Effect of Repeated Zirconium Citrate Injections on Distribution of Plutonium in the Rat.* (20468)

PATRICIA L. HACKETT. (Introduced by Austin M. Brues)

From the Biology Section, Radiological Sciences Department, General Electric Co., Richland, Wash.

Schubert has shown that early administration of zirconium citrate to rats injected with plutonium results in a marked decrease in the skeletal deposition of this element(1). An additional experiment by the same worker demonstrated that the skeletal content of plutonium was nearly independent of the amount of zirconium injected (with a minimum amount of zirconium between 5.1 and 12.3 mg/kg) and of the number of doses of zirconium given, although the author believed that prolonged treatment with zirconium citrate would probably effect actual removal of plutonium from bone(2).

Most of the previous information(1-3) on the administration of zirconium citrate to animals receiving plutonium has been derived from relatively short-term experiments (3-6 days). The purpose of this report is to present some preliminary data on the effect of divided doses of zirconium citrate over a longer period of time.

Methods. Sixteen Sprague-Dawley male rats with weights averaging 290 g were divided into 4 groups of 4 rats each. Rats in the zirconium toxicity control group received 15 injections (3 per week), intraperitoneally, of 4 ml of a "zirconium citrate" solution prepared according to Schubert(1) and containing 12.8 mg zirconium per ml. All rats in the remaining 3 groups received a single intraperitoneal injection of 27.3 μg of Pu (IV) in 2 ml of 2% citric acid solution adjusted to pH 5.6. One of these plutonium-injected groups did not receive zirconium injections. Zirconium citrate was administered to the second group as described above for the toxicity control group, the first injection being given 30 minutes after the animals had received plutonium. The last group received zirconium citrate injections commencing 32 days after the plutonium injection.

It was planned to sacrifice all animals 71 days after the plutonium injection, but 3 animals were killed early because of a respiratory infection and otitis media. Corresponding animals from other groups were sacrificed at the same time for comparison.

Upon sacrifice, the viscera were removed and the remainder of the animal autoclaved for 30 minutes at 15 lb steam pressure to facilitate separation of the skeleton. The loss of plutonium from the bone when this procedure was used was found to be insignificant. Skeleton, liver, spleen, and residual carcass were ashed with concentrated nitric acid and the resulting acid solution was pipetted onto planchets for counting(4).

Results and discussion. Fig. 1 shows the average weight curves for the 4 groups of rats. Two rats which exhibited large weight losses due to respiratory infection were excluded from the averages. It is evident that the zirconium citrate injections were accompanied by significant weight loss, and the weight gains followed termination of these injections. Histological study of the livers of the zirconium toxicity control rats showed no evidence of damage. These results are similar to those obtained by McClinton and Schubert(5).

Results of plutonium analysis are shown in Table I. Animals 1 and 9 should probably not be considered in comparisons, since they lost about 30% of their initial weight before sacrifice.

The previously reported effect of early zirconium citrate treatment on skeletal deposition of plutonium is clearly confirmed. A 6-fold reduction in skeletal deposition is seen in rats 5, 6, and 7, and about a 12-fold reduction in rat 8; however, the plutonium content of all the various tissues from rat 8 is uniformly low.

Perhaps the most interesting finding of this study was the very marked increase in retention of plutonium in the soft tissues (residual

* Based on work performed under contract W-31-109-Eng.-52 for the Atomic Energy Commission.

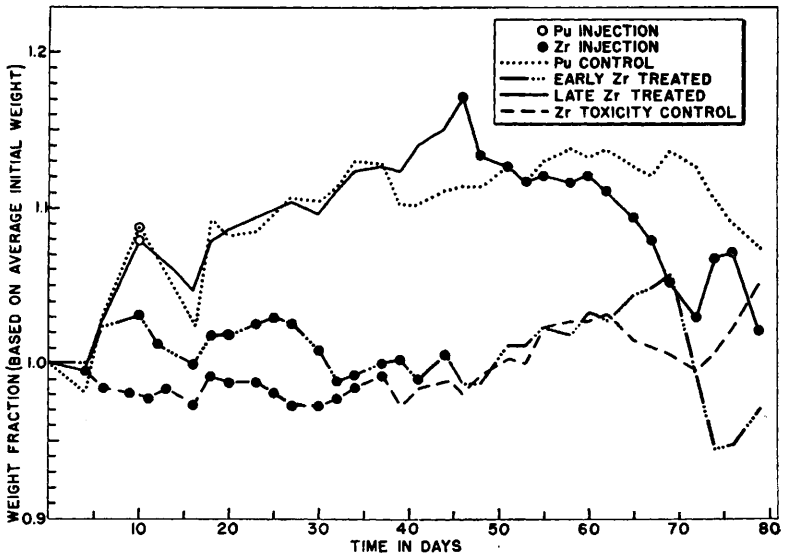

FIG. 1.

carcass) which resulted from the early zirconium citrate treatment. The 2 rats sacrificed during the zirconium citrate treatment (5 and 6) retained 6 to 9 times as much plutonium in the residual carcass as did the controls. Even a month after termination of the zirconium citrate treatment, rats 7 and 8 had 2 to 5 times as much plutonium in the residual carcass as did the controls. In preventing the skeletal deposition of plutonium, the long-term administration of zirconium citrate had also the positive effect of fixing the plutonium in the soft tissues. These findings are not in complete accord with later work from this laboratory(6); however, previous workers(7) have suggested that the eventual tissue distribution of the zirconium colloidal aggregates on which the circulating plutonium is adsorbed is a function of the colloid's particle size. It may be that minor variations in the procedure for preparing the zirconium citrate solution would result in a change of particle size.

