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## Effectiveness of DTPA in Removing Plutonium from the Pig.* (26553)

VICTOR H. SMITH, JOHN E. BALLOU, WILLIAM J. CLARKE AND ROY C. THOMPSON
(Introduced by L. Bustad)
*Biology Laboratory, Hanford Laboratories, General Electric Co., Richland, Wash.*

The superiority of diethylenetriaminepentaacetate (DTPA) over chelating agents previously employed for removal of internally deposited plutonium is attested by a number of reports of its effectiveness in small animals (1-5). Its effectiveness in the human has been reported by Norwood(6). To help fill the gap between the rather extensive data on the rat and the very limited experience with man experiments were performed employing miniature swine. These animals have a weight comparable to man, and appear from preliminary studies to metabolize plutonium in a manner similar to man(7). The experiments were designed to measure the effectiveness of DTPA when administered promptly following plutonium injection, and when administered after a long interval following plutonium injection.

*Methods.* The miniature swine employed were 13-15 month males of the Pitman-Moore strain, confined to metabolism cages throughout the experiment. In the prompt treatment experiment 6 animals weighing from 40 to 60 kg were injected intravenously with 25 $\mu$c of $Pu^{239}$, administered as the tetravalent citrate complex in pH 5 solution. One hour later, 3 of the animals were injected intravenously with 40 ml of a pH 7 solution containing 9 g of the calcium trisodium salt of DTPA. In the delayed treatment experiment two 15 month males weighing 62 and 75 kg, injected 2 months previously with approximately 175 $\mu$c of plutonium IV citrate, were given 5 consecutive daily intravenous injections of $CaNa_3DTPA$, one gram the first day and 2 g on each of the succeeding 4 days. Excreta were collected daily in both experiments; daily blood samples were obtained only in the delayed treatment experiment. All animals were sacrificed 6 or 7 days following treatment. Complete tissue analyses were performed on animals from the prompt treatment experiment.

*Results of prompt treatment.* Table I

TABLE I. Effect of Single Prompt Dose of DTPA on Excretion of Plutonium by Pigs.

| | Excretion (% dose/day) | | | |
|---|---|---|---|---|
| | Urine | | Feces | |
| Day post inj. | Control* | DTPA treated* | Control | DTPA treated* |
| 1 | .6 | 45. | .1 | 1.8 |
| 2 | .1 | 29. | .3 | 1.3 |
| 3 | .2 | 6.8 | .4 | 1.7 |
| 4 | .2 | .5 | .4 | .4 |
| 5 | .1 | 1.0 | .2 | .3 |
| 6 | .1 | .3 | .1 | .1 |
| Total | 1.3 (.9-1.9) | 83 (78-88) | 1.5 (1.2-2.0) | 5.6 (3.5-8.9) |

* Avg of results from 3 pigs (range in parentheses).

* Work performed under Contract between Atomic Energy Comm. and General Electric Co.

TABLE II. Effect of Single Prompt Dose of DTPA on Retention of Plutonium by Pigs.

| Tissue | Retention (% of dose/total tissue) Control* | DTPA treated* |
|---|---|---|
| Liver | 14. (11. –16. ) | .47 ( .32 – .55 ) |
| Kidneys | .20 ( .16 – .27 ) | .20 ( .19 – .20 ) |
| Spleen | .24 ( .14 – .28 ) | .013 ( .012 – .013 ) |
| Testes | .10 ( .08 – .12 ) | .009 ( .007 – .010 ) |
| Adrenals | .0017 ( .0015– .0019) | .0005 ( .0005– .0006) |
| Femur | 2.5 ( 1.7 – 3.4 ) | .29 ( .20 – .38 ) |
| Total skeleton | 57. (56.5 –57.6 )† | 5.9 ( 5.2 – 7.1 ) |
| Excreta: Urine | 1.3 ( .9 – 1.9 ) | 83. (78. – 88. ) |
| Feces | 1.5 ( 1.2 – 2.0 ) | 5.6 ( 3.5 – 8.9 ) |
| Total recovered | 74. (70. –78. )† | 96. (93. –100. ) |

* Avg of results from 3 pigs (range in parentheses). † Data available from only 2 pigs.

shows average daily excretion of plutonium by the DTPA treated and control pigs. Enhanced excretion is evident predominantly in the urine, with the major effect during the first 3 days after treatment.

