INFLUENCE OF EXPOSURE HISTORY ON MERCURY EXCRETION

Final Report

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Determination of whether the size of a dose of a given mercurial influences the kinetics of distribution and elimination of the mercury, based on the great differences in urinary mercury output observed among workers of the same group. Results of the study are considered applicable to strengthening the scientific basis for the bioassay monitoring portion of the occupational health surveillance program designed to prevent damage to exposed workers. The dose dependence of elimination kinetics is studied by injecting labeled mercury at various dose levels into rabbits and rats, measuring the elimination from the animal directly by total body counting techniques and indirectly by analysis of the daily urine and feces output of each animal.

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INTRODUCTION

The basic objective of this research was to learn whether or not the size of a dose of a given mercurial influences the kinetics of distribution and elimination of the mercury. The rationale for this line of inquiry is the great differences in urinary mercury output that have been observed among workers of the same group, differences too great to be lightly explained on the basis of "biological variability." The results obtained from this research would be applicable to strengthening the scientific basis for the bioassay monitoring portion of the occupational health surveillance program designed to prevent damage to exposed workers.

To attain this objective, it was proposed that the dose dependence of elimination kinetics be studied by injecting ^{203}Hg labeled mercury, either as $Hg(NO_3)_2$ or $HgCl_2$ at various dose levels into rabbits and rats; and then to measure elimination from the animal directly by total body counting technics and indirectly by analysis of the daily urine and feces output of each animal. Descriptions of our work, and the results, are summarized below.

EQUIPMENT

Two different whole body counters, one for rats and one for rabbits were designed, constructed, tested, and subsequently used.

The whole body counter for rats that was finally used (Figure 1), consisted of two horizontally opposed 1½" diameter x 1" thick NaI (Tl) scintillation counters, each shielded by 2½" lead. The rat, immobilized in a restrainer, was placed between the two detectors for counting. The output pulses from each detector were fed into a single channel analyzer whose window straddled the 0.279 MeV photopeak of ^{203}Hg. The output pulses from two single channel analyzers were counted by a single scaler. The background counting rate for the system, under our operating conditions, was about 9 c.p.m. The rats gave counting rates much higher than this background, on the order of tens of thousands of counts per minute when the experiment was started, and on the order of a hundred counts per minute at the end. The reliability of the counting system was verified by counting a rat 100 times (removing that rat from the counter and then replacing it after each count). A chi-square analysis of the data showed a distribution of counting rates that did not differ significantly from the expected distribution.

The whole body counter for rabbits is shown in Figure 2. It consists of a 1½" diameter x 1" thick lead shielded NaI (Tl) detector mounted on a carriage that slides in a frame in which the rabbit is slung. Depending on the collimator used and the position of the detector, either a small area or the entire animal could be viewed by the detector. The output pulses were processed by a single channel analyzer. Under the conditions used in these studies, the background was on the order of 5 c.p.m. The reliability of this system was determined in the same manner as the rat counter, and was found to give reproducible measurements.

METHOD FOR MERCURY DETERMINATION

Prior to our recent acquisition of an atomic absorption spectrometer, mercury was determined chemically by colorimetric measurement of a mercury-dithizone complex. This method, though accurate and reliable was relatively difficult, and not convenient for a large number of measurements. For this reason, we developed a relatively simple, reliable method for chemical determination of mercury in biological material to a level of 0.1 ppm. Our method is a variation of the colorimetric determination of the mercury-dithizone complex. The biological material is wet ashed with a mixture of HNO_3 and H_2SO_4 in order to destroy the organic matter. The mercury is oxidized to the +2 valence state by addition of excess $KMnO_4$ and then the excess permanganate is destroyed by $NH_2OH \cdot HCl$. The volume of the resulting solution is measured, then is used to titrate a measured volume of dithizone solution. The titration end point is a very sharp change in color from deep blue to bright yellow. The concentration of mercury in the titrating solution is read from a standard curve, Figure 3.

INTRAPERITONEAL VS. INTRAVENOUS INJECTION: Effect on Systemic Uptake

The influence of route of administration may be expected to influence the rate of systemic absorption of mercury, and hence, for a given dose, to influence the rate and route of elimination. For this reason we studied the influence of two routes of mercury administration, intraperitoneal and intravenous injection, and two dose levels, 20 and 200 micrograms of mercury, on concentration of mercury in the blood, kidneys and liver at various times after exposure.

Rats from a group of 96 female Holtzman albinos, whose mean weight was 159 $\pm$ 11 gm, were randomly assigned to one of the four treatment groups. Each rat was injected with the appropriate amount of ^{203}Hg tagged mercury as $HgCl_2$ in 0.5 ml of phosphate buffered (pH = 7.4) isotonic saline. After injection, the

FIGURE I

Whole Body Counter for Rats

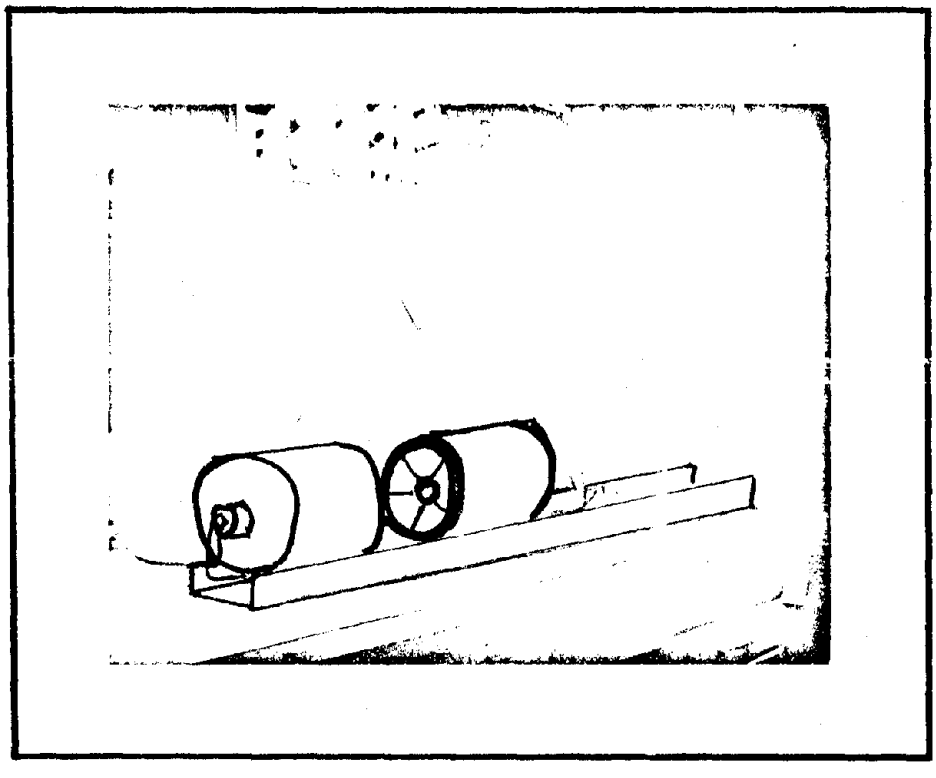
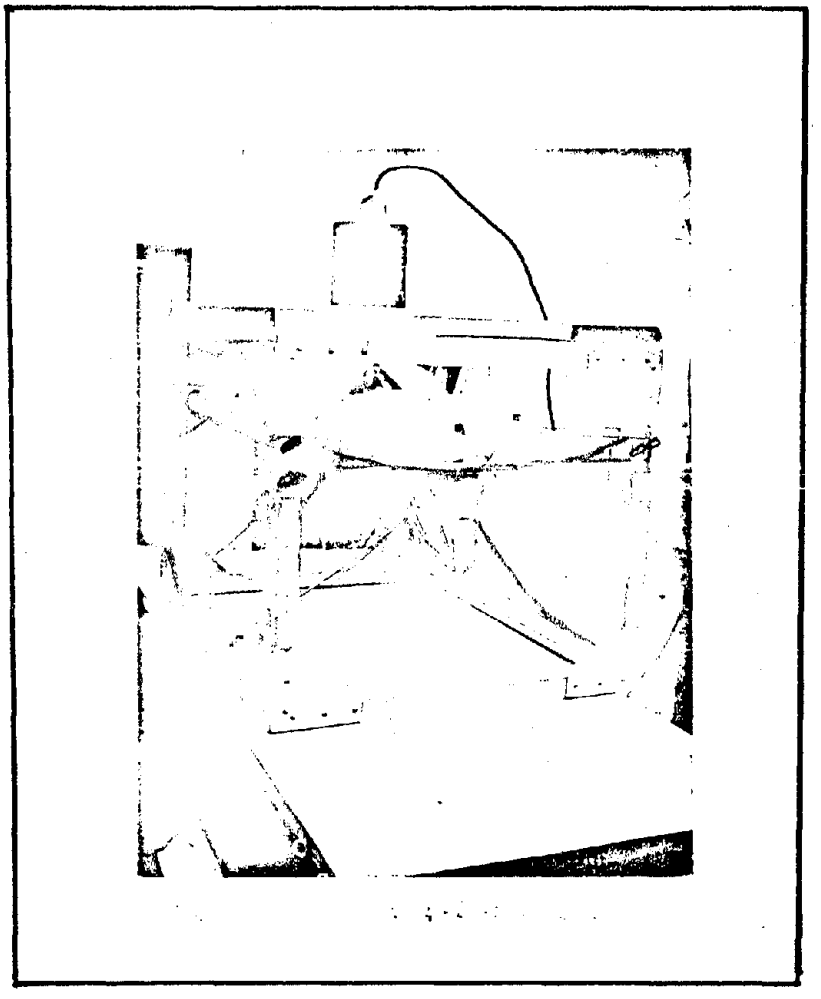


FIGURE 2

Whole Body Counter for Rabbits



rats were caged in threes, and were permitted food and drink ad libitum. The rats were sacrificed three at a time, by exsanguination, at time intervals of ½ to 96 hours post injection. After sacrifice, blood, kidneys, and liver were analyzed radiometrically for mercury, and the per cent injected dose in the kidneys and liver were calculated. The sensitivity of the analytical determination was $1.7 \times 10^{-4} \pm 10\%$ microgram Hg.

The results of the study are summarized in Tables I, II and III. The data show a dependence both on size of dose and on route of administration. The difference among the treatments was found to be significant at the 1% probability level. As was expected, the intravenously injected rats showed a greater per cent deposition in the kidneys and liver than the intraperitoneally injected rats. Also, the rate of absorption from the peritoneal cavity was greater for the 200 microgram dose, presumably because of the higher mercury concentration gradient in the case of the higher dose. The kidney data, when plotted graphically, Figure 4, show the clearance of Hg to be at least a two phase phenomenon. Furthermore, the short lived component is dose dependent. The clearance half time of the short lived component for the 200 microgram dose is on the order of 20 hours, while for the 20 microgram dose it is on the order of 35 hours. The long lived component, on the other hand, is not dose dependent.

Table I

Whole Blood
per cent of injected dose per ml whole blood

Hours after injection	20 µgm IP	20 µgm IV	200 µgm IP	200 µgm IV
¼	0.68	2.54	1.06	2.46
½	0.73	2.25	0.93	1.56
1	0.44	1.58	0.60	0.99
2	0.56	1.33	0.54	0.74
4	0.75	1.21	0.38	0.86
6	0.58	0.98	0.23	0.74
24	0.25	0.80	0.22	0.44
96	0.07	0.35	0.02	0.25

Table II

Kidneys
per cent of injected dose in kidneys

Hours after injection	20 µgm IP	20 µgm IV	200 µgm IP	200 µgm IV
¼	10.3	34.4	12.5	41.6
½	11.3	35.9	26.6	59.3
1	14.9	43.5	35.7	69.5
2	23.4	50.6	30.2	75.0
4	46.6	49.0	41.4	67.7
6	49.1	52.6	42.3	59.3
24	33.8	36.7	18.5	33.0
96	32.9	28.7	17.3	21.9

Table III
Liver
per cent of injected dose in liver

Hours after injection	20 µgm IP	20 µgm IV	200 µgm IP	200 µgm IV
¼	2.28	8.59		12.2
½	3.19	19.4		10.3
1	2.42	11.1		12.7
2	3.15	10.64		11.4
4	8.30	12.5		6.3
6	8.16	8.9	8.1	10.8
24	4.62	2.9	4.62	14.3
96	2.27	2.4	1.34	7.7

Fig. 3

Standard Curve for Mercury Determination

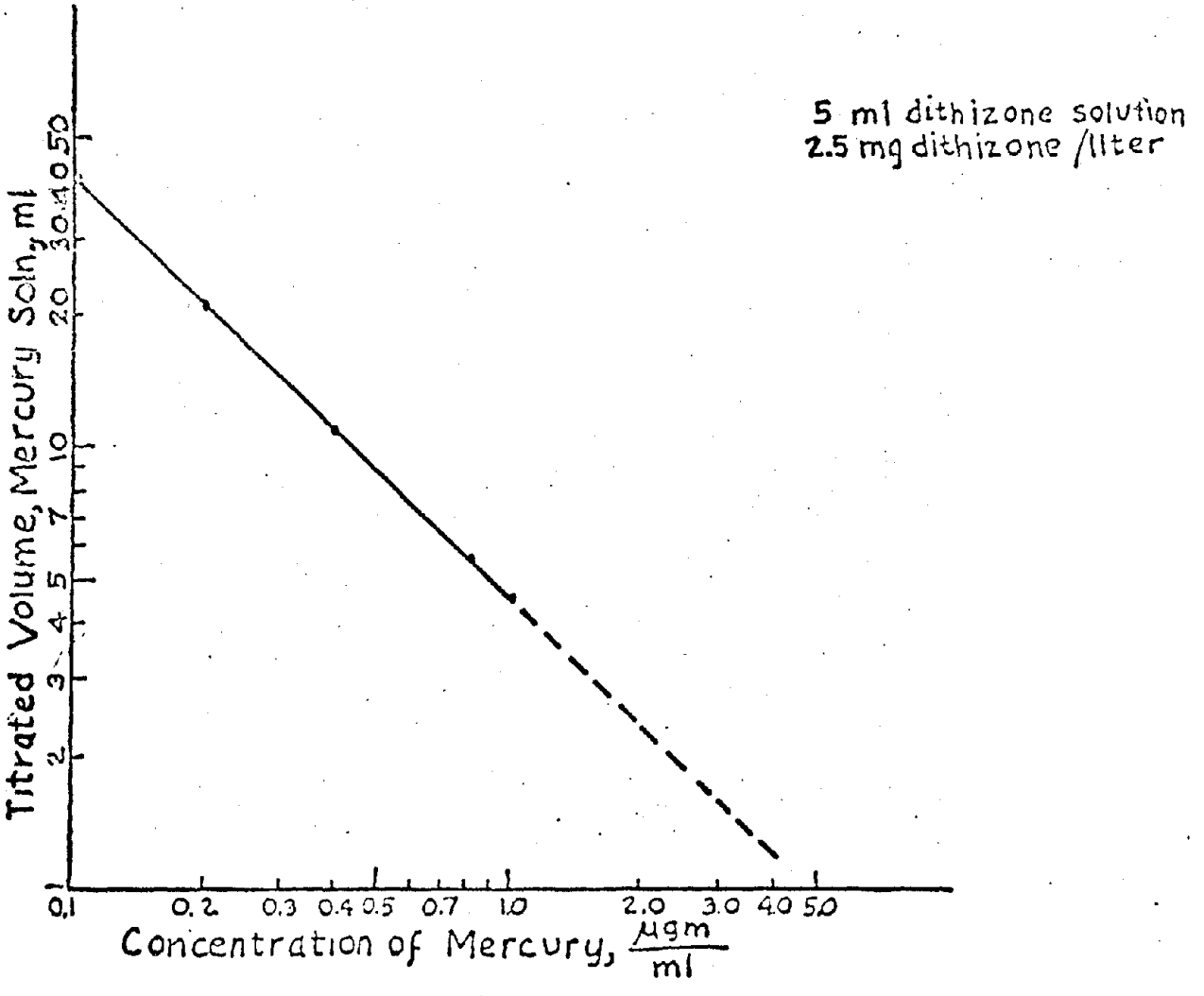
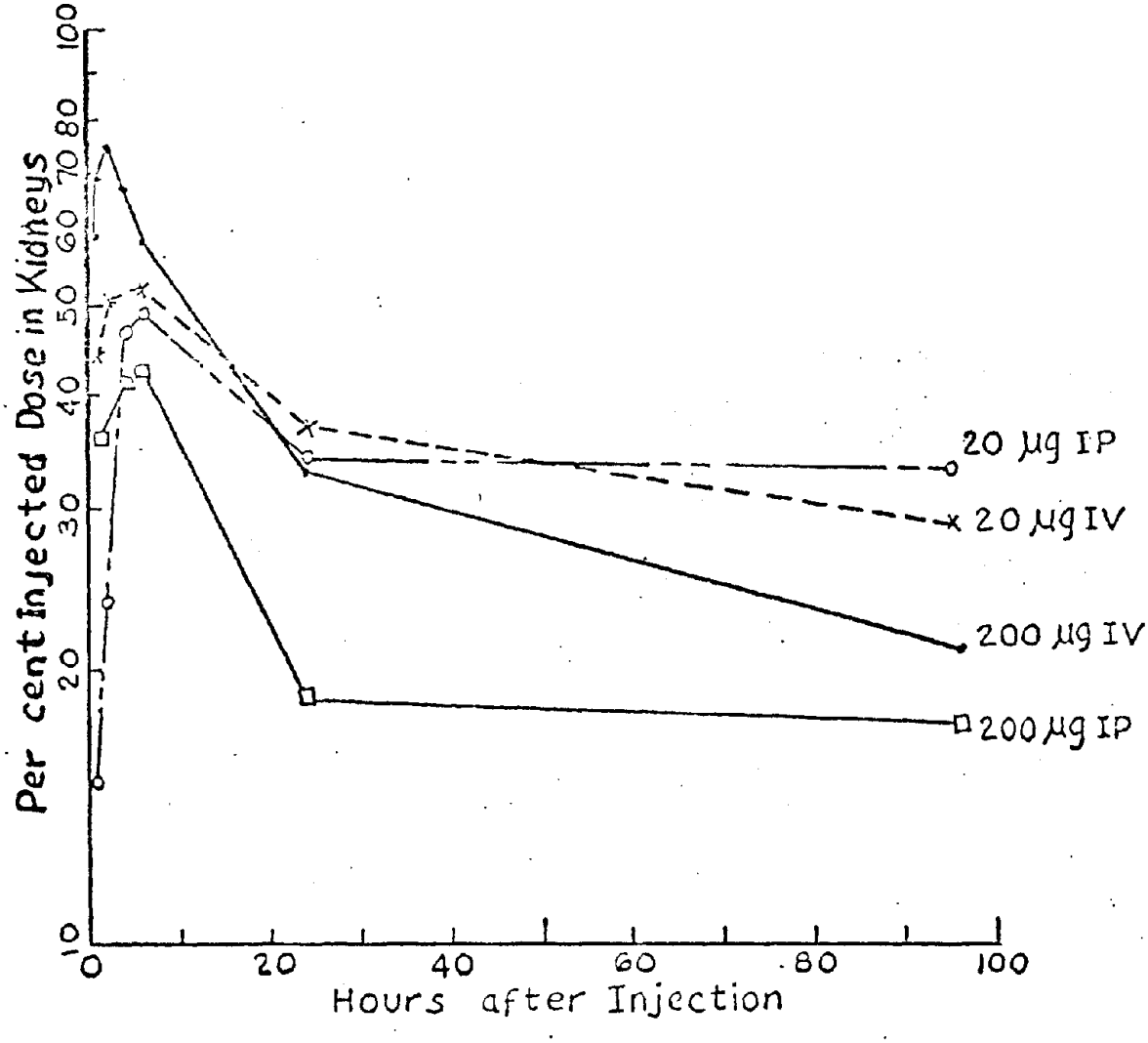


