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DISPOSITION OF INHALED ^{239}Pu CITRATE

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Abstract

Dogs administered oral, intravenous or inhaled ^{239}Pu citrate were sacrificed from 1 to 100 days after exposure to compare distribution and retention kinetics. Approximately 0.08% of the oral dose was retained after 3 days, distributed mainly between skeleton (50%) and liver (25%). After intravenous administration ^{239}Pu was retained principally in the liver (32-45% of the dose) and skeleton (29% of the dose) during the 100 day post injection period. Inhaled ^{239}Pu reached a peak concentration in blood after 6 hours and then decreased with clearance kinetics similar to those described by others for dogs and humans administered intravenous ^{239}Pu citrate. The lung, skeleton, liver, blood and intestines retained most of the inhaled ^{239}Pu 1 day after exposure. After 100 days 36% of the initial deposit was retained; 33% in the lung, 44% in the skeleton and 17% in the liver. Plutonium was concentrated by lymphatic tissue following all methods of administration and at all time intervals. Although the concentration in specific lymph nodes (hepatic, splenic and tracheobronchial) was among the highest of any tissue analyzed, the total amount deposited in a selection of 12 lymph nodes (~10 g tissue) was always less than 0.6% of the body burden.

DISCLAIMER

DISPOSITION OF INHALED ^{239}Pu CITRATE*

J. E. Ballou, J. F. Park and W. G. Morrow

Classical studies of plutonium metabolism have employed the citrate complex of plutonium administered intravenously to a variety of animals including man. The rationale for the choice of compound and method of administration has included the assumption that the clearance and translocation kinetics of injected plutonium citrate adequately represent the behavior of the solubilized fraction of more "naturally" incorporated plutonium. The validity of this assumption has been discussed in a recent review by Buldakov et al, 1969[1] who concluded that intravenous plutonium citrate probably comes closest to duplicating the tissue distribution pattern of plutonium absorbed following oral, percutaneous or inhalation exposure. In view of the scarcity of information on inhaled soluble plutonium compounds the present study was initiated as a further test of the assumed equivalency of intravenous, oral and inhaled plutonium citrate.

Method

Beagles were administered ^{239}Pu citrate prepared from ^{239}Pu nitrate stock solution according to the procedure outlined by Stover et al, 1959.[2] Briefly, the ^{239}Pu nitrate stock solution was reduced to Pu (III) with SO_2 then ^{239}Pu hydroxide was precipitated by addition of NaOH to the solution chilled in an ice bath. The ^{239}Pu hydroxide was recovered by centrifugation, washed with distilled water and solubilized with concentrated HNO_3 in the

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ice bath. The resulting solution was a bright green complex of ^{239}Pu (IV) nitrate containing approximately 4 mCi ^{239}Pu. The ^{239}Pu (IV) nitrate solution was transferred to a vial containing sufficient sodium citrate to neutralize the excess HNO_3 and the final dilution to 25 ml was made by adding equal amounts of 0.08 M citric acid and 0.08 M sodium citrate. The end product was a plutonium citrate solution of pH 3.5 that was 72 $\pm$ 3% ultrafilterable through Visking cellulose tubing.

Fourteen female beagle dogs 21-24 months of age, average weight approximately 10 kg were administered a single inhalation exposure to ^{239}Pu citrate aerosols generated with a Lauterbach generator. The aerosol had an aerodynamic median activity diameter of 1.45 μm with a geometric standard deviation of 2.75. Individual exposures to the aerosols were varied from 6 to 15 minutes in an attempt to adjust the dose to approximately 5 μCi deposited in each dog. Actual deposition ranged from 1.4 to 11.8 μCi as determined by total tissue and excreta analysis. Animals were sacrificed in groups of 2 after 1, 3, 7, 14, 30, 62 and 100 days.

Three male beagles, 19 months of age and 12 kg average weight were injected with 4.35 μCi of ^{239}Pu citrate (0.03 ml) in the cephalic vein. One dog was sacrificed at each interval 1, 30 and 100 days after injection.