Table II shows results of tissue analyses on DTPA treated and control pigs. It is evident that DTPA exhibits some selectivity in its effect on tissue plutonium deposition. As previously observed in rats(2), concentration of plutonium in the liver is most favorably affected, while concentration in the kidney is least affected. The low recovery of only 74% of total injected plutonium in the case of the control animals is probably due to sampling problems, and to analytical difficulties in assay of the very large tissue samples involved. Such difficulties would have much less effect on total recovery in the DTPA treated animals where tissue plutonium content was lower by an average factor of about 10. While all tissue concentrations may therefore be low by as much as 25%, the general conclusions with regard to the effectiveness of DTPA treatment are not altered.

TABLE III. Excretion of Plutonium by Pigs Administered DTPA 60 Days after Plutonium Deposition.

| Days since Pu inj. | DTPA admin. (g) | Pig 1 Urine ($\mu$c/day) | Pig 1 Feces ($\mu$c/day) | Pig 2 Urine ($\mu$c/day) | Pig 2 Feces ($\mu$c/day) |
|---|---|---|---|---|---|
| 57 | | .02 | .004 | | .004 |
| 58 | | .01 | .003 | .01 | .005 |
| 59 | | .02 | .004 | .01 | .005 |
| 60 | 1 | .01 | .003 | .01 | .005 |
| 61 | 2 | 1.2 | .04 | (lost) | 1.3 |
| 62 | 2 | 1.3 | .8 | 1.4 | .8 |
| 63 | 2 | .7 | 1.7 | 2.3 | 3.8 |
| 64 | 2 | .8 | 3.9 | 2.1 | 4.1 |
| 65 | 1 | 1.2 | 1.5 | 2.8 | .2 |
| 66 | | .2 | .9 | .7 | 2.6 |
| 67 | | .3 | 1.5 | .5 | 5.4 |
| 68 | | .3 | .4 | .10 | .8 |
| 69 | | .2 | .4 | (lost) | 2.3 |
| 70 | | .15 | (lost) | .2 | 5.6 |
| 71 | | .14 | (lost) | .5 | (lost) |
| Total excretion ($\mu$c) | | 6.6 | 11.2 | 10.6 | 26.9 |
| Total excreted (% of initially inj. dose) | | 4 | 7 | 5 | 14 |

Results of delayed treatment. Table III shows the data on plutonium excretion of 2 pigs for 3 days prior to treatment, for 5 days during treatment, and for a subsequent 7-day period following treatment. The pretreatment data serve as a control for the treatment period and indicate an increase in plutonium excretion during and following the treatment period by factors of approximately 40 and 100 in the urine, and factors of 300 and 500 in the feces. Total plutonium recovered in the excreta represents in the one case 11% and in the other case 19% of the plutonium injected 60 days prior to treatment. Of particular interest is the fact that the effect of treatment was evident for at least 7 days following cessation.

Daily blood analyses showed plutonium contents ranging from 12-46 $\mu\mu$c/g with no evidence of a correlation between plutonium level and time of DTPA administration.

*Discussion.* The enhanced excretion of plutonium obtained through both prompt and delayed treatment with DTPA is clearly of a magnitude to recommend consideration of such treatment in cases of human exposure

to plutonium. Reduction of plutonium deposition in bone by a factor of 10, and in liver by a factor of 30, as a consequence of treatment one hour following plutonium injection is a more dramatic observation than the 11 or 19% removal of plutonium occasioned by the delayed treatment. The effectiveness of the delayed treatment is perhaps the more surprising, and the more encouraging of the 2 observations, however, for the delayed treatment was successful in removing plutonium which had been firmly fixed in tissues whereas the plutonium removed by the prompt treatment was probably still present in large amount in the blood stream. Also, the DTPA dosage employed in the delayed treatment was a conservative one, comparable to those which have been employed without untoward effects in man(6,8); while the single dose administered in the prompt treatment experiment was somewhat larger than would probably be considered appropriate for man.

The difference in pattern of plutonium excretion in the 2 experiments is particularly noteworthy. With prompt treatment, increased excretion is primarily *via* the urine, while delayed treatment results in large increases in both urinary and fecal excretion, with fecal excretion predominant. One may speculate that the fecal plutonium is that which is removed *via* the bile from liver. This is consistent with observations of delayed DTPA treatment of plutonium bearing rats, where more than three-fourths of the plutonium in the liver was removed, and only about one-fourth of that in bone removed(2). A reciprocal relationship between plutonium appearing in the feces and that retained in the liver of rats during treatment with DTPA has also been noted by Fried *et al.*(9). The predominance of fecal excretion of plutonium was not observed by Norwood in his human cases(6). These, however, were treated at much longer intervals following plutonium deposition.