Fig 4
Renal Uptake and Clearance
of
Mercury as $HgCl_2$



DISTRIBUTION OF MERCURY AMONG THE BLOOD FRACTIONS AND SERUM PROTEINS

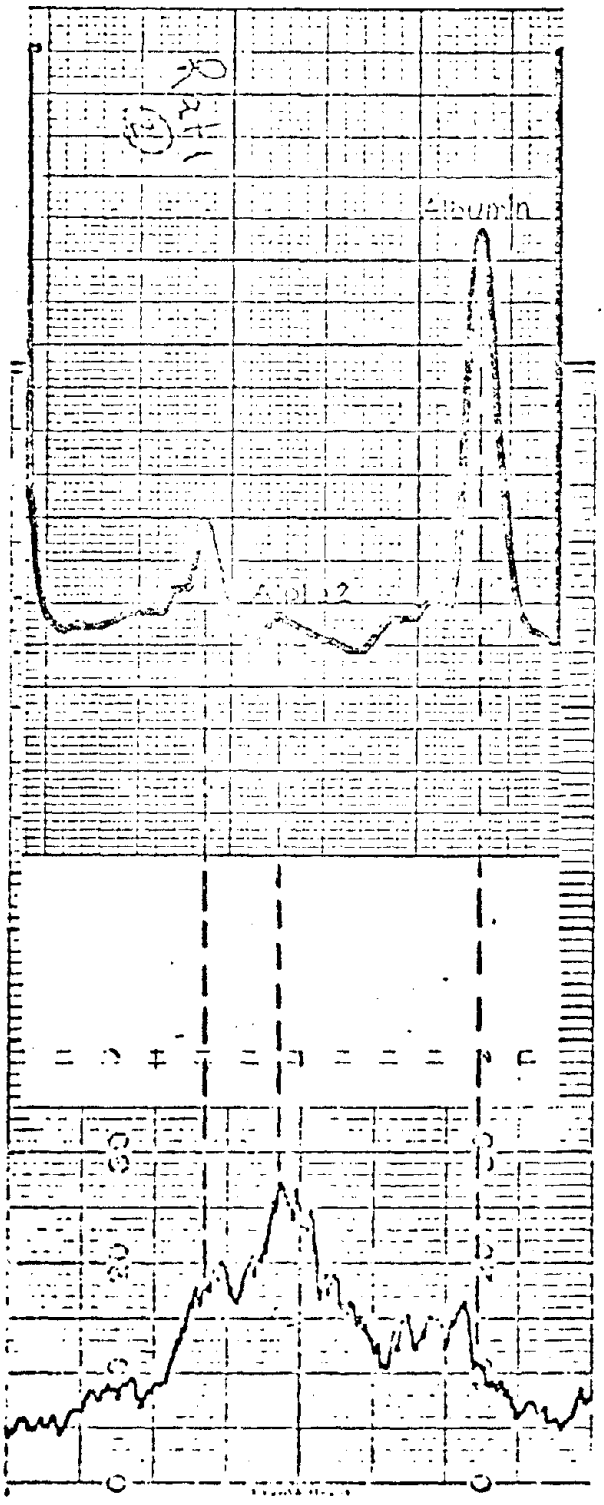
The distribution of mercury, intravenously administered, among several blood components: a) erythrocytes and plasma, and b) serum proteins, was studied at three different dose levels: 1) 0.12 mg per kg, 2) 0.65 mg per kg and 3) 1.2 mg per kg.

The general technics employed in each of these treatments were similar. The rats were injected with 0.5 ml solution of ^{203}Hg tagged $HgCl_2$ in saline solution. The rats were then serially sacrificed, by exsanguination through the heart, over time periods up to 16 days. The blood was divided into two portions; one portion was heparinized for determination of mercury in the whole blood and in the red blood cells. The mercury content was determined by suspending a known volume of blood in a standard counting volume of isotonic saline, and then counting the gamma ray emission with a single channel gamma ray spectrometer. The red blood cells from a known volume of blood were spun down, separated from the plasma, washed three times with isotonic saline, then resuspended in a standard volume of isotonic saline for counting.

The second portion of blood was allowed to clot in order to produce serum. The total protein concentration in the serum was determined colorimetrically by means of the biuret reaction, and the mercury content of the serum was determined radiometrically. To check the distribution of serum mercury between the serum proteins and the aqueous fraction, the protein in a serum sample was precipitated by acetone, the precipitate and supernatant were separated by filtration, and then both the precipitate and the supernatant were counted. In this way, we learned that essentially all the mercury in the serum was bound to the serum proteins. The serum proteins were separated by placing four lambdas of serum on a cellulose acetate strip, immersing the strips in barbital buffer at pH 8.4, and electrophoresing for one hour at 300 volts. Replicate strips were run for each separation. After staining, two strips were cleared for graphical protein determination with a densitometer and for graphical mercury determination with a 4 pi windowless radiochromatogram scanner, Figure 5. Two other strips were cut, after staining, into the albumin, alpha 1 globulin, alpha 2, beta and gamma globulins. These pieces were then counted in a 2 pi windowless gas flow counter for determination of mercury. Following the counting procedure, the dye was eluted with 0.1 N NaOH, and the amount of eluted dye from each fraction was determined spectrophotometrically at 500 millimicrons. From these measurements, we calculated the percent dis-

Fig 5

In-Vivo Distribution of Mercury Among Serum Proteins



tribution of the mercury in each protein fraction and the amount of mercury associated with each of the protein fractions. In the case of the 20 and 200 microgram groups, in addition to determining the distribution of the mercury among the blood components, the amount of mercury in the kidneys, liver, spleen, brain and skeleton were determined by digesting these organs and counting the mercury in the digest. The weights of the animals, and the number of animals used at each treatment level are shown below. In all cases, the rats used were female Holtzman albinos.

Injected dose	Treatment level	No. of rats	Weight, grams
20 μgm	0.12 mg/km	30	158 ± 5
80 μgm	0.65	21	122 ± 8
200 μgm	1.2	30	167 ± 7

The difference in weight between the middle group and the other two groups should be noted. The experiment was done in three steps, using groups of animals that had been purchased separately for the respective treatment level. The first treatment given was the middle level, that is, the 0.65 mg/kg dose. When we were ready for the next treatment level (the 0.12 mg/kg dose), rats of the same weight distribution as used in the first treatment were not available. Rather than wait (our high specific activity mercury, which is available only on certain dates, was decaying) until the supplier would have the rats available, we used larger rats.

RESULTS

The results of the blood studies are summarized in the Tables IV, V and VI; the clearance of mercury from the blood is shown by the curves in Figure 6. The data show certain consistant systematic distributions as well as an apparent anomaly: the percent of the dose per ml whole blood within one hour after injection is highest in the middle treatment level, 2.8% per ml and lowest for the highest treatment level, 0.98% per ml. A plausabile explanation for this apparent discrepancy is based on the smaller weight of the group of rats showing the anomaly and the weights of the others. The smaller rats, having less massive organs, may have absorbed the circulating mercury more slowly than the larger rats, thus accounting for the higher concentration of mercury in their blood. This explanation is supported by the fact that, on the whole, the blood concentrations of the lowest and hightest treatment levels were not very much different from those found in the I P versus I V study.

Several striking factors emerge from the study. First is the fact that serum albumin is not the main carrier of mercury. Whereas it is widely held that almost all the mercury in the fluid portion of the blood is bound to the albumin, our data show that most of this mercury is carried by the globulins.

Table IV

Distribution of mercury among blood components

20 μgm Hg as $HgCl_2$

Time	% dose / ml bld	RBC / whole blood	μgm Hg / ml plasma	Albumin % Hg	Albumin moles Hg / mole x10^{-4}	Alpha 1 % Hg	Alpha 1 moles Hg / mole α1 x10^{-4}	Alpha 2 % Hg	Alpha 2 moles Hg / mole α2 x10^{-4}	Beta % Hg	Beta moles Hg / mole β x10^{-4}	Gamma % Hg	Gamma moles Hg / mole γ x10^{-4}
3/4hr	1.61	0.77	0.0798	12.0	1.09	20.5	25.5	38.2	58.3	17.5	10.97	11.8	14.9
1½ hr	1.27	0.76	0.0917	15.4	1.33	19.5	29.5	34.7	64.4	16.2	9.70	14.1	18.4
3 hr	0.86	0.68	0.0970	18.3	1.44	17.0	25.9	32.2	56.1	16.6	9.25	15.8	14.3
6 hr	0.60	0.44	0.1298	18.0	2.18	12.0	27.4	27.9	63.2	16.3	10.36	25.6	21.3
12 hr	0.28	0.53	0.0485	6.0	.23	15.7	15.0	43.3	29.0	15.0	0.29	19.9	9.8
24 hr	0.16	0.43	0.0354	12.3	.40	14.3	6.6	38.9	17.5	14.6	0.23	19.9	4.3
48 hr	0.13	0.52	0.0175										
96 hr	0.06	0.64	0.0074										

Weight of rats = 158 ± 5 grams
dose = 0.12 mg/kg

Table V

Distribution of mercury among blood components

80 μgm Hg as $HgCl_2$

Time	% dose / ml bld	RBC / whole blood	μgm Hg / ml plasma	Albumin		Alpha 1		Alpha 2		Beta		Gamma	
				% Hg	moles Hg / mole $\times 10^{-4}$	% Hg	moles Hg / mole $\times 10^{-4}$	% Hg	moles Hg / mole $\times 10^{-4}$	% Hg	moles Hg / mole $\times 10^{-4}$	% Hg	moles Hg / mole $\times 10^{-4}$
1 hr	2.80	0.68	0.700	16.8	2.55	19.9	26.5	27.6	33.2	17.2	12.1	18.6	21.6
2 hr	2.28	0.51	0.723	21.9	2.48	20.2	30.9	25.9	44.3	13.1	8.27	18.8	20.4
4 hr	1.69	0.62	0.509	18.3	1.83	21.8	19.0	31.8	33.1	13.4	5.09	14.2	12.5
8 hr	1.86	0.60	0.590	30.1	3.14	19.3	16.4	23.0	34.1	17.7	7.83	12.3	13.5
16 hr	0.34	0.40	0.163	30.5	0.69	17.0	4.4	31.4	13.7	16.7	2.74	11.7	4.15
24 hr	0.37	0.41	0.230	26.5	1.03	16.3	7.4	34.6	17.7	10.2	1.71	12.6	6.50
72 hr	0.14	0.42	0.062	15.6	0.17	21.5	1.8	38.9	3.6	9.0	0.41	17.6	1.76
120 hr	0.10	3.36	0.053	25.8	0.28	16.0	1.4	26.0	2.8	17.9	0.85	12.9	1.52

Weight of rats = 122 ± 7 grams

dose = 0.66 mg/kg

Table VI

Distribution of mercury among blood components

200 μgm Hg as $HgCl_2$

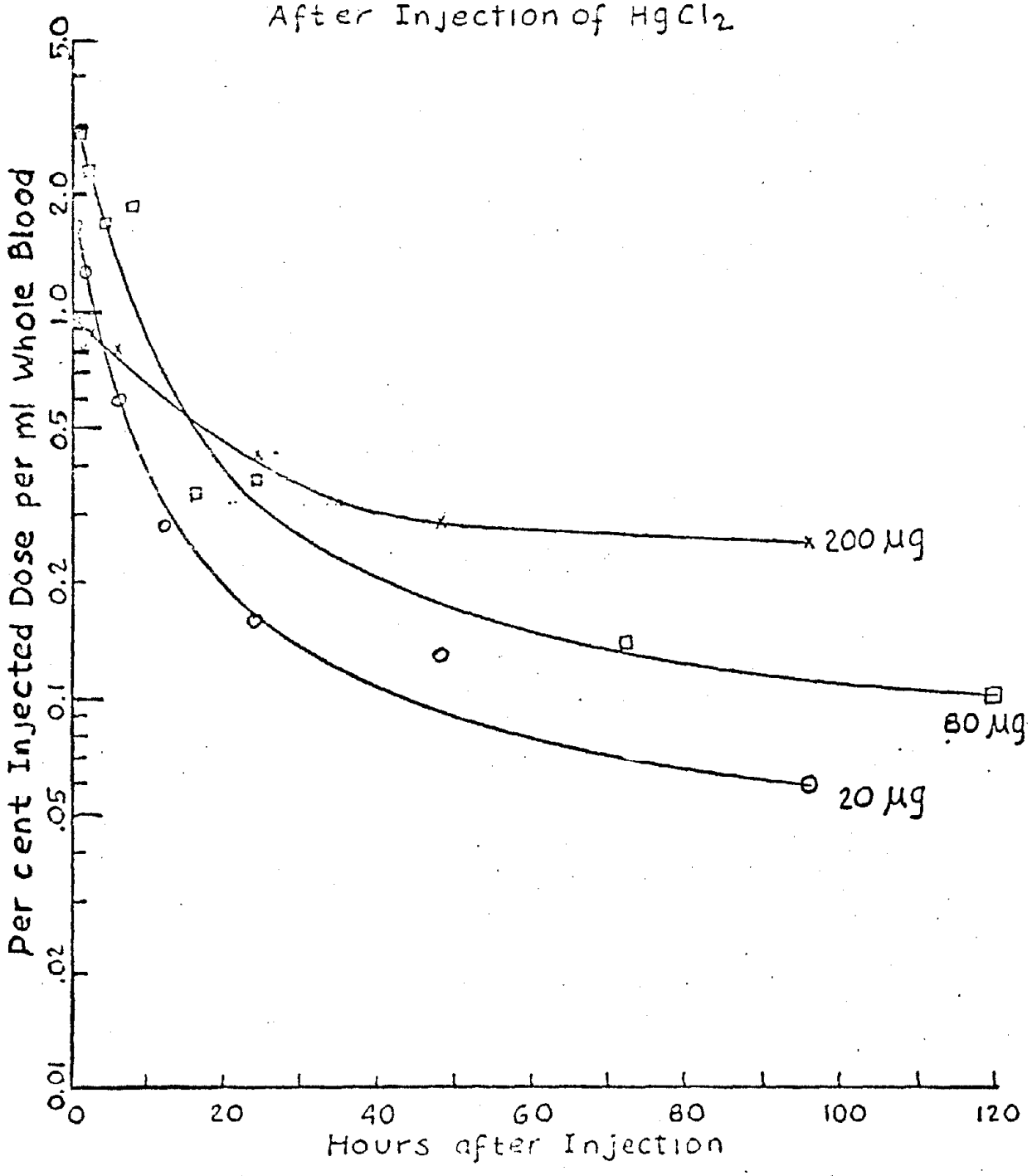
Time	% dose / ml bld	RBC / whole blood	μgm Hg / ml plasma	Albumin % Hg	Albumin moles Hg / mole x10^{-4}	Alpha 1 % Hg	Alpha 1 moles Hg / mole $\alpha 1$ x10^{-4}	Alpha 2 % Hg	Alpha 2 moles Hg / mole $\alpha 2$ x10^{-4}	Beta % Hg	Beta moles Hg / mole β x10^{-4}	Gamma % Hg	Gamma moles Hg / mole γ x10^{-4}
3/4 hr	0.[illegible]	0.724	0.722	17.8	11.78	17.63	126.7	27.73	327.3	14.56	54.4	22.57	131.7
1½ hr	0.783	.745	0.733	14.2	11.15	15.45	103.6	21.20	244.5	15.85	55.2	33.25	188.5
3 hr	0.901	.757	0.765	13.8	9.12	14.33	125.1	21.70	296.7	15.10	49.9	35.03	158.0
6 hr	0.821	.616	1.033	18.5	19.01	23.43	256.7	22.70	309.7	14.20	71.1	21.13	180.6
12 hr	0.607	.523	0.903	19.2	17.95	20.73	165.0	19.40	248.3	16.23	56.0	24.33	149.0
24 hr	0.428	.450	0.882	26.0	29.30	15.93	134.0	17.67	179.3	15.77	64.3	24.56	129.7
48 hr	0.289	.354	0.715	28.6	40.50	9.55	76.1	17.55	176.0	16.05	75.6	30.85	184.0
96 hr	0.254	.228	0.708	36.8	33.60	10.93	58.0	15.03	124.7	7.83	27.0	39.33	138.5