A single female beagle weighing 12 kg and 99 months of age was administered by mouth 580 μCi ^{239}Pu citrate in gelatin capsules. The dog was killed 3 days after ^{239}Pu ingestion.

In all cases plutonium citrate was administered within 3 hours after preparation and without further dilution. The animals were housed in metabolism cages where either daily or pooled urine and feces samples

were collected. Blood samples were taken periodically for radiochemical analysis and for examination of hematologic changes indicative of radiation effects. The dogs were sacrificed by exsanguination under sodium pentobarbital anesthesia and subjected to detailed necropsy where more than 40 tissues and organs were removed for plutonium analysis. Tissue and excreta samples were prepared for alpha scintillation counting by a combined dry ash-wet ash procedure.(3)

Results and Discussion

Changes in the peripheral blood picture, notably a depression in the total number of white cells, was the only clinical evidence of plutonium effect after injection or inhalation of ^{239}Pu citrate. Variations in the total white cell count and corresponding changes in the major white cell elements are shown for a female beagle administered inhaled ^{239}Pu citrate in Figure 1. The white cell depression was a consistent finding and appeared to be due principally to a decrease in the segmented neutrophils. Although lymphocytes were also depressed in this animal, the most sensitive cells were the segmented neutrophils both in the early postexposure stimulation and in the later depression seen at more extended time intervals. Animals in this study occasionally showed early lymphocytosis rather than the increased neutrophil count, however, the later changes were fairly consistent with a neutrophil depression. Others have observed a marked lymphopenia as the earliest indicator of plutonium oxide inhalation.(4) Inhaled plutonium nitrate on the other hand produced a generalized leukopenia similar to that observed here with plutonium citrate.(5) The greater

translocation of the soluble compounds from the lung and the resulting more generalized distribution of the radiation dose in other soft tissues and the skeleton probably accounts for the different hematologic effects. The radiation dose following inhaled $^{239}PuO_2$ was confined largely to the lung and the thoracic lymph nodes.

Plutonium was rapidly translocated from the lung to the blood after inhalation of plutonium citrate (Figure 2). Dog 290, which was catheterized and bled during the 15 minute exposure period, showed blood values of about 1/20 the maximum concentration by the time the exposure was terminated. In general the plutonium level in blood approached a plateau value after 6 hours.

Plutonium was cleared from the blood more slowly after inhalation exposure than after intravenous administration (Figure 3). This is probably not a true physiological difference in the mechanism of blood clearance but rather is a consequence of the continuing mobilization of plutonium from the reservoir in the lung to the blood following inhalation exposure. Similarly, the comparatively low blood levels observed during the early time intervals after inhalation exposure are probably an artefact of the route of administration. It is perhaps more significant that less than order of magnitude differences were observed among the studies in Figure 3 considering the different species and different methods of administration employed. The usual laboratory procedure of injecting plutonium citrate appears to give a reasonably good estimate of the blood level at least for the first 100 days after inhalation of a soluble plutonium compound such as the citrate complex.

The pattern of urinary excretion was also closely similar for inhaled and injected Pu citrate (Figure 4) presumably because plutonium concentrations in blood and the mechanisms of transport and clearance were comparable. The actual data points shown for two inhalation dogs varied considerably from day to day, which is not unusual for this type of study, but the reason is not readily apparent. Injected plutonium citrate appeared to give a satisfactory representation of the urinary excretion after inhaled plutonium citrate in dogs as well as after injected plutonium citrate in humans.

The distribution of plutonium in tissues was significantly influenced by the method of administration as shown in Table 1. About 0.08% of the oral dose was absorbed from the gastrointestinal tract and was deposited mainly in the skeleton and liver. Intravenous plutonium citrate was deposited more extensively in the liver than in skeleton in contrast to earlier studies[2] which showed greater deposition in skeleton. It is probable that physical-chemical differences in the injection solutions account for this discrepancy. A large fraction of the inhaled plutonium citrate was retained in the lung at all time intervals. The plutonium translocated from the lung was distributed between skeleton and liver not unlike the pattern after oral ingestion or after intravenous injection of monomeric plutonium citrate solutions.[2] Both the inhaled and injected dose were retained to about the same extent over the 100 day experimental period.