An encouraging finding was that of the sustained action of DTPA during the week following delayed treatment. Urinary excretion of plutonium decreased rather abruptly upon cessation of treatment, but remained a factor of 10 or more higher than pretreatment levels. Fecal excretion of plutonium during the week following treatment was nearly as high, or in some cases actually higher than during the treatment period. Substantial additional amounts of plutonium might have been excreted had the animals not been sacrificed when they were. Lindenbaum and Schubert have recently shown that the less pronounced sustained effects of DTPA treatment in rats are a reflection of an increased ultrafiltrability of the tissue deposited plutonium(10). This sustained effect of DTPA has also been noted by Norwood in man and is much more pronounced than in the case of treatment with EDTA(6). As a hypothesis one might propose that the sustained elimination of plutonium occurs principally from the liver and may be due to: (1) ability of DTPA (to a greater extent than EDTA) to penetrate the reticulo-endothelial cells in which the plutonium is retained as a colloid-sized aggregate, and (2) slow action of this intracellular DTPA in solubilizing these plutonium aggregates. In support of such a hypothesis, Foreman, using labeled chelating agents, has shown that a small fraction of administered DTPA is retained for long periods and that this fraction is substantially greater than the fraction of EDTA which is similarly retained(5). Schubert and Fried have shown in experiments with rats that thorium when administered in ionic form is more promptly removed by DTPA treatment than when administered in colloidal form (11). The colloidal thorium is deposited predominantly in the reticulo-endothelial system and its excretion occurs only gradually and at an increasing rate as treatment is continued over a period of several days.

The observation during the delayed DTPA treatment that blood levels of plutonium were not increased, despite, on certain days, an increase of a hundred-fold or more, in rate of urinary excretion of plutonium, remains unexplained. Quite possibly the chelated plutonium which reaches the blood from the tissues is so rapidly excreted that it exerts little influence on total blood plutonium levels, even though it may, in total quantity, greatly surpass the amount of plutonium in

the blood at any given moment. The further study of blood plutonium levels in relation to administration of chelating agents would seem to offer much promise of enlightenment with regard to the mechanisms involved in the therapeutic effectiveness of these agents.

*Summary.* A single intravenous dose of diethylenetriaminepentaacetate (DTPA) one hour after plutonium administration resulted in excretion of approximately 90% of the plutonium. As compared with untreated controls, retention of plutonium in the skeleton was reduced by a factor of 10 and retention in the liver was reduced by a factor of 30. Two pigs treated with 5 consecutive daily injections of DTPA excreted 11 and 19% of the plutonium injected 60 days prior to treatment.

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## Histidine Decarboxylase Activity of Basophils from Chronic Myelogenous Leukemic Patients. Origin of Blood Histamine.* (26554)

WILLIAM J. HARTMAN, WILLIAM G. CLARK AND SHIRLEY D. CYR
*Veterans Administration Hospital, Sepulveda, Calif.; and Department of Physiological Chemistry, Medical Center, University of California, Los Angeles*

It is well established that relatively large amounts of histamine are present in tissue mast cells(1-3), and leukocytes(4-6), especially the granulocytes and probably largely the basophils(4,7).† Eosinophils have also been claimed to contain histamine(7).

A polemic has existed since the first report of Code in 1936(4) regarding the origin of histamine in the body. Some argued that it originated solely from ingestion with food and from decarboxylation of histidine by intestinal flora, and others asserted that it also originated by the action of a tissue decarboxylase. The bulk of evidence supports the concept that tissue mast cells of mammalian organisms decarboxylate histidine to histamine(1-3,11) although some workers have denied this(8). Only a few investigators have ruled out bacterial decarboxylation in their *in vitro* studies. These are Werle, *et al.*(12) who used sterile precautions and tested for contamination after incubation; Gustafsson, Kahlson and Rosengren who worked with germ-free rats(9); and Schindler, Day and Fisher who worked with sterile

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† Whether or not the blood basophils are related to mast cells is not settled.