Weight of rats = 167 ± 7 grams

dose = 1.2 mg/kg

Fig. 6

Blood Levels of Mercury
After Injection of $HgCl_2$



Furthermore, the relative distribution among the proteins seems to be dependent on the size of the dose and the time after exposure. Thus, for example, the data from two lowest treatment groups show varying values for the fraction of the mercury in the albumin, but no clear cut dependence on time. In the case of the highest dose level however, a definite, systematic dependence on time after exposure may be seen. (An analysis of variance shows this time-dependence to be significant at the 95% confidence level.) The fraction of the serum mercury in the albumin increased from 17.8 percent 3/4 hour after injection to 36.8 percent 96 hours after exposure. Over the same time interval, the mercury in the alpha I and alpha II fraction decreased. It should be noted that the increase in relative amount of mercury in the albumin was accompanied by an absolute increase, from about 11 moles Hg per mole albumin to about 34 moles Hg per mole albumin. The alpha I and alpha II globulins' mercury content decreased, in absolute quantity, while the beta and gamma globulins had about the same amount of mercury during the 96 hours after injection (at the next sacrifice date, 192 hours after injection, the mercury content of the separate proteins was too low to permit resolution into the various fractions). These data imply a transfer of mercury from the alpha globulins to the albumin with increasing time (until at least 96 hours) after injection. This inference is reinforced by the fact that the concentration of mercury in the plasma 96 hours after exposure was about the same as that 3/4 hour after exposure, while the concentration of mercury in the whole blood decreased significantly. That this decrease of mercury in the blood of the rats in the highest treatment level is due to a loss of mercury from the erythrocytes is shown by the decreasing ratio of mercury in the red blood cells to mercury in the whole blood with increasing time after exposure.

REVERSIBILITY OF MERCURY BINDING BY SERUM PROTEINS

The study of the distribution of mercury among the serum proteins suggested a transfer of mercury from the alpha globulin to the albumin with increasing time after exposure. The reversibility of mercury binding to these proteins was checked by equilibrium dialysis technics using ^{203}Hg tagged $HgCl_2$. A dialysis bag containing 10 ml of a 1% solution of the protein was suspended in 900 ml of an appropriate solution of $HgCl_2$. Twenty-four hours later, after equilibrium was attained, the mercury inside and outside the dialysis bag was determined. The bag was then rinsed and suspended in pure buffered isotonic saline and the mercury was allowed to equilibrate a second time after which the mercury concentration inside and outside the bag was again determined. The first run was made at a temperature of $12^{\circ}C$ using 1% human serum albumin and $HgCl_2$, at a pH of 6.8, at initial concentrations ranging from $10^{-4}M$ to $10^{-8}M$. Subsequent runs were made at $12\text{-}13^{\circ}C$ using human and rat albumin, alpha globulin, and gamma globulin, with $HgCl_2$ at concentrations of $10^{-4}M$ to $10^{-8}M$, but at a pH of 7.4

The results of the first run, in which the pH was slightly acid, are shown in Figure 7. All the data fall on a single, smooth curve, thereby showing that, under conditions of the experiment, the binding of mercury by albumin is readily reversible.

Reversibility of the mercury protein binding was found to be sharply affected by pH. Subsequent runs, in which the pH was controlled at 7.4, showed the mercury to be essentially irreversibly bound - over a period of 24 hours - to the proteins.

Tables VII, VIII, IX, X, XI, XII and Figures 8 and 9, which are characteristic of the data in these tables, show little or no changes in the number of moles of mercury bound per mole of protein when, after the first equilibrium, the bag containing the free mercury-protein bound mercury was immersed in saline and allowed to equilibrate a second time. The data show rat albumin and human albumin, as well as rat and human alpha globulin, and rat and human gamma globulin to be similar in their mercury binding capacity. The binding capacities of these proteins, however, differed. Under any given set of conditions, the alpha globulins bound more mercury than the albumin, and the gamma globulins bound more mercury than the alpha globulins.

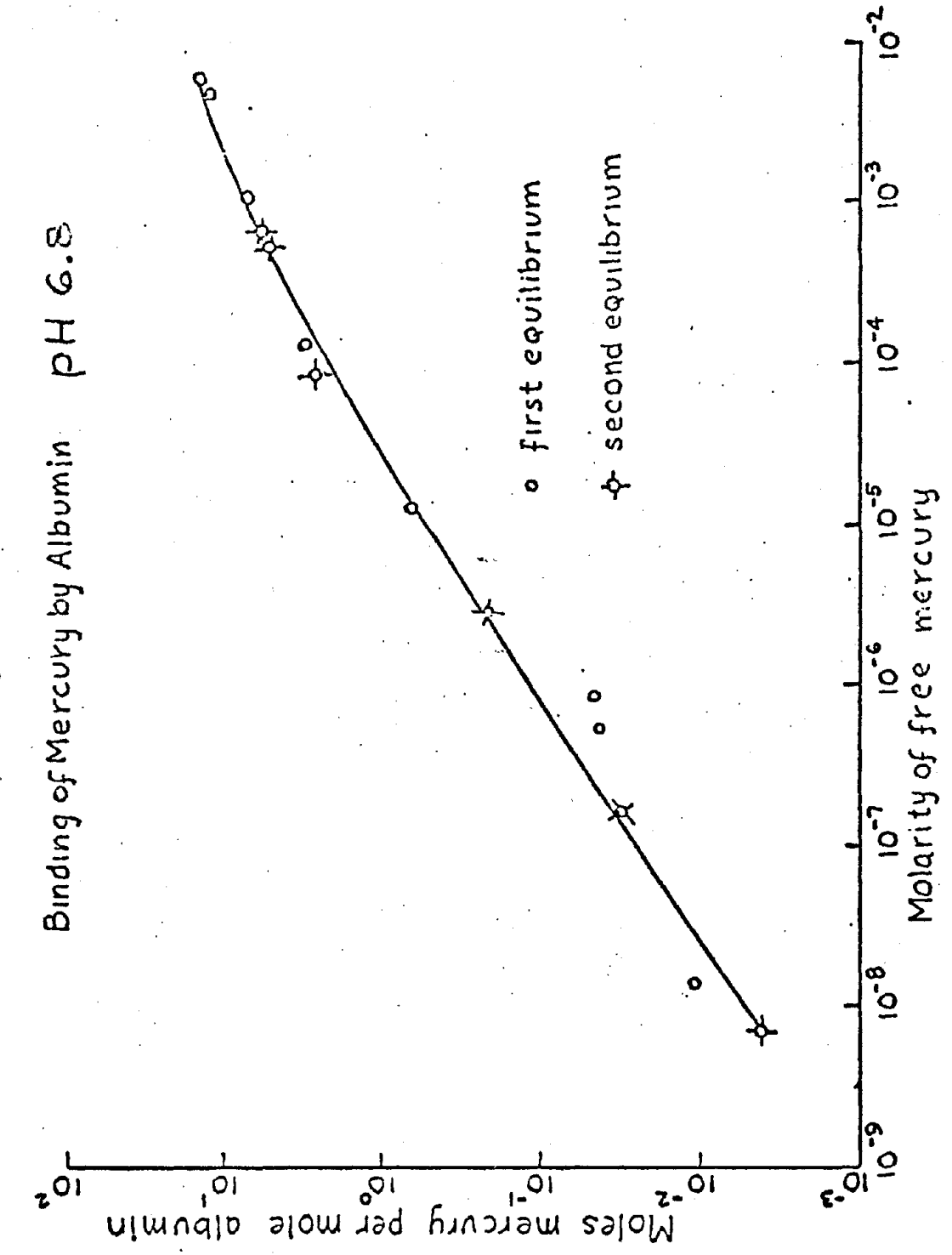


Fig 7

Binding of Mercury by Albumin pH 6.8

Table VII

Human Albumin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
6.44×10^{-5}	8.4×10^{-7}	9.43	9.55
3.0×10^{-5}	5.9×10^{-7}	5.24	5.09
5.5×10^{-6}	1.1×10^{-7}	1.09	1.05
2.85×10^{-6}	9.4×10^{-8}	0.572	0.496
5.95×10^{-7}	3.5×10^{-8}	0.134	0.119
2.39×10^{-7}	2.55×10^{-8}	0.082	0.0744
3.1×10^{-8}	8.4×10^{-9}	0.0131	0.0104
1.05×10^{-8}	1.19×10^{-9}	0.00591	0.0057
3.8×10^{-9}	2.43×10^{-10}	0.00107	0.0010

Table VIII

Rat Albumin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
6.1×10^{-5}	8.55×10^{-7}	8.66	8.35
3.86×10^{-5}	3.22×10^{-7}	2.27	2.2
6.1×10^{-6}	1.65×10^{-7}	0.95	0.874
3.9×10^{-6}	7.65×10^{-8}	0.37	0.355
7.3×10^{-7}	2.92×10^{-8}	0.099	0.085
2.82×10^{-7}	1.95×10^{-8}	0.054	0.0446
3.95×10^{-8}	4.68×10^{-9}	0.012	0.0095
2.04×10^{-8}	3.76×10^{-9}	0.0059	0.0048
5.4×10^{-9}	2.87×10^{-10}	0.00044	0.00044

Table IX

Human Alpha Globulin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
6.7×10^{-5}	1.7×10^{-6}	28.5	27.3
3.55×10^{-5}	6×10^{-7}	17.2	17.1
5.7×10^{-6}	1.3×10^{-7}	3.15	2.99
2.96×10^{-6}	1.16×10^{-7}	1.47	1.24
6×10^{-7}	8.3×10^{-8}	0.40	0.35
3.22×10^{-7}	5.6×10^{-8}	0.236	0.18
4.1×10^{-8}	6.8×10^{-9}	0.046	0.037
1.78×10^{-8}	4.1×10^{-9}	0.024	0.020
4.3×10^{-9}	1.9×10^{-9}	0.0042	0.0041

Table X

Rat Alpha Globulin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
7.1×10^{-5}	1×10^{-6}	19.5	18.8
2.2×10^{-5}	2.6×10^{-7}	16.4	14.2
6.47×10^{-6}	1.1×10^{-7}	1.95	1.80
2.87×10^{-6}	6.85×10^{-8}	0.89	0.74
6.1×10^{-7}	5.6×10^{-8}	0.266	0.221
2.74×10^{-7}	2.5×10^{-8}	0.166	0.141
4.9×10^{-8}	6.1×10^{-9}	0.039	0.034
1.77×10^{-8}	2.9×10^{-9}	0.018	0.016
4.8×10^{-9}	2.6×10^{-10}	0.0037	0.0036

Table XI

Human Gamma Globulin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
6.6×10^{-5}	3.3×10^{-6}	243.4	230.4
3.15×10^{-5}	1.3×10^{-6}	124.8	126.0
5×10^{-6}	1×10^{-7}	28.7	28.6
2.1×10^{-6}	7.7×10^{-8}	17.9	16.5
6.54×10^{-7}	2.4×10^{-8}	3.93	3.45
2.32×10^{-7}	2.2×10^{-8}	1.88	1.68
1.63×10^{-7}	2.1×10^{-9}	0.296	0.284
9.1×10^{-9}	4.3×10^{-10}	0.116	0.113
2.63×10^{-9}	1.1×10^{-10}	0.045	0.042

Table XII

Rat Gamma Globulin - Mercury Binding

Free Hg Molarity		Moles Hg bound / mole protein	
1st equilibrium	2nd equilibrium	1st equilibrium	2nd equilibrium
6.7×10^{-5}	6.3×10^{-6}	163.0	138.7
4.3×10^{-5}	1.1×10^{-6}	33.6	30.5
7.2×10^{-6}	4.1×10^{-7}	16.7	14.2
3.85×10^{-6}	1.35×10^{-7}	8.1	6.85
1.36×10^{-6}	5.7×10^{-8}	2.4	1.58
2.56×10^{-7}	1.85×10^{-9}	1.58	1.29
3.8×10^{-8}	4.8×10^{-9}	0.318	0.264
1.87×10^{-8}	1.93×10^{-9}	0.1 42	0.123
3.4×10^{-9}	2.5×10^{-10}	0.027	0.023