It was an unexpected finding that inhaled plutonium citrate was retained so tenaciously, i.e. about 25% of the estimated initial lung burden

was found in lung after 100 days. This may be due in part to the relatively high percentage of polymerized plutonium in the solutions (about 25% was not ultrafilterable) but is more probably to be expected as a consequence of neutralization and the formation of colloids in the lung environment. In either case the concentration in lung was disproportionately high after inhalation exposure (see Table 2). The concentration of plutonium in turbinates (this sample included all soft tissue and bone that could be scraped from the nasal cavity) was explained initially on the basis of physical trapping because of the method of exposure. The amount retained at later time intervals as well as the high concentration after intravenous injection implies that plutonium may be selectively deposited in turbinates possibly because of the well developed vascularization of this tissue.

It is noteworthy that lymph nodes concentrated plutonium following all 3 routes of administration. Without exception the concentration in specific nodes was among the highest of any tissue analyzed. The values for a selection of 12 nodes taken from different anatomical locations ranged over several orders of magnitude as indicated in parenthesis in Table 2. Either the hepatic or tracheobronchial nodes accounted for the highest concentrations within the range of values. The lymph nodes which concentrated the maximum amount of plutonium were always closely related anatomically to major sites of plutonium deposition. It was noted after intravenous injection that the prescapular nodes immediately anterior to the injection site contained exceptionally large amounts of plutonium.

The concentration in these nodes was not included in Table 2. The dog sacrificed 30 days after intravenous injection gave anomalous values for tissues distribution because of a poor injection which left about 1/3 of the dose in the perivascular tissue at the injection site. Approximately 8% of the dose was recovered in the prescapular nodes of this dog.

Conclusions and Implications

Although the small number of animals employed in this study limits the interpretation of the results it seems clear that intravenous plutonium citrate would be a reasonable choice as the model system to describe plutonium citrate metabolism experimentally. Intravenous plutonium citrate appeared to produce clinical effects and show blood clearance, deposition and excretion kinetics that were fairly representative of incorporation by more normal routes of entry. Major deviations from this pattern of agreement were due to artefacts of the method of administration. That is, inhalation exposure led to greater relative retention in the lung while the distribution of intravenous plutonium citrate was possibly influenced by the well known physical-chemical variables of the injection solutions. Plutonium levels in blood and urine were not greatly effected by these rather large differences in tissue distribution. Bioassay for exposure to soluble plutonium could presumably be interpreted on the basis of past studies with injected plutonium citrate. It is not yet clear if this would apply also to exposures to plutonium nitrate, a soluble compound of practical industrial hygiene concern, however preliminary results from our laboratory suggest that inhaled citrate and nitrate salts of plutonium are translocated to about the same extent from the dog lung.

The tendency for plutonium to concentrate in lymph nodes, particularly after ingestion or inhalation exposure, emphasizes the role of the lymphatic system in normal plutonium clearance and the potential for plutonium to concentrate in relatively small tissue volumes. The accumulation of plutonium in regional lymph nodes following subcutaneous injection is reported elsewhere in this symposium. It seems probable therefore, that the highest radiation doses may be absorbed in lymph nodes following industrial exposure to plutonium. Although the significance of this dose to only a small fraction of the total lymphatic system is not clearly understood in terms of the long-term effects of plutonium incorporation, it is of interest that tumors involving lymph nodes have been described in dogs following exposure to plutonium by both inhalation[6] and injection.[7] Future investigations, in particular of the low level, long-term type described by Park in this symposium[8] may go a long way towards establishing the priority of radiation to the skeleton, lung and lymph nodes. At the present time the well established effects in bone and lung would seem to require first consideration for these tissues as critical organ for radiation hazard considerations.

