*(5).

A comparison of these studies with those of Cohn *et al.*(3) are of interest in that the effect they observed was much greater than that noted in the experiments described in this paper. However, it should be kept in mind that in the studies by Cohn *et al.*(3) the

TABLE IV. Distribution of 0.12 $\mu$c of $Pu^{238}$ in Tissues of the Rat 50 Days after I.V. Inj. Values expressed as % of dose/organ and g wet wt and corrected to 100% recovery. Average recovery of the 10 control animals was 82.3% and 90.0% for Versene treated animals. 162 mg Versene given daily by I.P. from 25th to 50th day following Pu inj.

| | Control | | | | Versene | | | |
|---|---|---|---|---|---|---|---|---|
| | % per organ | Mean* stand. error | % per g | Mean* stand. error | % per organ | Mean* stand. error | % per g | Mean* stand. error |
| Spleen | .91 | ± .05 | 1.60 | ± .17 | .45 | ± .07 | .67 | ± .10 |
| Blood | .15 | <.01 | .01 | <.01 | .14 | <.01 | .01 | <.01 |
| Liver | 5.25 | .44 | .63 | .07 | 4.90 | .67 | .47 | .06 |
| Kidney | .68 | .07 | .37 | .05 | .65 | .02 | .32 | .01 |
| G.I. tract | .29 | .04 | — | — | .16 | .02 | — | — |
| Skeleton | 61.2 | 1.13 | 3.29 | .16 | 52.0 | 1.08 | 2.74 | .09 |
| Muscle | 1.06 | .18 | .01 | <.01 | .80 | .09 | <.01 | — |
| Balance | 3.68 | .15 | — | — | 1.51 | .18 | — | — |
| Skin | .98 | .04 | .03 | <.01 | .54 | .11 | .02 | <.01 |
| Urine | 6.33 | .40 | — | — | 15.2 | .40 | — | — |
| Feces | 19.5 | 1.11 | — | — | 24.1 | .68 | — | — |

* See footnote, Table II.

Versene compounds as well as the radio-yttrium were given by intraperitoneal injection. It is entirely possible that in their studies a large fraction of the radio-yttrium was chelated before it was absorbed. Thus their experiments and those described here are not entirely comparable.

With current information available it would appear that Versene is of very limited practical values for the treatment of chronic plutonium poisoning. The relatively small increase of urinary excretion after this radio-element has been deposited in the skeleton together with the large amount of Versene which appears necessary would seem valid reasons for not giving this substance as a practical therapeutic agent. Moreover, in the rat, the administration of Versene necessary to secure a significant amount of urinary excretion is in the toxic range. The immediate parenteral 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 might be made, careful consideration should be given to the problems of acute and chronic toxicity of Versene compounds in man.

*Conclusion.* 1. Oral administration of Versene in the rat at a toxic range did not produce a marked depletion of plutonium in soft tissues and had but little effect upon skeletal content. 2. When given parenterally large amounts of Versene depleted the skeletal content to a significant degree. 3. Under these conditions the absorption of plutonium from the intramuscular site of injection was increased. 4. These experiments indicate that it would appear that the use of Versene in chronic human plutonium poisoning offers little promise. 5. The prompt administration of Versene in large amounts might conceivably be of benefit when plutonium has entered the body by cuts or abrasions.

The authors wish to express their appreciation for the technical assistance of Miss Margaret Gee, Miss Marilyn Hemenway and Miss Helen Johnson.

1. Bersworth Chemical Co., Framingham, Mass., Technical Bulletin, *Pharmacology, Physiology, Biochemistry and Toxicity of Versenes.*

2. Popovici, Antoinette, Geschickter, Charles, Reinovsky, Albert and Rubin, Martin, PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 415.

3. Cohn, S. H., Gong, J. K., and Fishler, M. C. *Studies on the Treatment of Internal Radioactive Contamination.* II. *Prevention of Deposition of a Fission Product by Salts of Ethylene Diamine Tetra-Acetic Acid.* USNRDL-358.

4. Foreman, Harry and Hamilton, Joseph G. *The Use of Chelating Agents for Accelerating Excretion of Radioelements.* UCRL-1351.

5. Scott, Kenneth G., Axelrod, Dorothy, Crowley, Josephine and Hamilton, Joseph G., *Arch. Path.*, 1949, v48, 31.

Received March 24, 1953. P.S.E.B.M., 1953, v83.