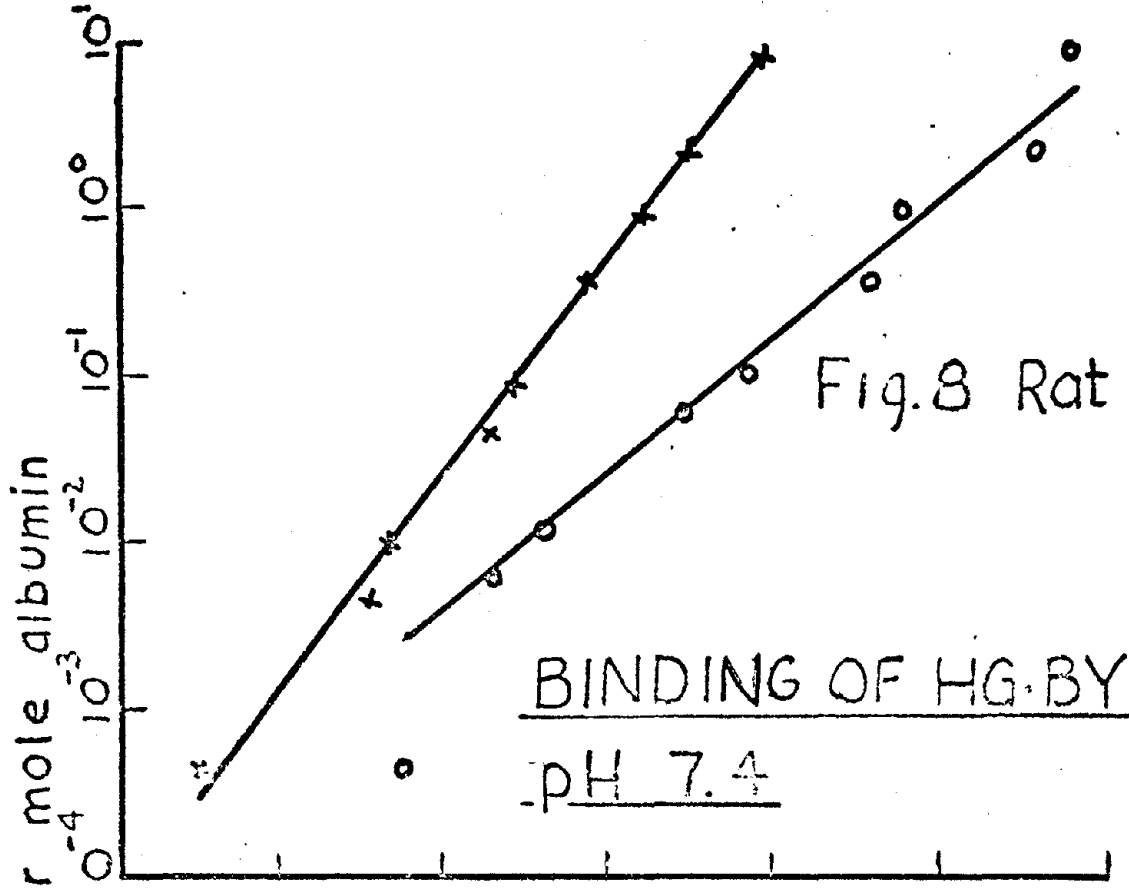


Fig. 8 Rat

BINDING OF HG·BY ALBUMIN

pH 7.4

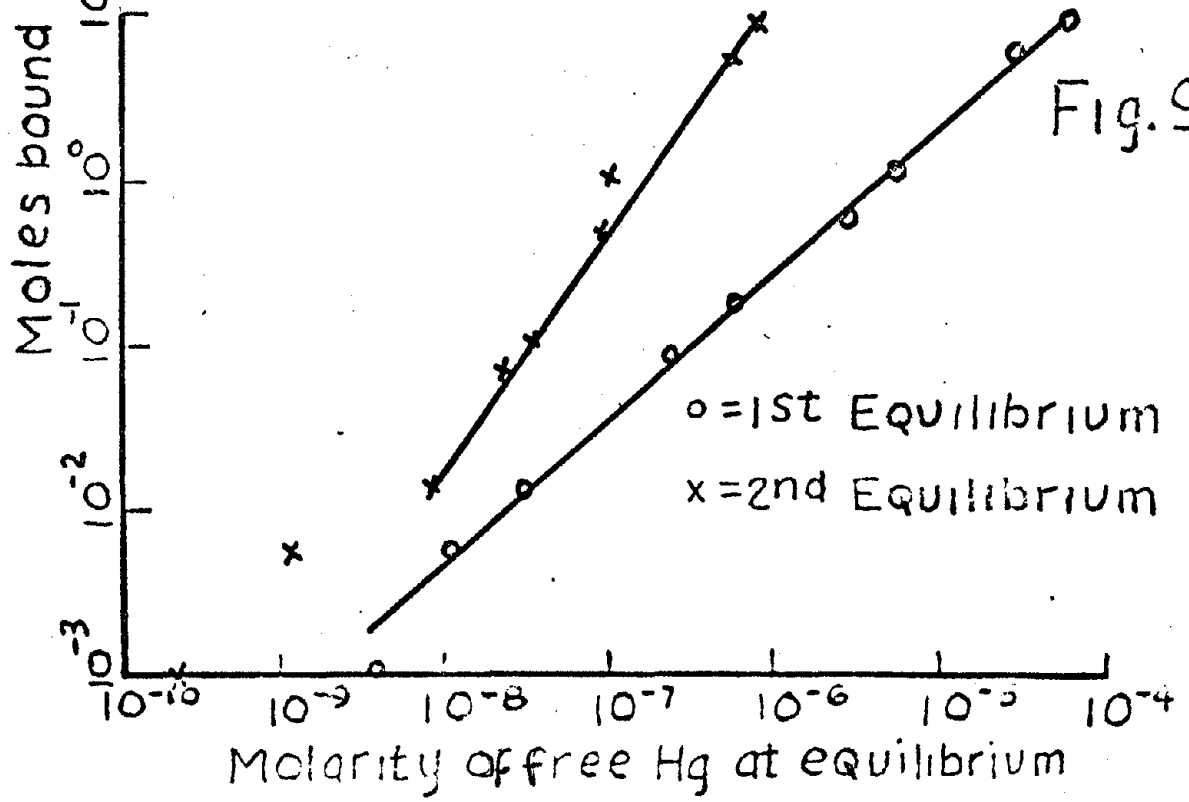


Fig. 9 Human

EFFECT OF MERCURY ON CARBONIC ANHYDRASE ACTIVITY: IN-VITRO VS. IN-VIVO

Our work on the distribution of mercury among blood fractions showed sharp differences between in-vitro and in-vivo distributions. Since much of the data on which engineering control limits are based are derived from in-vitro studies, the correlation between in-vivo and in-vitro results was further checked through the use of carbonic anhydrase inactivation by mercury. This biological indicator was chosen because of the ralatively large amount of mercury found in the erythrocytes within the first 96 hours after exposure, and because carbonic anhydrase is stored in the erythrocyte.

The effects of mercury on carbonic anhydrase inhibition were studied by measuring the time necessary for the formation of carbonic acid in an uncatalyzed saturated aqueous CO_2 solution, in a solution of the enzyme and saturated CO_2 either with or without added $HgCl_2$, and in-vivo using carbonic anhydrase taken from rats that had or had not been injected with $HgCl_2$. The mechanism of inhibition by mercury presumably is by chemical binding of the mercury to active sites on the enzyme; or alternatively, by displacing the Zn in the carbonic anhydrase. [The affinity of metals for the SH^- group of serum albumin decreases in the order of $HG^{++} > Hg^{++} > Ph^{++} > Cd^{++} > Zn^{++} > Ca^{++} > Mg^{++}$.] To learn whether or not a relationship exists between inhibition of carbonic anhydrase and mercury bound to the enzyme, hemoglobin from rats injected with mercury was separated electro-phoretically, and the carbonic anhydrase fraction was checked by radiometric technics for presence of mercury.

For the uncatalyzed reaction, 2.0 ml of a saturated solution of CO_2 in distilled water was injected into a test tube containing 2.0 ml Veronal buffer at pH 8.1, 1 ml distilled water, and 10 drops bromthymol blue indicator. The color changes rather sharply from blue to yellow at a pH of 6.5. The injection, with a hypodermic syringe, was very rapid; a stopwatch was activated when the syringe plunger reached the end of its travel. The test reaction was carried out in an ice bath using reagents that had also been kept in the ice bath. The catalyzed reaction time was measured using the same technics, except that in place of the 1 ml distilled water that had been injected into the test media, 1 ml of carbonic anhydrase (purchased from the Pentex Corporation) in distilled water, 0.025 mg/ml, was injected. The effect of mercury on the catalyzed reaction time was studied by

diluting the carbonic anhydrase with 10^{-4}M aqueous $HgCl_2$ solution instead of with pure distilled water.

For the in-vivo studies, carbonic anhydrase that had been extracted from the red blood cells of control rats and from the rats that had been injected intravenously with 200 µgm Hg as $HgCl_2$ was tested. The erythrocytes were washed three times with isotonic saline, the packed cell volume was accurately measured, and then the cells were lysed by resuspending them in doubly distilled water (1.5 times the volume of the packed cells). Ten lambdas of the lysate were diluted into 20 ml doubly distilled water to make the carbonic anhydrase test solution.

As expected, in vitro addition of mercury to the enzyme-substrate system increased the reaction time, thereby confirming that mercury mixed in vitro with carbonic anhydrase does, in fact, inhibit the action of the enzyme. However, no inhibitory effect was found among any of 29 rats that had been injected with 200 µgm mercury. The mean reaction time for the controls, (rats that had not been treated with mercury) was 52 $\pm$ 1 second, while the mean reaction time for the experimental animals was 50 $\pm$ 5 seconds. Measurement of the radiochromatograms showed that the carbonic anhydrase contained no detectable mercury. Apparently the mercury, though bound to the red cells, does not penetrate the cell membrane.

This study shows that in-vitro measurements on carbonic anhydrase inactivation or inactivation of other enzymes may be meaningless in the context of mercury exposure of a whole animal, where certain barriers may exist to prevent the mercury from reaching a site of toxic action. Such studies must be carried out using the whole animal if we wish to ascertain the hazard to a certain biological system from the mercury (or any other toxic substance), since hazard depends on the probability of the toxic substance arriving at the site of its toxic action, as well as on the nature of the toxic action.

RELATIONSHIP BETWEEN SIZE OF DOSE AND CLEARANCE KINETICS:

I. IN THE RAT

Four groups of rats, whose mean weight was 199 grams, were intraperitoneally injected with ½ cc ^{203}Hg tagged $HgCl_2$ at dose levels of 10, 50, 100 and 500 micrograms mercury. After injection, the rats were housed in Cass metabolism cages in groups of two, and serially sacrificied, four at a time, over a period of 100 days. At the times of injection and sacrifice, and periodically during the rats' lifetimes, each rat was counted in the total body counter to determine the body burden. Urine and feces were collected daily during the experiment for the 16 rats that were scheduled to be kept for 50 days or more. The rats were sacrificied by exsanguination through a heart puncture, and the blood, lungs, pancreas, adrenals, liver, kidneys, spleen, brain, and femur were taken for mercury analysis. Urine and feces were collected daily from all the rats, and were analyzed for mercury.

Total body counting data shown in Figure 10 were found to be best fitted by a three component exponential equation of the form

$$y = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

Numerical values for each of the parameters are listed in Table XIII. By analogy with the proposed mathematical model for mercury clearance (Cember, A.I.H.A.J., 30:367-371, 1969) the longest lived component is thought to represent the kidneys, the intermediate component represents the liver and the short lived component represents the rest of the body.

The parameters for the longest lived component were found to be a function of the dose for the 50, 100, and 500 μg per kg dose levels:

$$A = 108\ d^{-0.227}$$

$$\bar{\alpha} = 0.0315 - (0.515/d)$$

where d = dose level, μg/kg.

A functional dependence on dose for the parameters of the other two components did not emerge from the data. The 10 μg per kg dose level did not follow this functionality; the clearance rate fell between that of the 50 and 100 μg/kg dose levels, while the initial compartmental contents was the same as that for the 50 μg/ kg. dose.

The cumulative amounts of mercury eliminated via the urine and feces for each of the four dose levels are shown in Figures 11, 12, 13 and 14. In all cases the data were fit by the equation

FIGURE 10

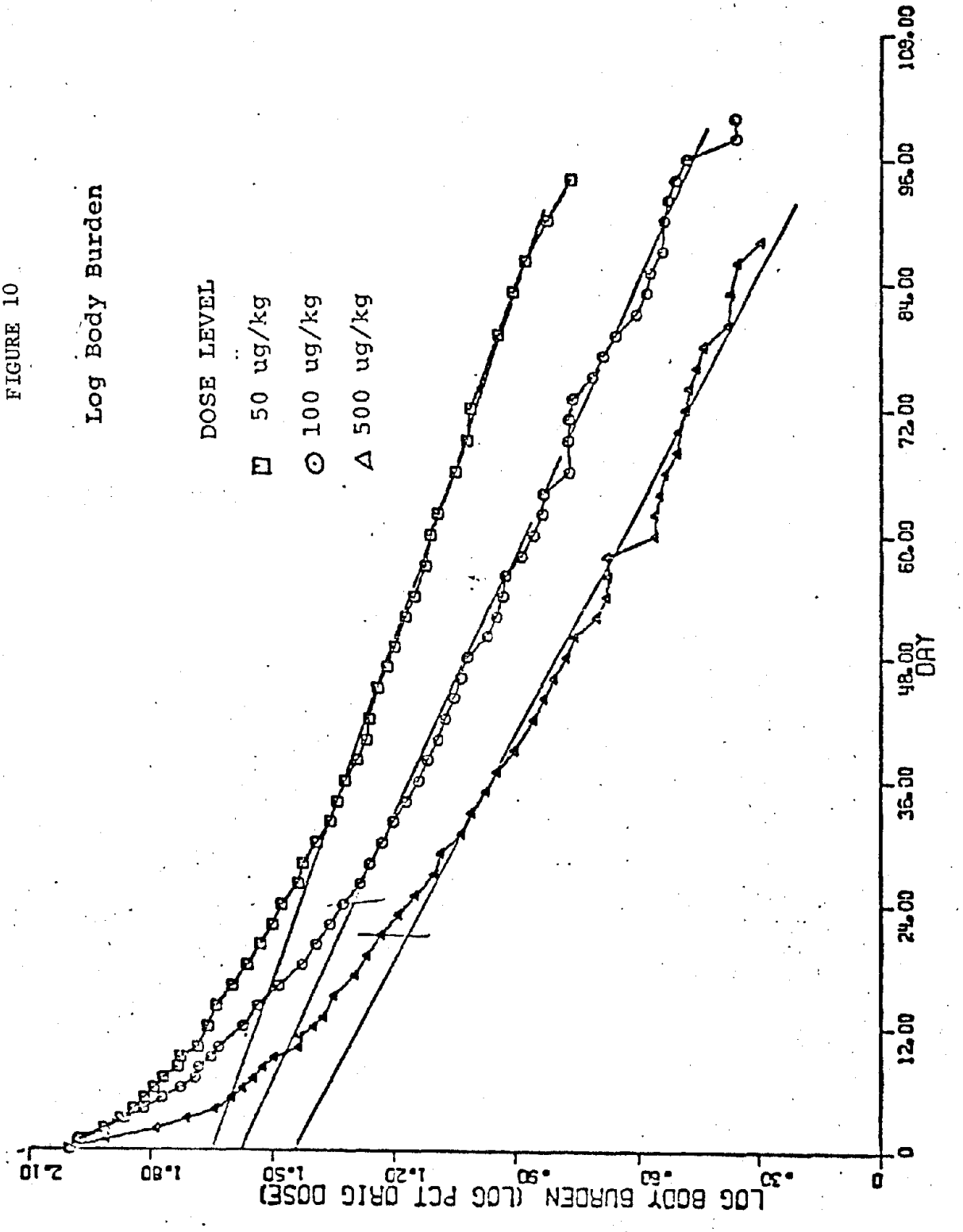

Log Body Burden
DOSE LEVEL
□ 50 ug/kg
⊙ 100 ug/kg
△ 500 ug/kg
LOG BODY BURDEN (LOG PCT ORIG DOSE)
2.10
1.80
1.50
1.20
.90
.60
.30
0
12.00
24.00
36.00
48.00
60.00
72.00
84.00
96.00
108.00
DAY

TABLE XIII

Parameters for whole body clearance of mercury

General Form: $y = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$

Dose	A	α	B	β	C	γ
10 ug/kg	44.9	0.024	34.2	0.09	22.8	0.49
std dev.	1.115	0.0015	1.36	0.0095	1.22	0.049
Half Life		28.6d		7.7		1.43
50	45.0	0.0209	44.0	0.122	10.7	0.614
	1.08	0.0009	1.42	0.014	1.38	0.21
Half Life		33.1		5.75		1.13
100	37.4	0.0269	59.9	0.148	4.3	0.325
	1.16	0.00152	1.26	0.0106	2.17	0.512
Half Life		25.7		4.64		2.16
500	26.5	0.0301	27.6	0.1105	42.8	0.61
	1.24	0.0024	1.25	0.01	1.68	0.095
Half Life		23.0		6.2		1.14

FIGURE 11

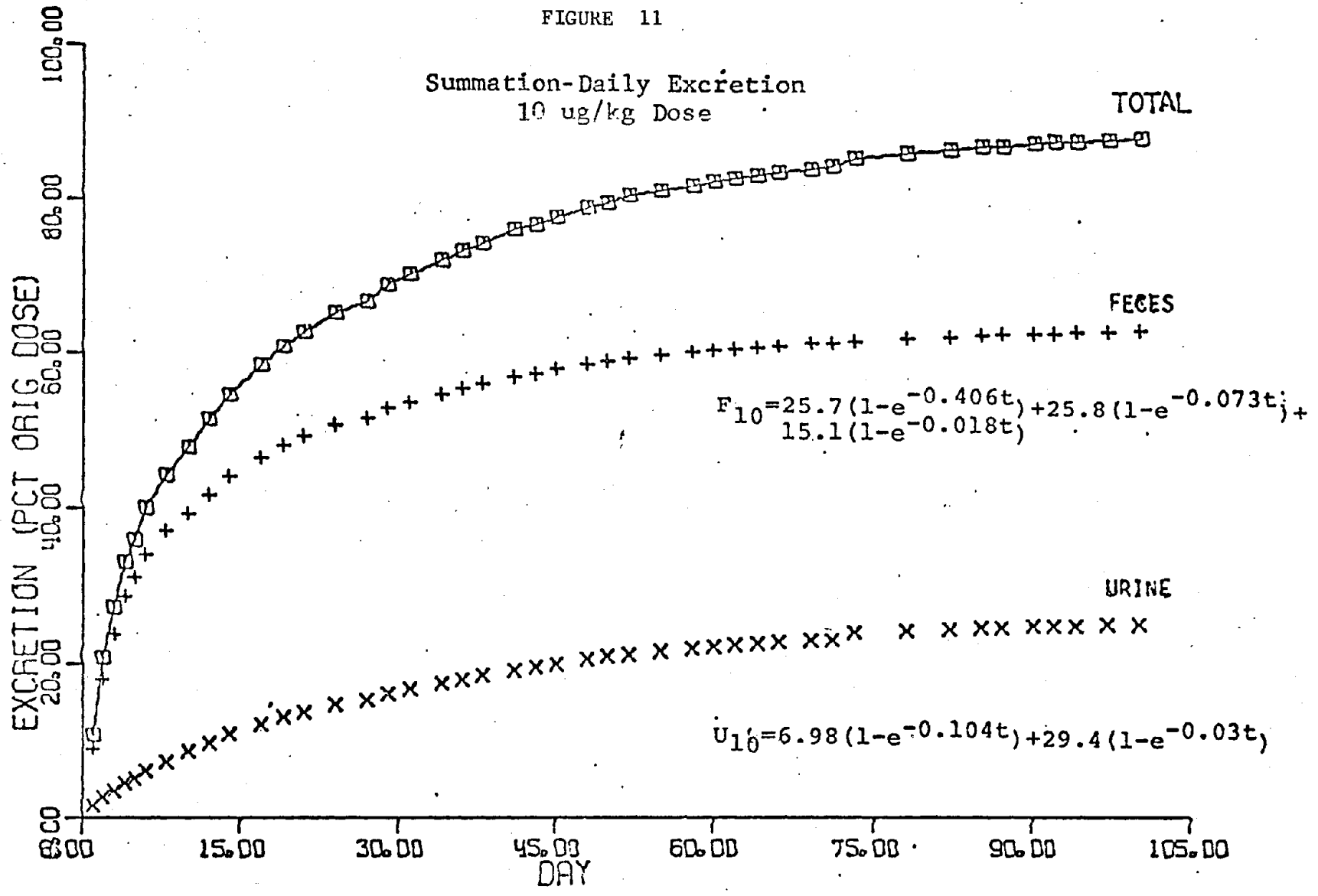

Summation-Daily Excretion
10 ug/kg Dose
TOTAL
FECES
URINE
$F_{10}=25.7(1-e^{-0.406t})+25.8(1-e^{-0.073t})+15.1(1-e^{-0.018t})$
$U_{10}=6.98(1-e^{-0.104t})+29.4(1-e^{-0.03t})$
EXCRETION (PCT ORIG DOSE)
0.00
20.00
40.00
60.00
80.00
100.00
0.00
15.00
30.00
45.00
60.00
75.00
90.00
105.00
DAY