TABLE 1

Disposition of ^{239}Pu Administered as ^{239}Pu Citrate by Several Routes. (Values are the range of values observed during the given time intervals)

	Percent of Administered Dose*		
	Oral (3 days post)	Intravenous (1-100 days post)	Inhalation (1-100 days post)
Lung	0.0008	0.8-1.6	11-72
Turbinates	0.0002	0.16-0.21	0.08-2.3
Liver	0.02	32-45	1-18
Skeleton	0.04	29	5-56
Lymph Nodes	0.005	0.2-0.3	0.05-0.6
Total Animal	0.083	85-99	86-96

*Dose for inhalation dogs was computed as the total amount deposited less the amount excreted during the first 6 days.

Table 2

CONCENTRATION OF ^{239}Pu IN TISSUES AFTER ^{239}Pu CITRATE ADMINISTRATION

Route of Administration	Days After Administration	^{239}Pu Concentration (pCi/gram)				
		Turbinates	Lung	Liver	Skeleton	Lymph Nodes*
Oral	3	83	47	366	291	194 (77-723)
Intravenous	1	1099	1026	4861	1539	2327 (268-6865)
	30	1654	57	777	2352	909 (208-30780)
	100	1099	406	6433	1332	1463 (821-4629)
Inhalation	1	5904	54752	248	389	628 (97-17150)
	3	3327	16656	794	783	496 (83-5512)
	7	5356	13687	412	1024	400 (56-6767)
	14	5499	15823	1618	1426	832 (243-7496)
	30	2619	10014	310	958	778 (140-13770)
	62	1685	14498	2084	1770	1658 (169-37150)
	100	4872	18980	2824	2006	3721 (58-48170)

*Values are the average of 12 different lymph nodes with the range shown in parenthesis.

List of Tables and Figures

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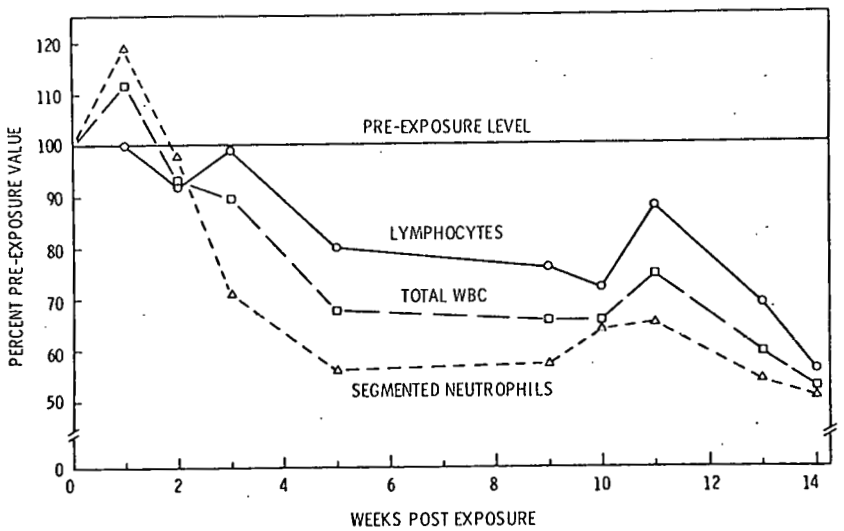
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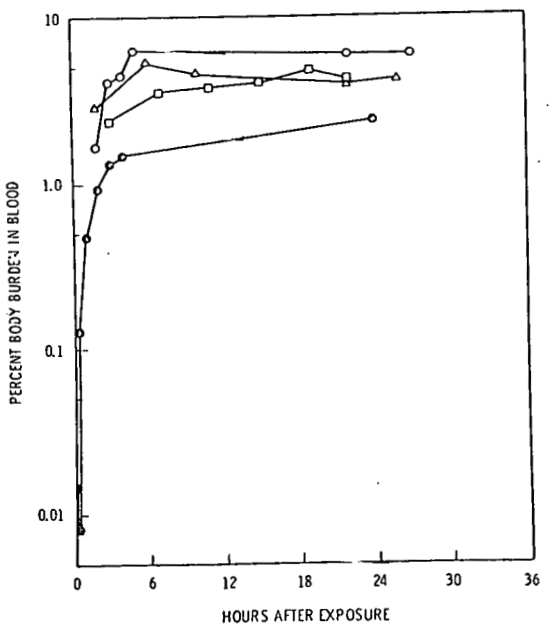
110
100
90
80
70
60
50
0
PRE-EXPOSURE LEVEL
LYMPHOCYTES
TOTAL WBC
SEGMENTED NEUTROPHILS
0
2
4
6
8
10
12
14
WEEKS POST EXPOSURE