Because of the enhanced soft tissue retention of plutonium, the effectiveness of the zirconium citrate treatment in decreasing the

TABLE I. Retention and Distribution of Plutonium.

Group	Animal No.	Time of sacrifice (days after Pu inj.)	No. Zr injections	Total mg Zr injected	% of injected plutonium retained				
					Liver	Spleen	Skeleton	Residual carcass	Total
Pu control	1*	20	—	—	3.4	.29	46	6.9	57
	2†	62	—	—	1.7	.39	66	4.7	73
	3	71	—	—	3.1	.45	62	5.0	70
	4	71	—	—	4.1	.44	70	4.6	79
Early Zr treated	5‡	20	9	463	7.3	.52	11	44	63
	6§	27	12	617	4.0	.22	11	31	46
	7	71	15	771	4.0	.53	11	23	39
	8	71	15	771	2.1	.14	5.1	10	19
Late Zr treated	9‖	56	9	463	.89	.11	29	5.7	36
	10¶	62	11	565	2.3	.47	63	4.6	70
	11	71	15	771	4.0	.25	58	7.6	70
	12	71	15	771	—	.18	53	10	—

* Died of respiratory infection after large wt loss. † Died suddenly of respiratory infection. ‡ Sacrificed early for comparison with control #1. § Sacrificed after development of respiratory infection. ‖ Sacrificed after large wt loss for comparison with rat #5. ¶ Sacrificed early for comparison with control #2.

total plutonium body burden is much less dramatic than is its effect on skeletal deposition. By a comparison of the total injected dose retained in the Pu controls and the early zirconium treated animals it appears, however, that this plutonium retained in soft tissue may be lost from the body at a considerably faster rate than plutonium deposited in the skeleton. It also seems evident that the soft tissue plutonium is not redistributed to the skeleton after termination of the zirconium citrate treatment (rats 7 and 8).

The late zirconium citrate treatment had perhaps a slight effect both in decreasing the amount of skeletally deposited plutonium and in increasing the amount in the residual carcass. Further studies would be required to establish the significance of these effects. No differences of particular significance were observed in the plutonium content of livers and spleens of rats in the different groups.

Summary. Serial zirconium citrate injections during the month immediately following the administration of plutonium were found to effect a 6-fold reduction in the skeletal deposition of plutonium and to increase the retention of plutonium in the soft tissues. Therapy with zirconium, commencing a month after the plutonium injection, was found to have little, if any, effect on the distribution of plutonium.

Sincere appreciation is extended Dr. Joseph Katz, Mrs. Willa D. Oakley and the Radiochemistry Laboratory for their assistance during the course of this work.

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Received June 30, 1953. P.S.E.B.M., 1953, v83.

Use of Baby Chicks for Isolation of Leptospires. (20469)

WARREN G. HOAG, WILLIAM S. GOCHENOUR, JR., AND ROBERT H. YAGER.

From the Veterinary Division, Army Medical Service Graduate School, Washington, D. C.

Guinea pigs(1), embryonated hens' eggs (2), Syrian golden hamsters(3), and laboratory white mice(4) have been employed in the isolation of leptospires from blood, tissues, and other body fluids. Their employment for this purpose has been limited by factors of cost, availability, and facilities appropriate for their maintenance. The successful cultivation of leptospires in embryonated hens' eggs suggested the possible utilization of baby chicks for recovery of these organisms.

Methods. Two-day-old chicks were inoculated intraperitoneally with 0.5 ml of 5-day-old culture of *L. pomona,* S91,(5) in Fletcher's semi-solid medium(6). Subsequent culture generations were lethal for 20 g golden hamsters. Heart's blood cultures were made in both Schuffner's modification of Verwoort's medium(6) and Fletcher's semi-solid medium on the 4th, 7th, 8th, 9th, 10th, 11th, 14th, and 21st days post-inoculation.

Groups of chicks were sacrificed on the 9th, 11th, 14th, and 21st days post-inoculation. Kidney and liver emulsions in 0.85% NaCl solution were cultured separately on both leptospiral mediums. These cultures were incubated at 30°C for 28 days and examined by darkground microscopy at 7-day intervals.

Serums were obtained by clot-squeeze separation from blood drawn by cardiac puncture on the 9th, 11th, 14th, and 21st days post-inoculation. These serums were examined for presence of leptospiral antibodies by the