FIGURE 12

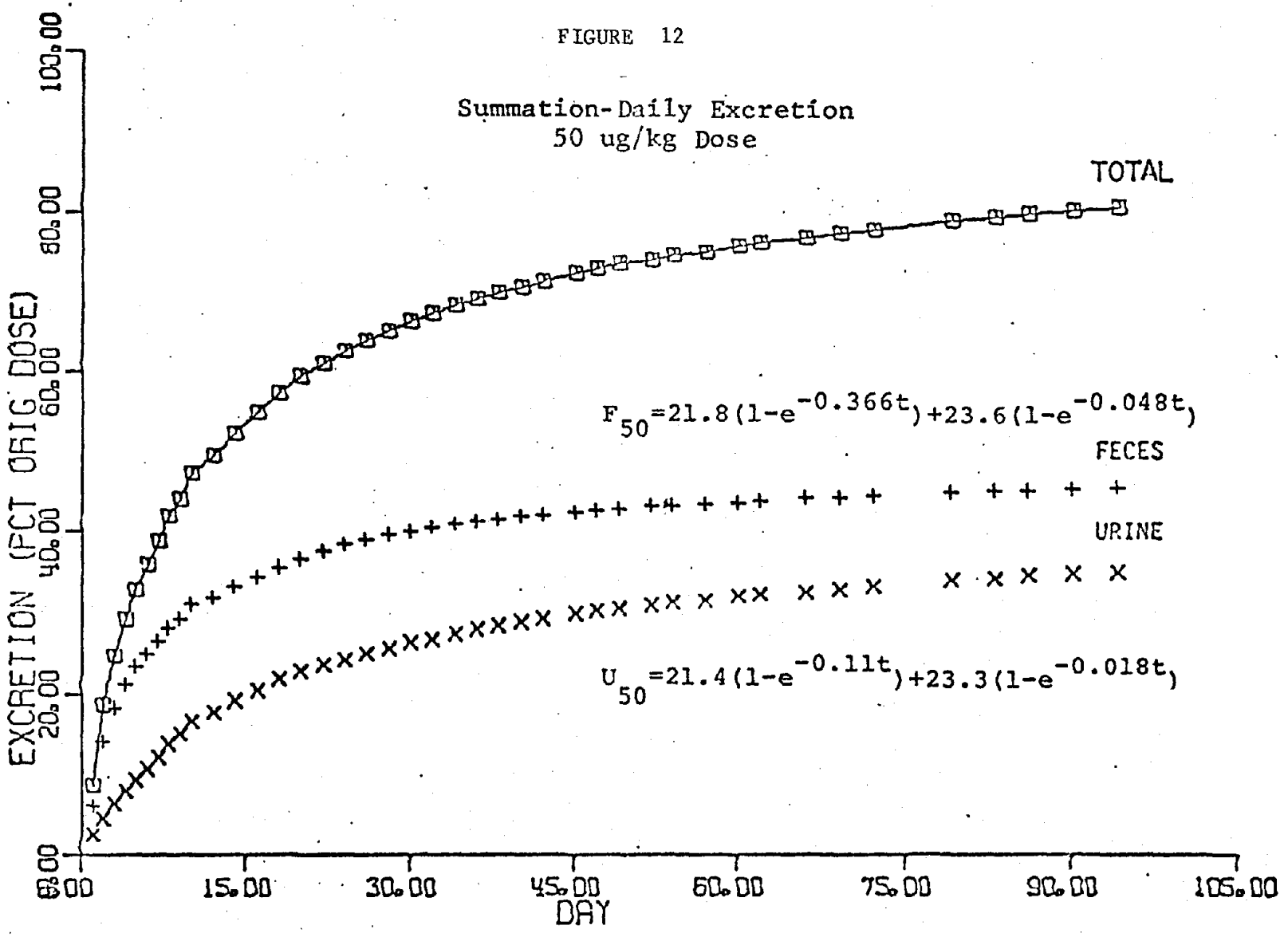

Summation-Daily Excretion
50 ug/kg Dose
TOTAL
$F_{50}=21.8(1-e^{-0.366t})+23.6(1-e^{-0.048t})$
FECES
URINE
$U_{50}=21.4(1-e^{-0.11t})+23.3(1-e^{-0.018t})$
EXCRETION (PCT ORIG DOSE)
100.00
80.00
60.00
40.00
20.00
0.00
0.00
15.00
30.00
45.00
60.00
75.00
90.00
105.00
DAY

FIGURE 13

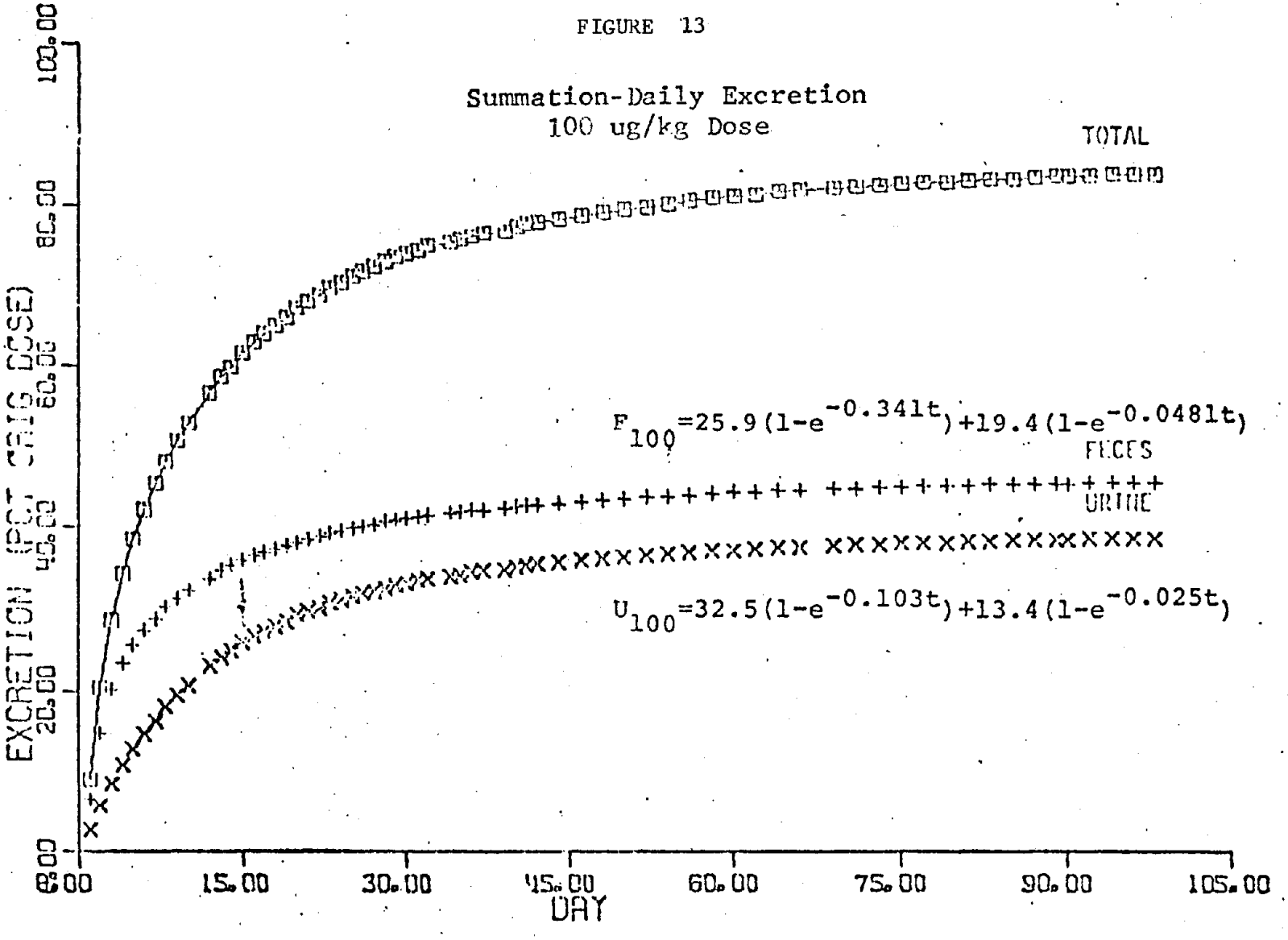
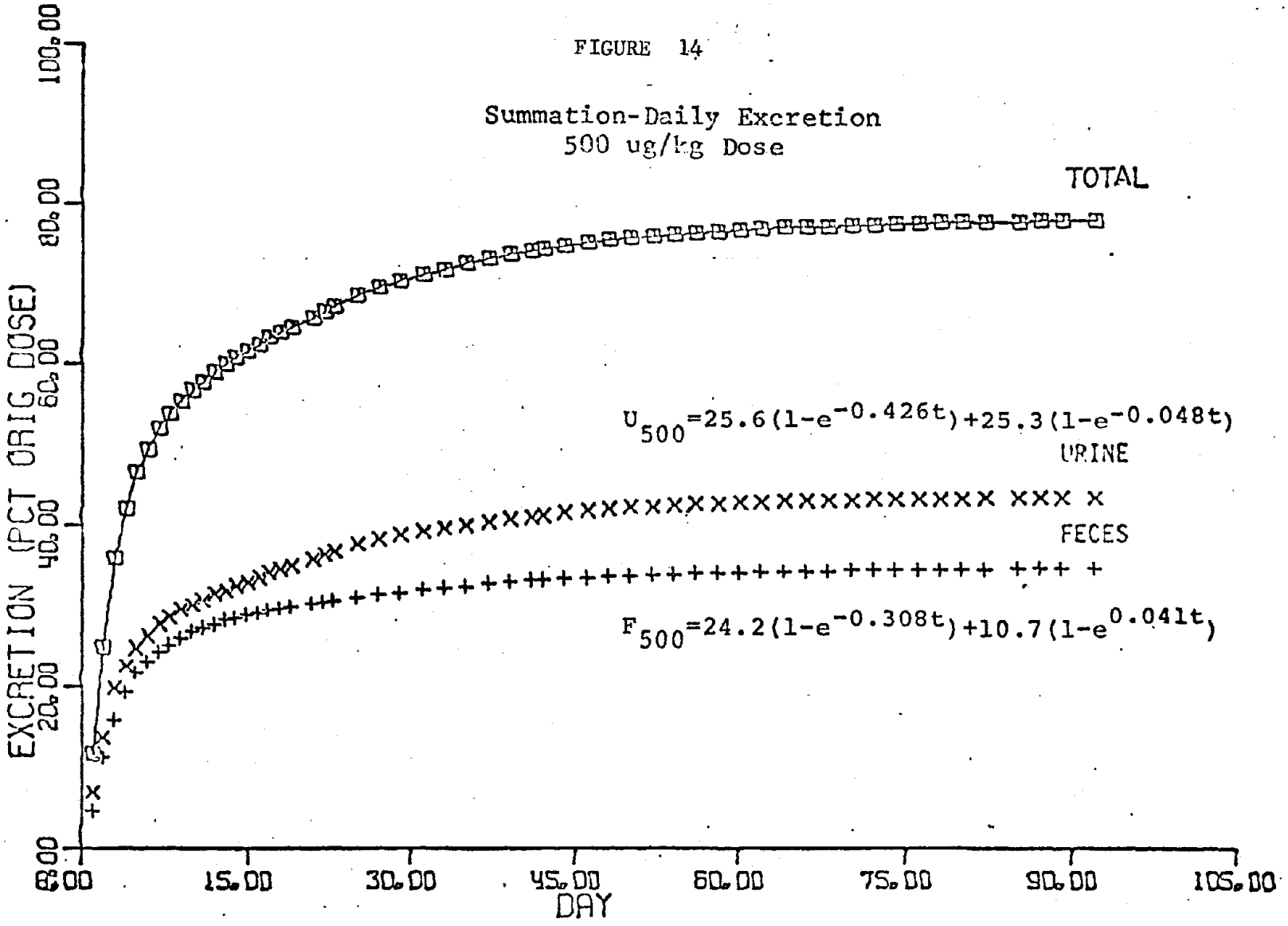

Summation-Daily Excretion
100 ug/kg Dose
TOTAL
$F_{100}=25.9(1-e^{-0.341t})+19.4(1-e^{-0.0481t})$
FECES
URINE
$U_{100}=32.5(1-e^{-0.103t})+13.4(1-e^{-0.025t})$
EXCRETION (PCT ORIG DOSE)
0.00
20.00
40.00
60.00
80.00
100.00
0.00
15.00
30.00
45.00
60.00
75.00
90.00
105.00
DAY

FIGURE 14


Summation-Daily Excretion
500 ug/kg Dose
TOTAL
$U_{500}=25.6(1-e^{-0.426t})+25.3(1-e^{-0.048t})$
URINE
FECES
$F_{500}=24.2(1-e^{-0.308t})+10.7(1-e^{0.041t})$
EXCRETION (PCT ORIG DOSE)
100.00
80.00
60.00
40.00
20.00
0.00
0.00
15.00
30.00
45.00
60.00
75.00
90.00
105.00
DAY

$$y/y_{max} = k_1 e^{-\lambda_1 t} + k_2 e^{-\lambda_2 t} + ... + k_n e^{-\lambda_n t}$$

For the 10 μg per kg dose, the data were best fit by a three component equation; for all the other dose levels, two component equations were found to give the best fit. The 50 μgm per kg data are typical of the excretion data. At this dose level, about 44.7% of the injected dose was found in the urine and 45.4% was found in the feces. The urinary mercury seems to come from two functional compartments, one in which 21.4% of the injected dose was deposited, and which empties at a rate of 11.0% $\pm$ 0.31% per day, and a second in which 23.3% of the injected dose was deposited and which clears at a rate of 1.8% $\pm$ 0.14% per day. Of the two storage depots from which the feces appear to come, 21.8% of the injected dose was deposited in a compartment that cleared at a rate of 36.6% $\pm$ 1.8% per day, while 23.6% of the injected dose was cleared at a rate of 4.8% $\pm$ 0.2% per day.

The relationship between increasing proportion of mercury eliminated in the urine and increasing dose should be noted. This finding supports previously reported findings of this same phenomenon (Cember: The influence of the size of the dose on the distribution and elimination of inorganic mercury, $Hg(NO_3)_2$, in the rat, A.I.H.A. Journal, 23: 304-313, 1962). The increasing rate of Hg elimination with increasing dose we attribute to the cytotoxic effects of mercury bound to the tubular epithelium in the kidney. At low doses, few cells bind cytocidal quantities of mercury. Mercury elimination in the urine then is due to a small amount of mercury filtered through the kidney, and the non-cytocidal mercury bound to the tubular epithelium, and eliminated during the course of the normal turnover of the epithelial cells. As the mercury dose increases, the increasing amounts of mercury bound to the tubular epithelium become progressively more cytotoxic rapidly killing more cells and causing them to be sloughed off into the urine in increasing numbers. In this study, we found 86% of the urinary mercury to be bound to cellular debris.

The tendency of the kidney retention curves, Figures 15, 16, 17 and 18, supports the urine excretion curves. The 500 and 100 microgram animals showed two compartment kidney retention curves that were fitted by the following equations:

$$K(500) = 11.5e^{-0.45t} + 9.5\, e^{-0.026t}$$

$$K(100) = e^{-0.21t} + 21e^{-0.03t},$$

where K = percent of injected dose per gram kidney at time t days after exposure.