PERCENT BODY BURDEN IN BLOOD
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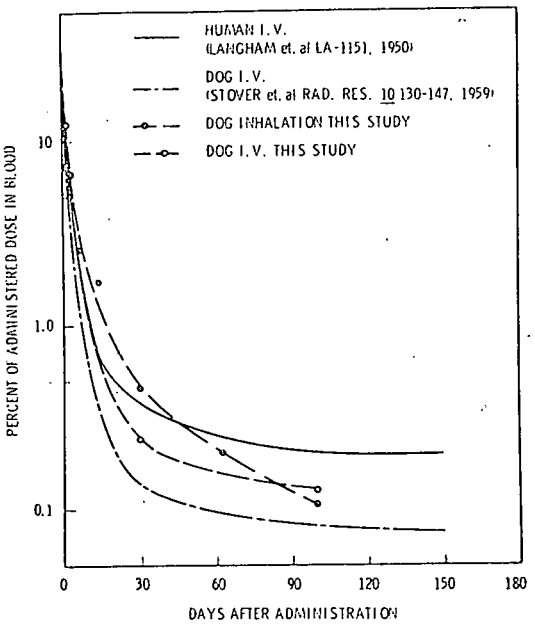
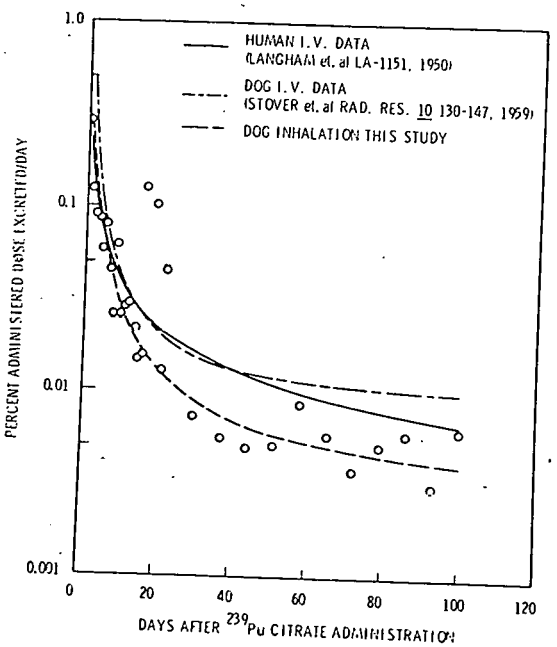
0.1
0.01
0
6
12
18
24
30
36
HOURS AFTER EXPOSURE


HUMAN I.V.
(LANGHAM et. al LA-1151, 1950)
DOG I.V.
(STOVER et. al RAD. RES. 10 130-147, 1959)
DOG INHALATION THIS STUDY
DOG I.V. THIS STUDY
PERCENT OF ADMINISTERED DOSE IN BLOOD
10
1.0
0.1
0
30
60
90
120
150
180
DAYS AFTER ADMINISTRATION


HUMAN I.V. DATA
(LANGHAM et. al LA-1151, 1950)
DOG I.V. DATA
(STOVER et. al RAD. RES. 10 130-147, 1959)
DOG INHALATION THIS STUDY
PERCENT ADMINISTERED DOSE EXCRETED/DAY
1.0
0.1
0.01
0.001
0
20
40
60
80
100
120
DAYS AFTER ^{239}Pu CITRATE ADMINISTRATION