FIGURE 15

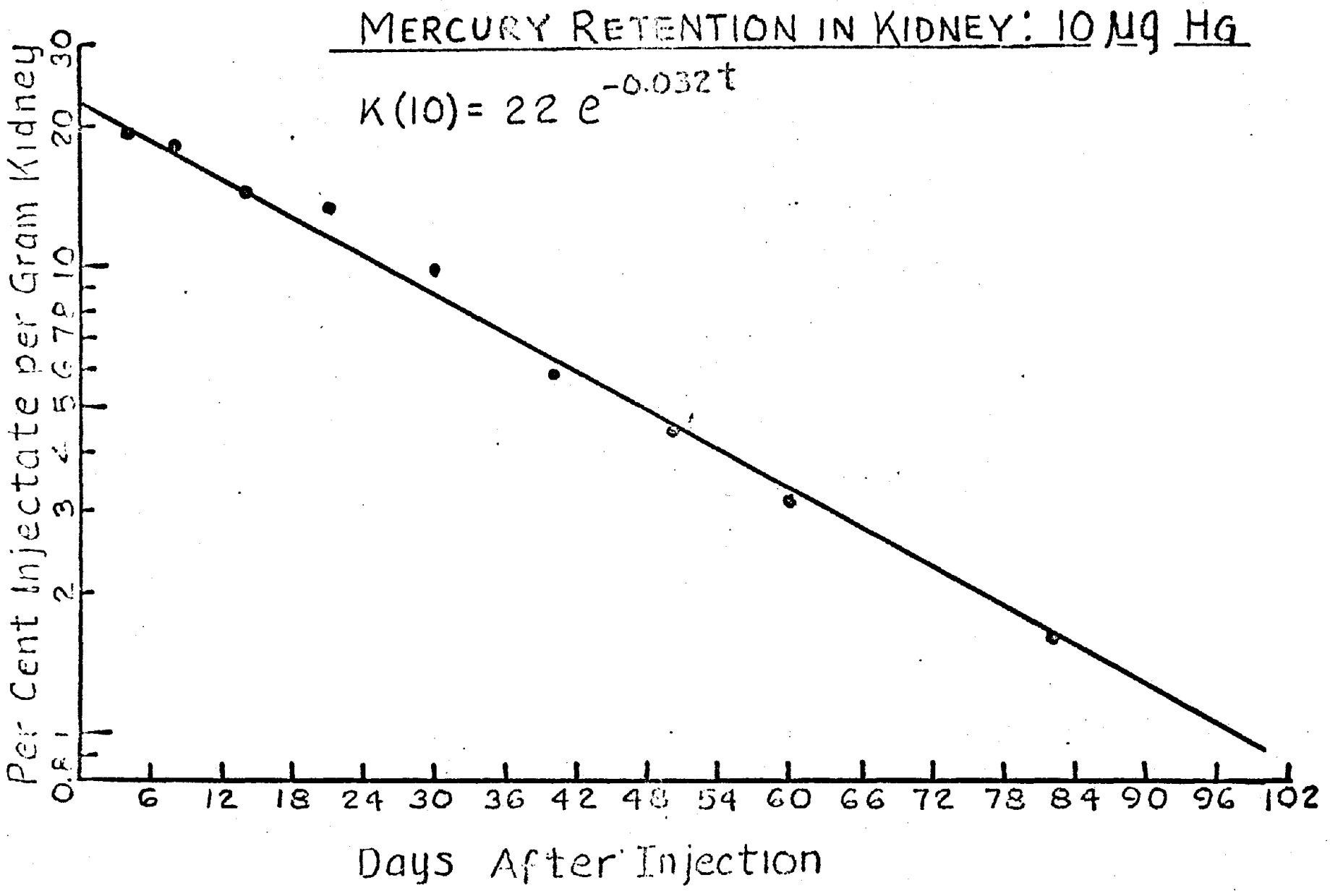

MERCURY RETENTION IN KIDNEY: 10 Mg Hg
K(10) = 22 e^-0.032t
Per Cent Injectate per Gram Kidney
0.1
2
3
4
5
6
7
10
20
30
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Days After Injection

FIGURE 16

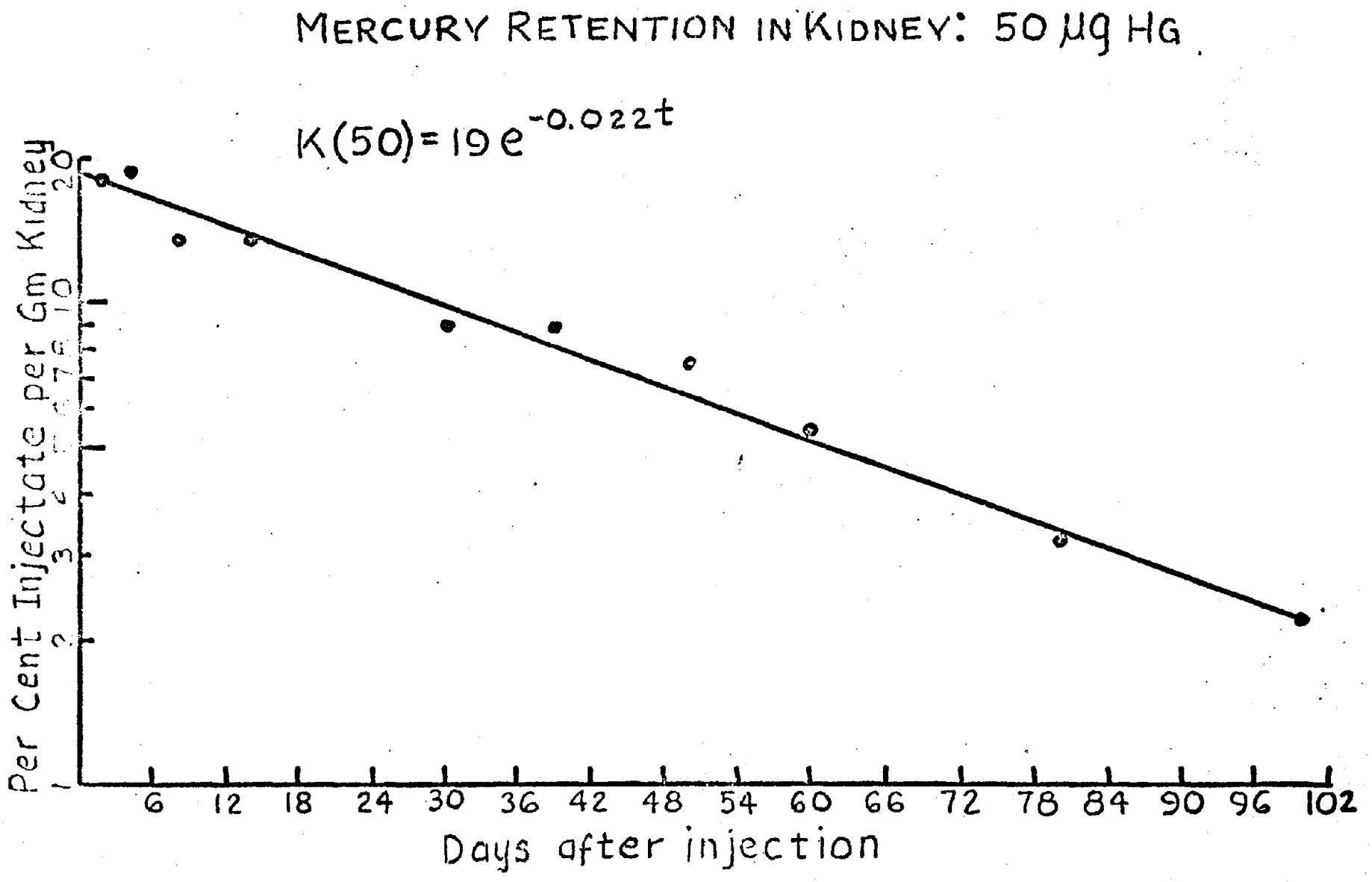

MERCURY RETENTION IN KIDNEY: 50 µg Hg
K(50) = 19 e^-0.022t
Per Cent Injectate per Gm Kidney
20
10
3
2
1
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Days after injection

FIGURE 17

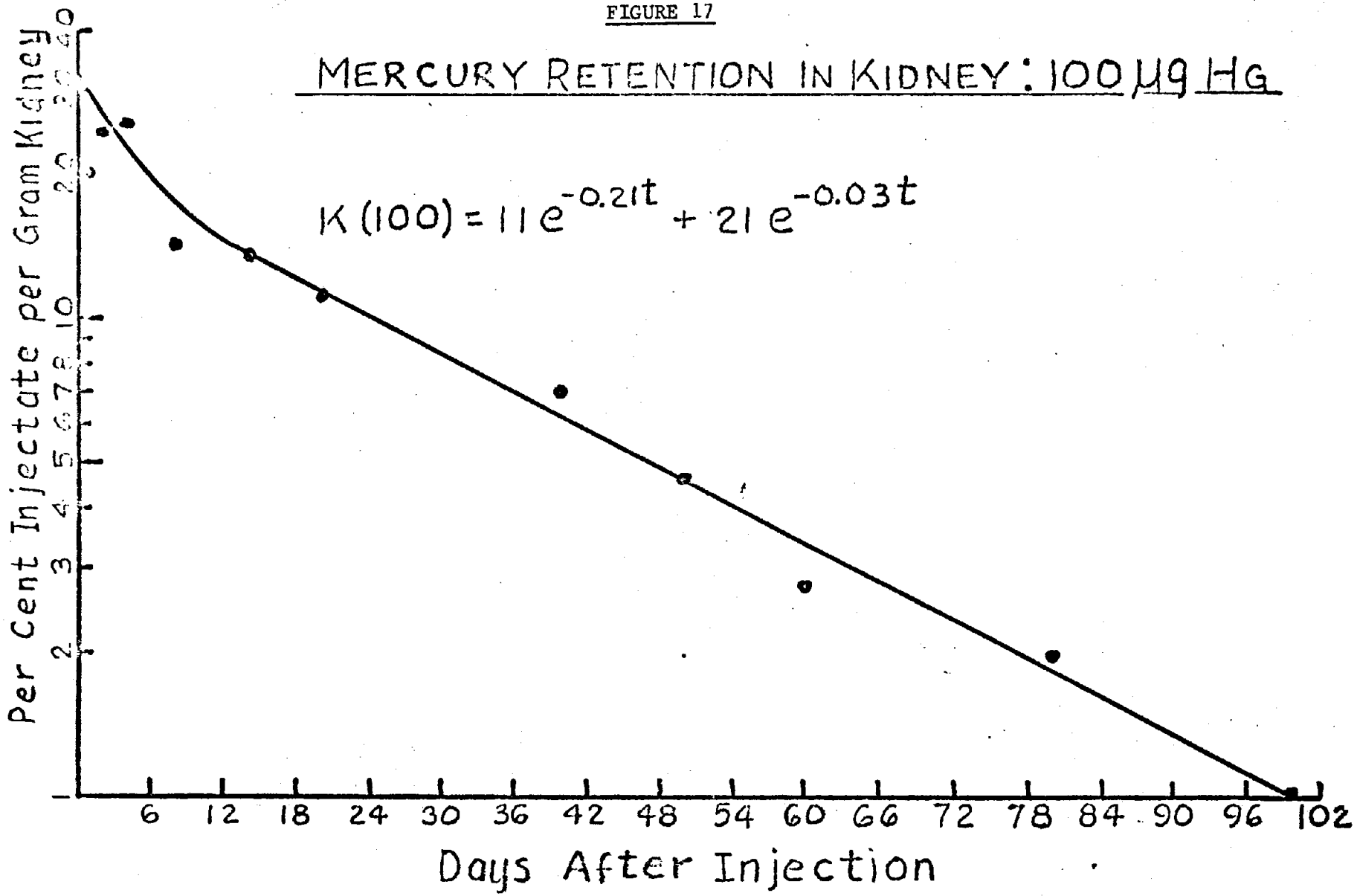
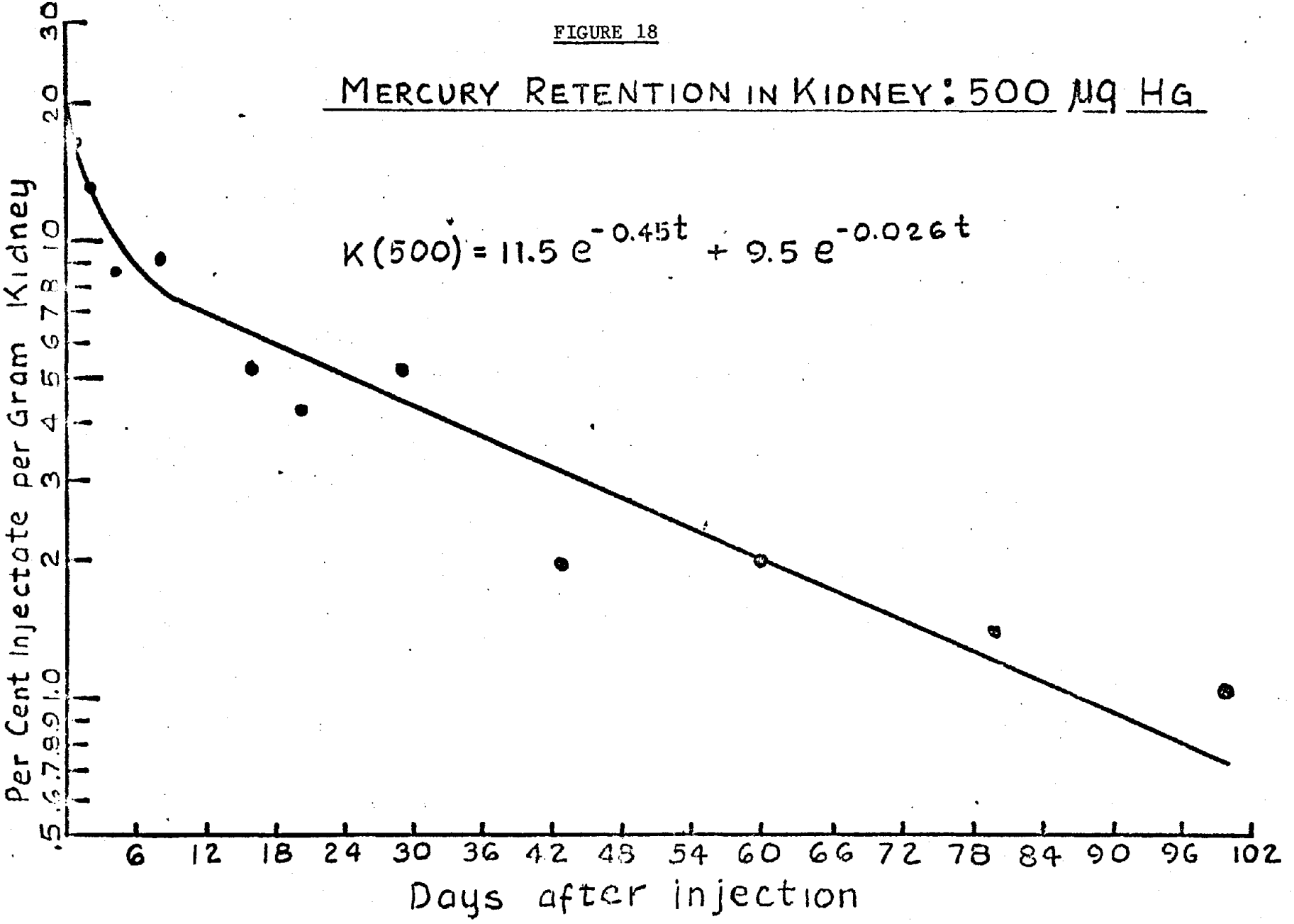

MERCURY RETENTION IN KIDNEY: 100 µg Hg
K(100) = 11 e^-0.21t + 21 e^-0.03t
Per Cent Injectate per Gram Kidney
2
3
4
5
6
7
8
10
20
30
40
6
12
18
24
30
36
42
48
54
60
66
72
78
84
90
96
102
Days After Injection

FIGURE 18


MERCURY RETENTION IN KIDNEY: 500 µg Hg
K(500) = 11.5 e^-0.45t + 9.5 e^-0.026t
Per Cent Injectate per Gram Kidney
.5 .6 .7 .8 .9 1.0 2 3 4 5 6 7 8 10 20 30
6 12 18 24 30 36 42 48 54 60 66 72 78 84 90 96 102
Days after injection

In these two instances, the short lived component is thought to represent the turnover of cells killed after binding cytocidal amounts of mercury, while the long lived components are thought to represent the elimination of mercury due to the "natural" turnover of cells in the nephron to which non-cytocidal quantities of mercury had been bound.

The kidney retention data from the two lower doses, 50 and 10 micrograms Hg respectively, were fitted by the following single component exponential equations:

$$K(50) = 19e^{-0.022t}$$

$$K(10) = 22e^{-0.032t}$$

The kidney retention equations are reasonably consistant with each other. In the case of the two higher dose levels, the short lived compartment included the same fraction of the injected dose, about 11%. However, the high dose resulted in a faster clearance rate, 45 per cent per day, than the 21 per cent per day observed in the case of the 100 microgram treatment level. The long lived compartments too show quantitative similarities. They all empty at a rate of about 2-3 per cent per day, while all but the highest dose group received about 22 per cent of the injected dose.

2. IN RABBITS

Three groups of three animals each were randomly chosen from a group of nine female New Zealand rabbits whose average weight was 1.85 kg (range 1.67-1.96 kg). Each group of three was treated, by injection into the ear vein, with either 20, 200 or 2000 micrograms mercury as ^{203}Hg tagged $HgCl_2$ in 1 ml isotonic saline at a pH of 7.4. In all cases, the total amount of radioactive tracer mercury was the same, 17.8 microcuries. Several minutes after injection, each rabbit was placed into the whole body counter for measuring the deposition of mercury in the kidney. (Preliminary measurements showed that deposition in the kidney was exceedingly fast, and that a maximum level was reached within minutes after injection.) Sequential measurements of the deposited mercury were made daily for the duration of the experiment. Before the first counting measurement was made, the kidneys were located with a hand held G.M. probe. The scintillation detector in the whole body counter was then adjusted at the point over the area of the kidneys that gave the maximum counting rate (about 20,000 cpm while background was about 10 cpm), and the center point was marked with indelible ink (the area over the kidneys had been shaved before the rabbits were injected). That this point remained reasonably stable is shown by Figures 19,20,21 and 22 which are longitudinal and transverse scans on the same rabbit 1 day and 14 days after injection.

FIGURE 19

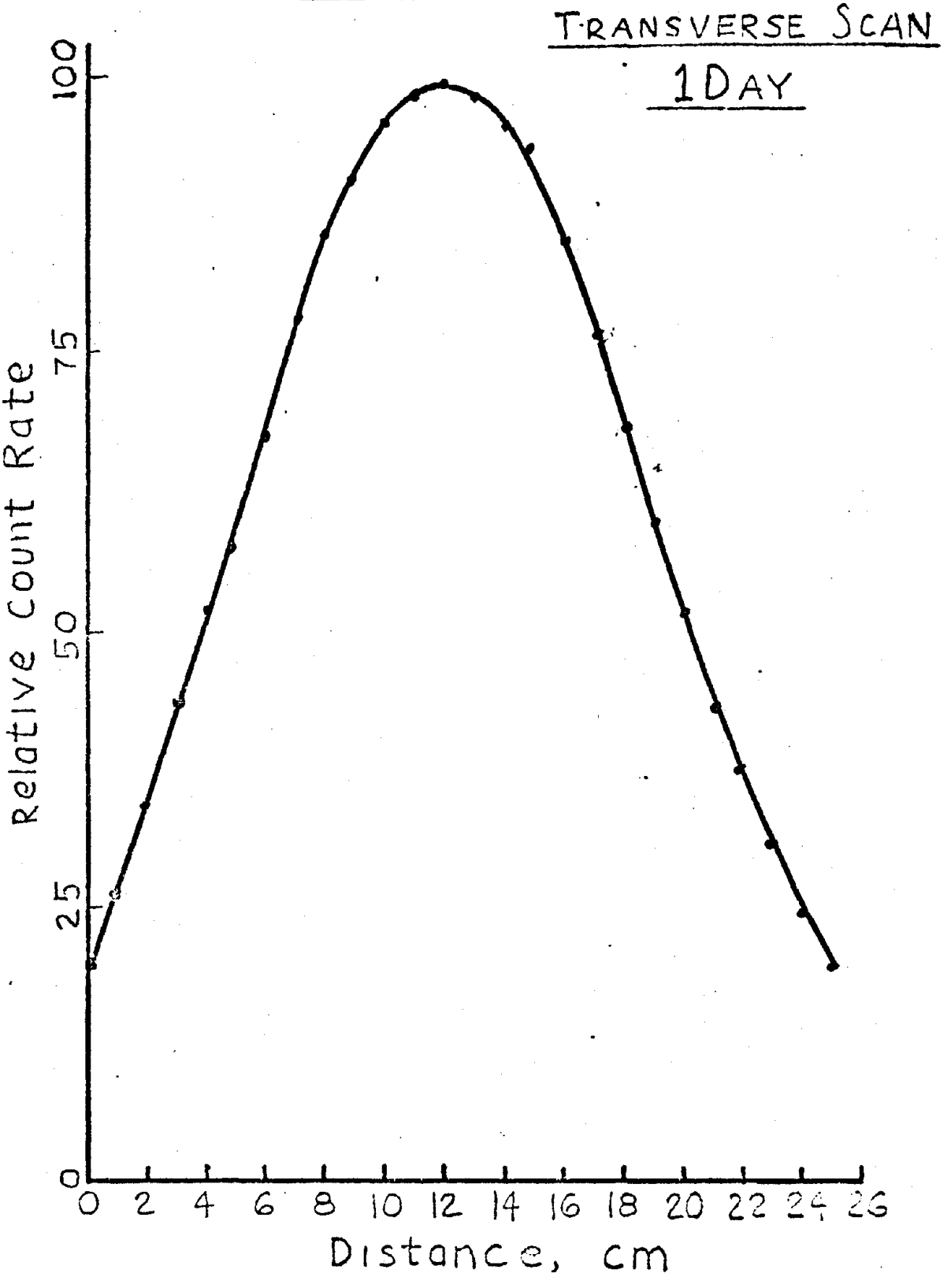

TRANSVERSE SCAN
1 DAY
Relative Count Rate
100
75
50
25
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Distance, cm

FIGURE 20

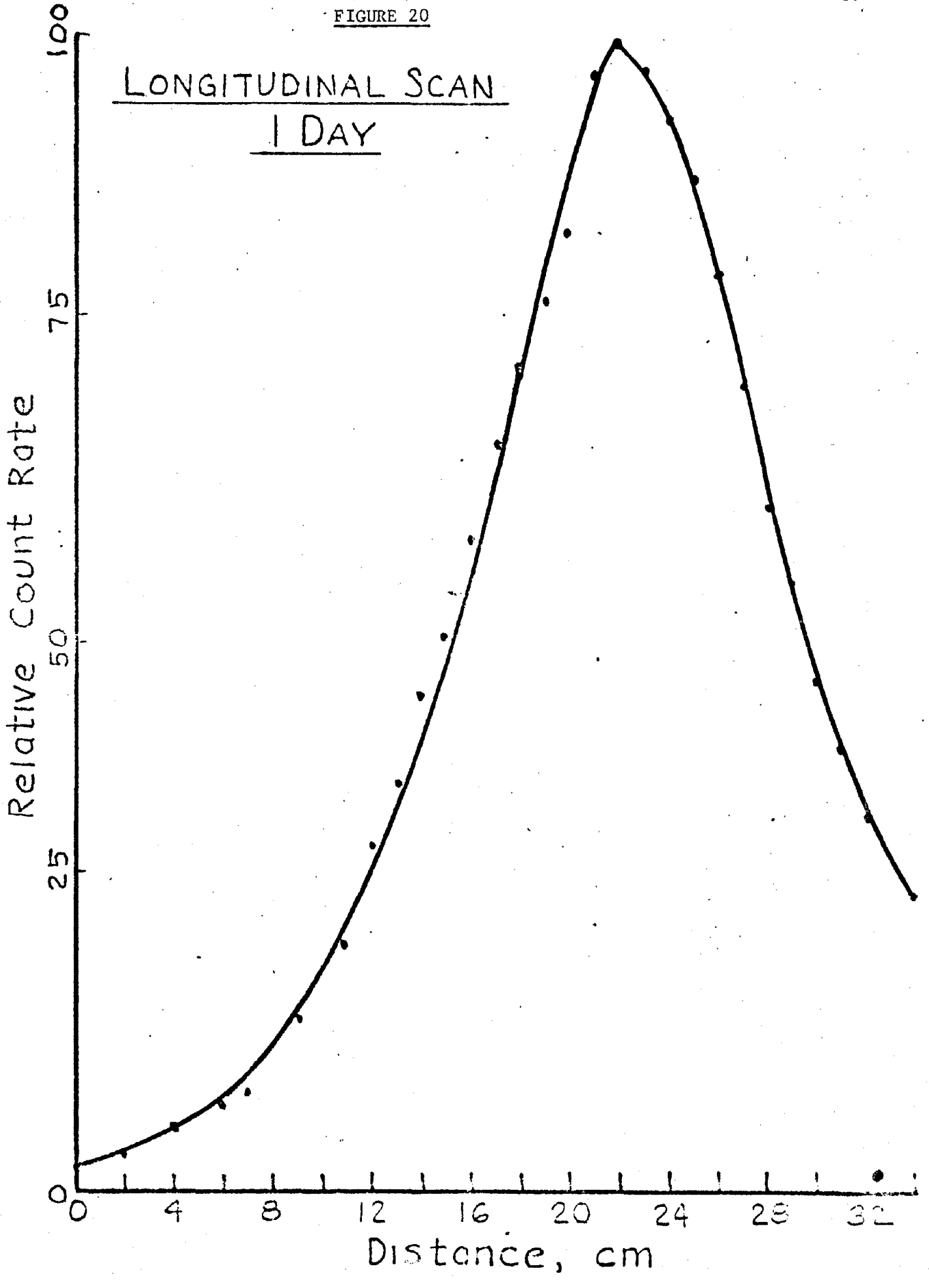

LONGITUDINAL SCAN
1 DAY
Relative Count Rate
0
25
50
75
100
0
4
8
12
16
20
24
28
32
Distance, cm

FIGURE 21

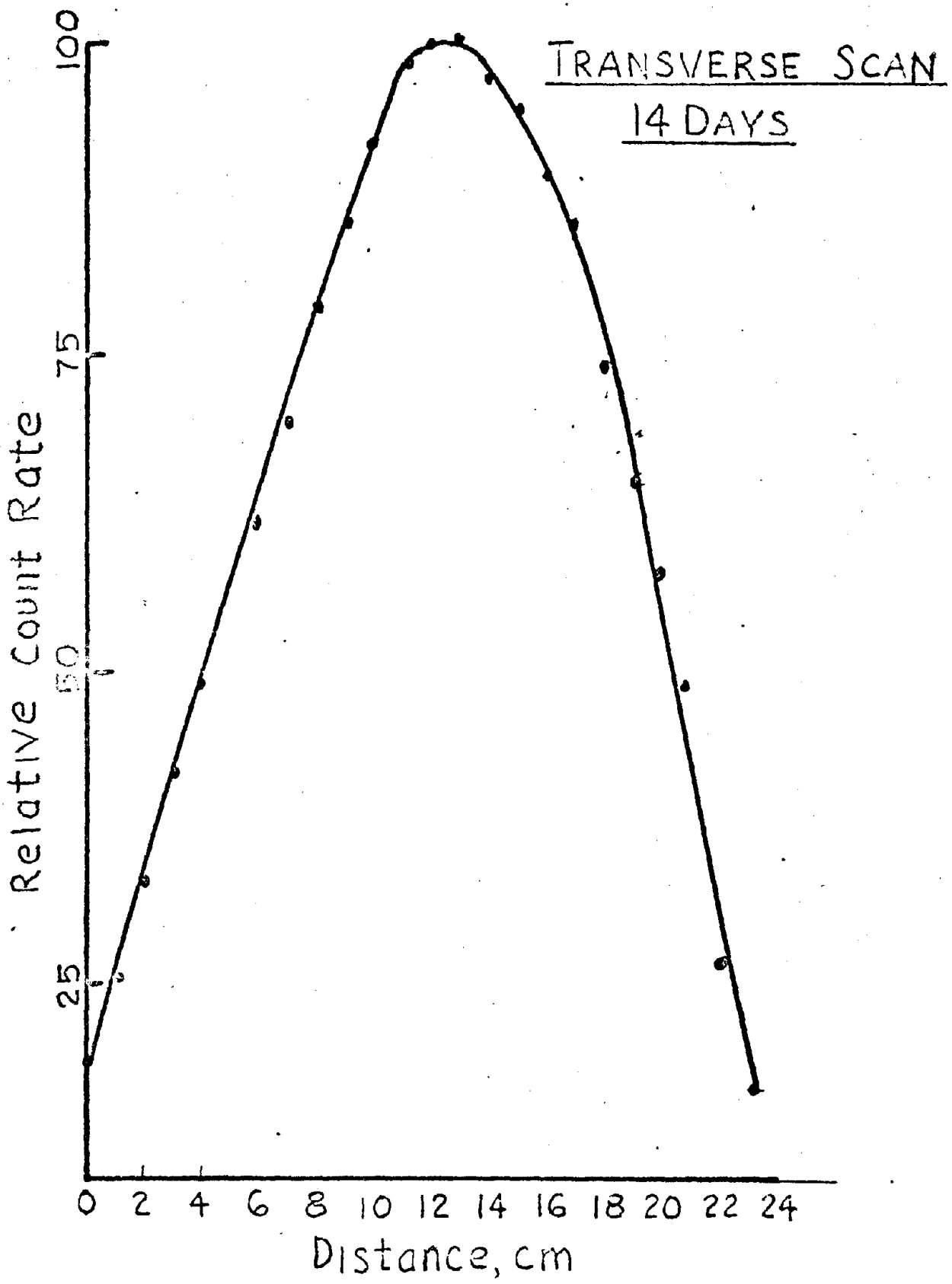
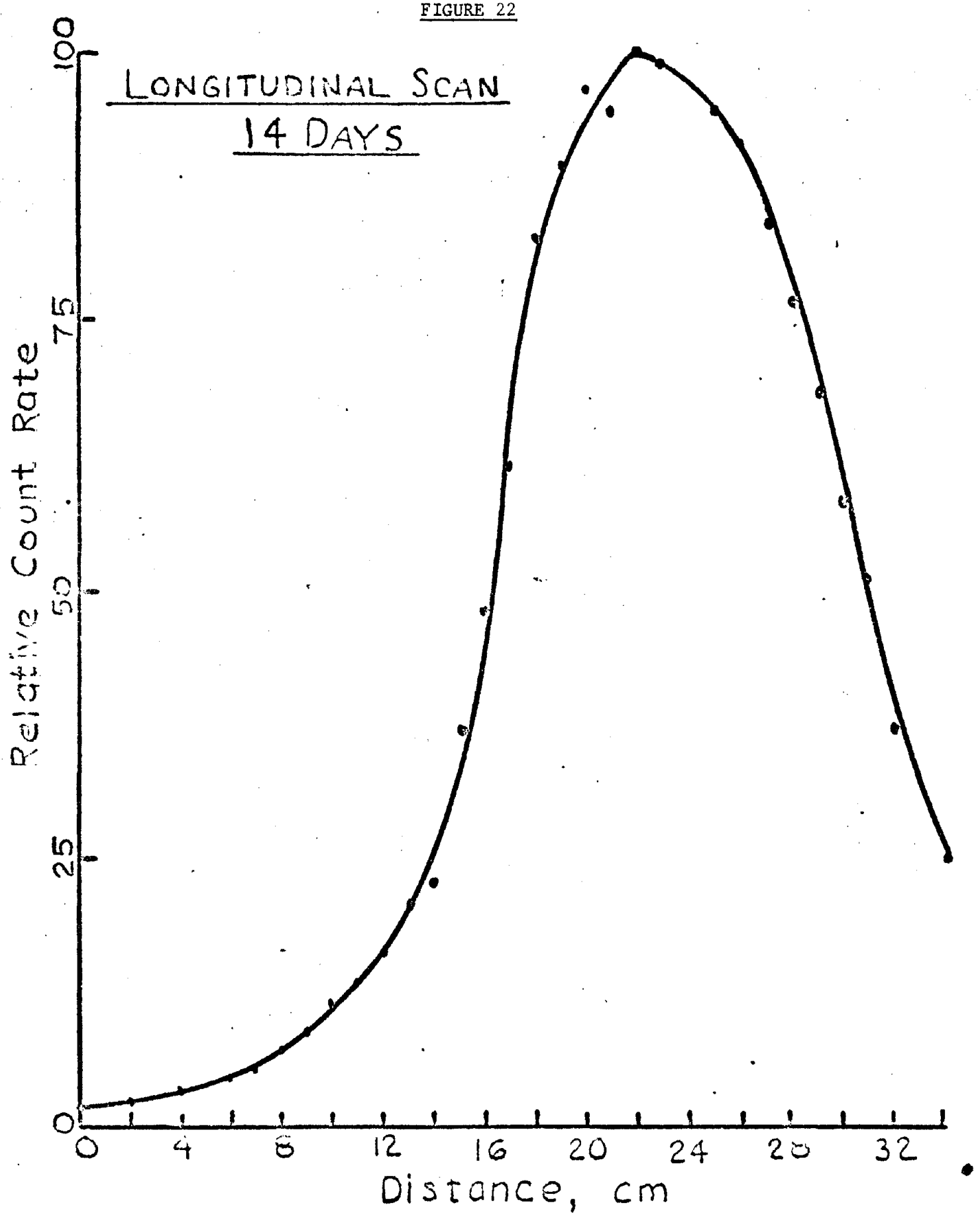

TRANSVERSE SCAN
14 DAYS
Relative Count Rate
100
75
50
25
0 2 4 6 8 10 12 14 16 18 20 22 24
Distance, cm

FIGURE 22


LONGITUDINAL SCAN
14 DAYS
Relative Count Rate
100
75
50
25
0
0
4
8
12
16
20
24
28
32
Distance, cm

A standard solution of ^{203}Hg was counted, in a fixed geometry, after each kidney count. Kidney clearance calculations were based on the ratio of the net standard count rate to the net kidney count rate. This permitted implicit correction for radioactive decay of the ^{203}Hg as well as providing a check on the stability of the counting system.

After injection, each rabbit was placed into a metabolism cage, and was permitted food and drink ad libitum. Urine and feces were collected daily for mercury analysis by gamma ray counting. After collection, the sample was spiked with non-radioactive mercury to prevent loss of the tagged mercury. The volume of urine was measured, 15 ml was transferred to a test tube, and the mercury was counted in a 2" diameter x 2" thick Na(Tl) well counter. Total mercury in the urine was calculated according to the total volume. Before the start of the main experiment, rabbit urine, spiked with known amounts of radiomercury, was poured into the metabolism cage and collected. Twenty-four hours later, the pan of the cage was rinsed with dilute HCl, and the rinse was collected and added to the urine. Measurements showed that 86.5% of the mercury was collected. All urinary mercury data were corrected by this factor. The feces were collected, spiked with non-radioactive mercury, and digested in nitric acid for twenty-four hours. The tagged mercury in the feces was determined after measuring the total volume of digest, then counting a 15 ml aliquot. A standard solution containing 1% of the amount of injected mercury was counted before each urine or feces measurement. All samples were counted for a time sufficiently long that the accuracy of the net counting rate was equal to or better than 10% at the 96% confidence level.

The results of this study are summarized in Figures 23,24 and 25, which show the whole body retention curves; and Figures 26, 27 and 28, the cumulative urine and feces excretion curves for the 20, 200 and 2000 microgram treatment levels, respectively.

The whole body retention curves for the 20 and 200 micrograms are qualitatively similar. Both sets of data were found to fit two compartment exponential curves:

$$B(20) = 21\, e^{-0.17t} + 79\, e^{-0.045t}$$

$$B(200)) = 44\, e^{-0.13t} + 56\, e^{-0.039t}$$

In this instance, the dose range from 20 to 200 micrograms mercury had little or no effect on the clearance rates of the two components. The clearance half time for the more rapidly clearing compartment was about 4½ days, while the other compartment had a clearance half time of about 16½ days.

The body retention curve for the highest dose level, 2,000 micrograms, was different from the curves for the two lower dose levels. The data were fitted by a three component exponential curve:

$$B(2000) = 8\, e^{-0.9t} + 62\, e^{-0.11t} + 30\, e^{-0.015t}$$

The cumulative excretion data were found to fit multi-component exponential curves.

$$U(20) = 29.3\left(1-e^{-0.077t}\right) + 29.3\left(1-e^{-0.027t}\right)$$

$$F(20) = 27\left(1-e^{-0.14t}\right) + 14.5\left(1-e^{-0.023t}\right)$$

$$U(200) = 41.3\left(1-e^{-0.115t}\right) + 27.6\left(1-e^{-0.014t}\right)$$

$$F(200) = 20.8\left(1-e^{-0.154t}\right) + 10.3\left(1-e^{-0.013t}\right)$$

$$U(2000) - 7.7\left(1-e^{-1.64t}\right) + 18.8\left(1-e^{-0.075t}\right) + 22.4\left(1-e^{-0.0091t}\right)$$

$$F(2000) = 34\left(1-e^{-0.146t}\right) + 17.5\left(1-e^{-0.0045t}\right)$$

where U(d) and F(d) represent the cumulative per cent of the injected mercury eliminated in the urine and feces respectively for each treatment level. As in the

FIGURE 23

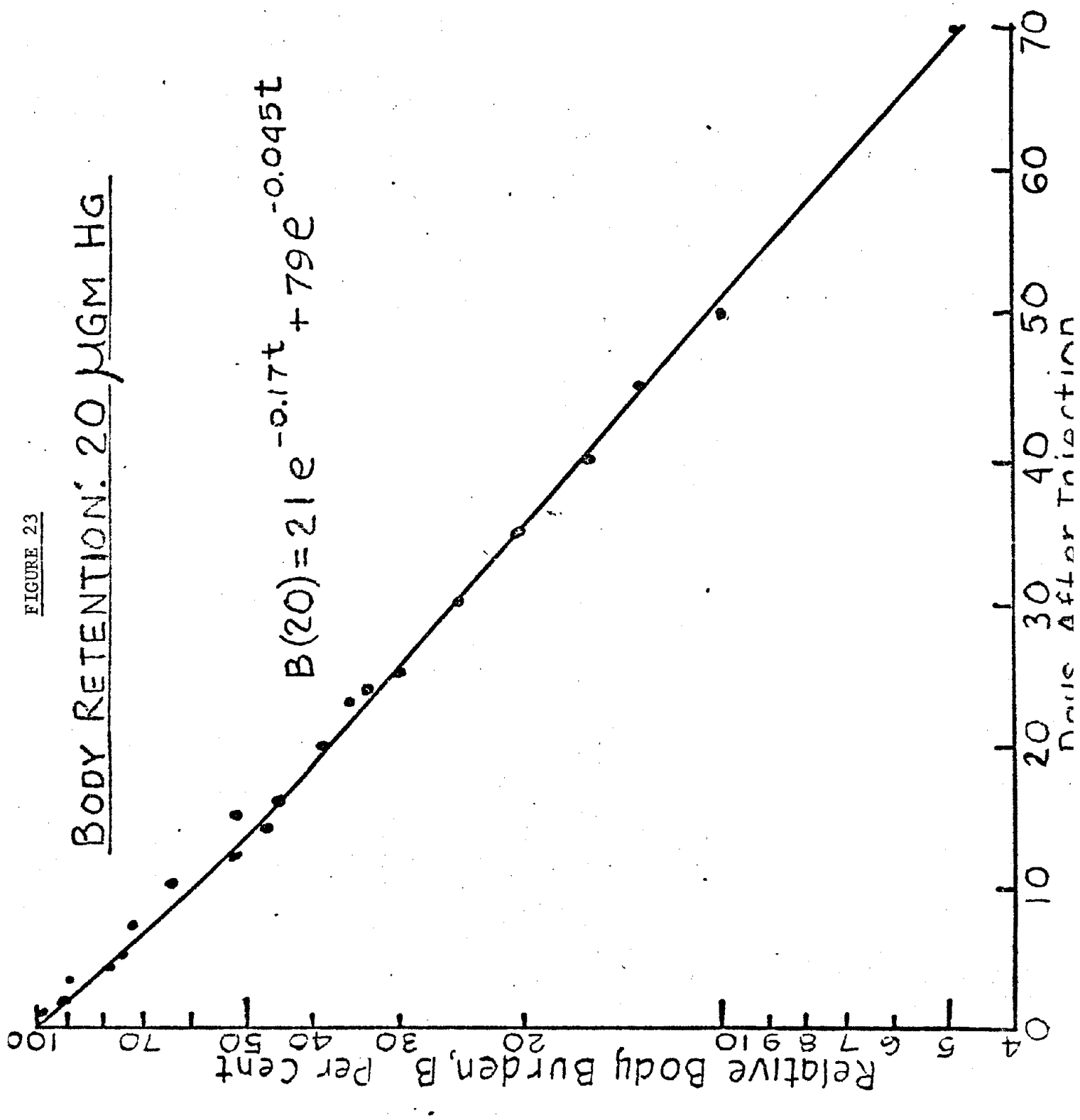

BODY RETENTION: 20 μGM HG
B(20) = 21e^-0.17t + 79e^-0.045t
Relative Body Burden, B Per Cent
100
70
50
40
30
20
10
9
8
7
6
5
4
0
10
20
30
40
50
60
70

FIGURE 24

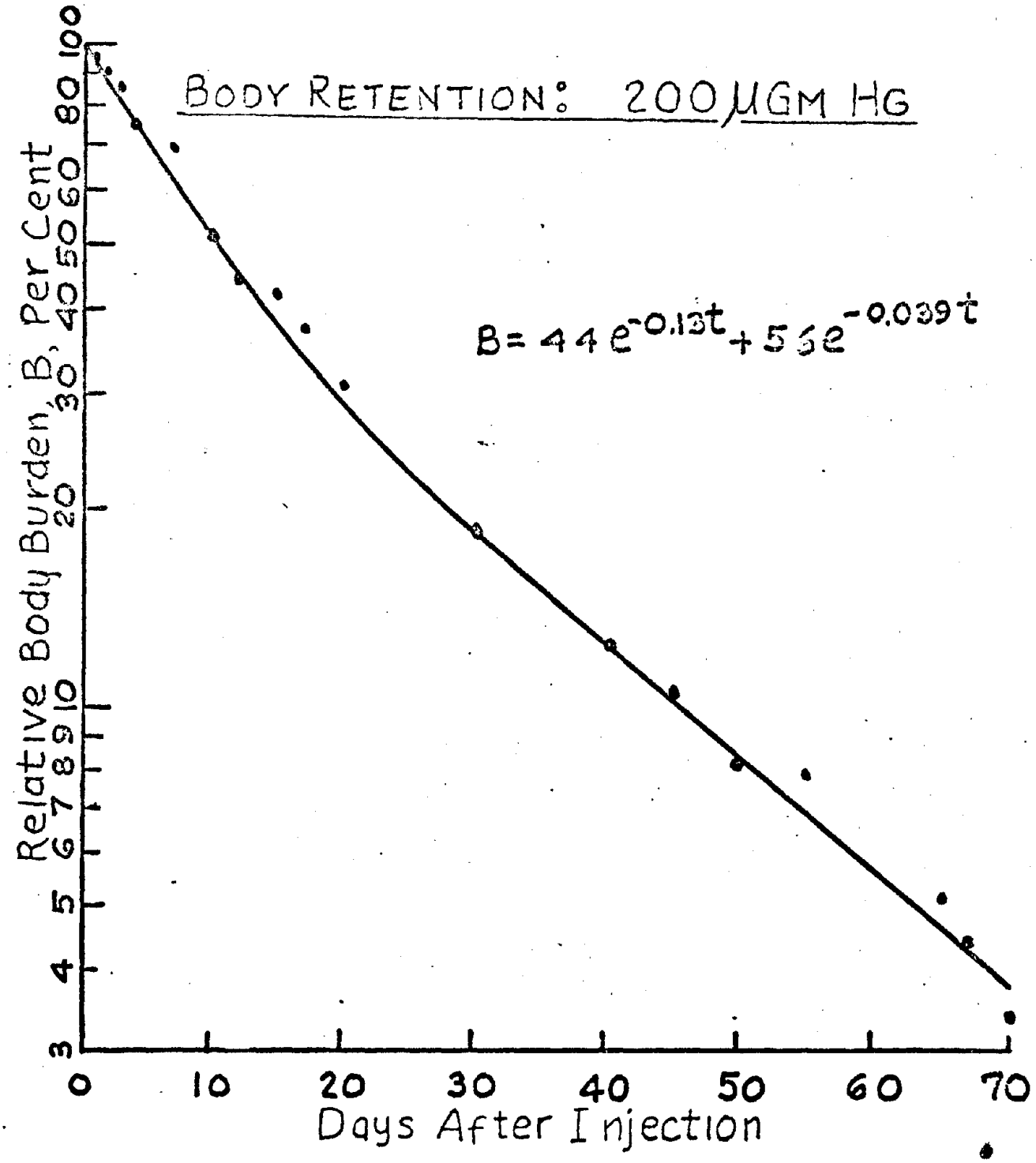

BODY RETENTION: 200 µGM HG
B = 44 e^-0.13t + 56 e^-0.039t
Relative Body Burden, B, Per Cent
100
80
60
50
40
30
20
10
9
8
7
6
5
4
3
0
10
20
30
40
50
60
70
Days After Injection

FIGURE 25

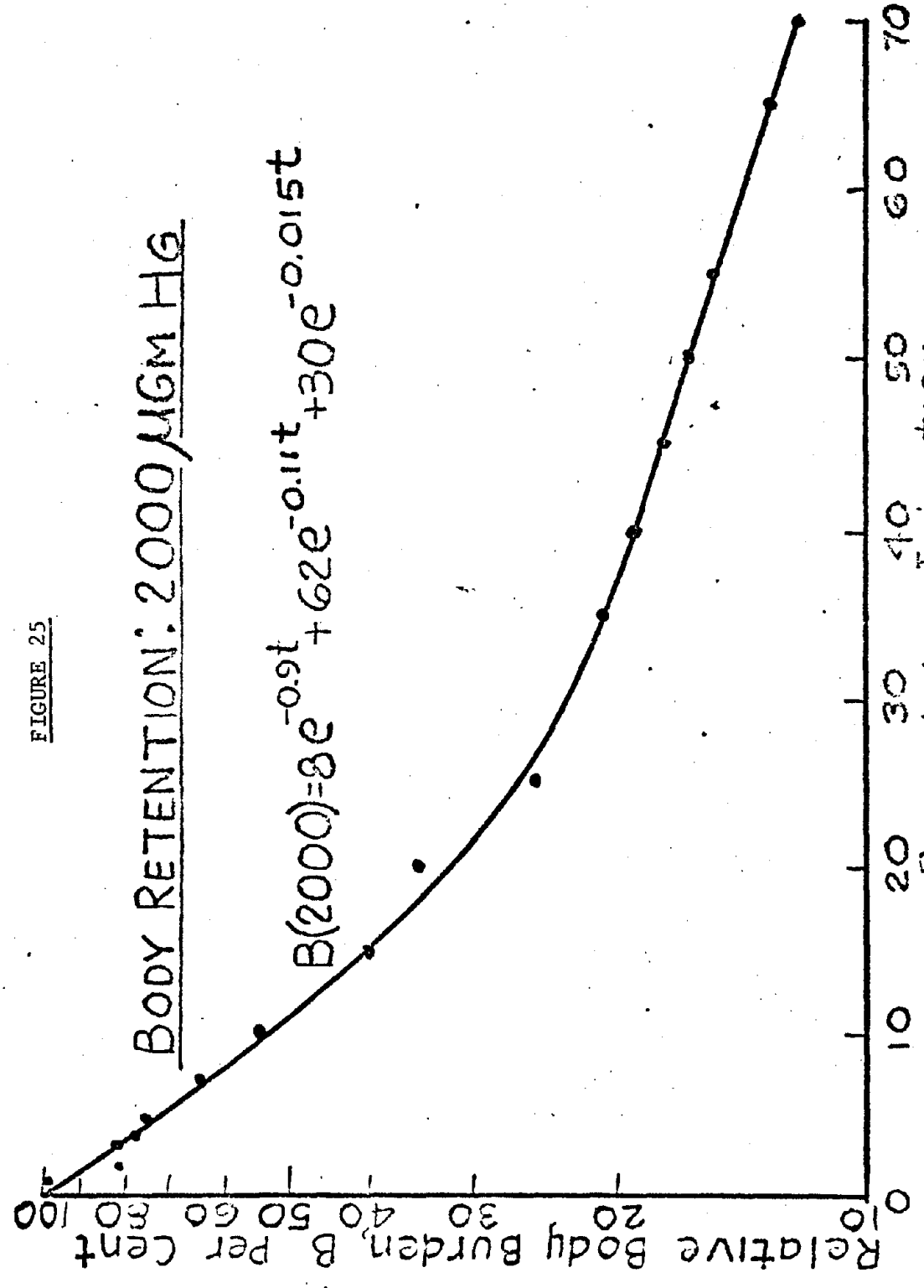

BODY RETENTION: 2000 μGM Hg
B(2000)=3e^-0.9t + 62e^-0.11t +30e^-0.015t
Relative Body Burden, B Per Cent
100
80
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Days After Injection

FIGURE 26

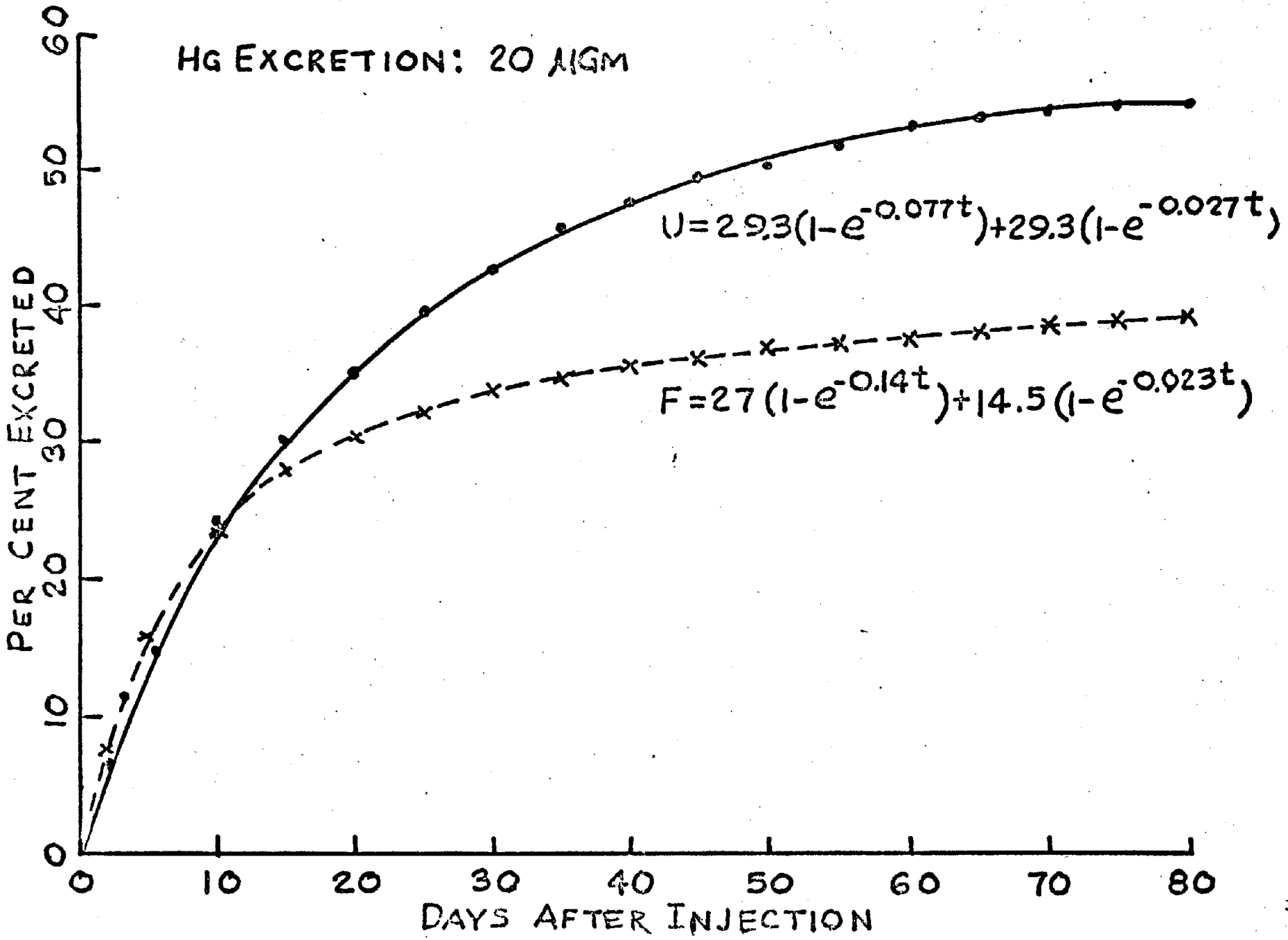

HG EXCRETION: 20 MGM
U=29.3(1-e^-0.077t)+29.3(1-e^-0.027t)
F=27(1-e^-0.14t)+14.5(1-e^-0.023t)
PER CENT EXCRETED
0
10
20
30
40
50
60
0
10
20
30
40
50
60
70
80
DAYS AFTER INJECTION

FIGURE 27

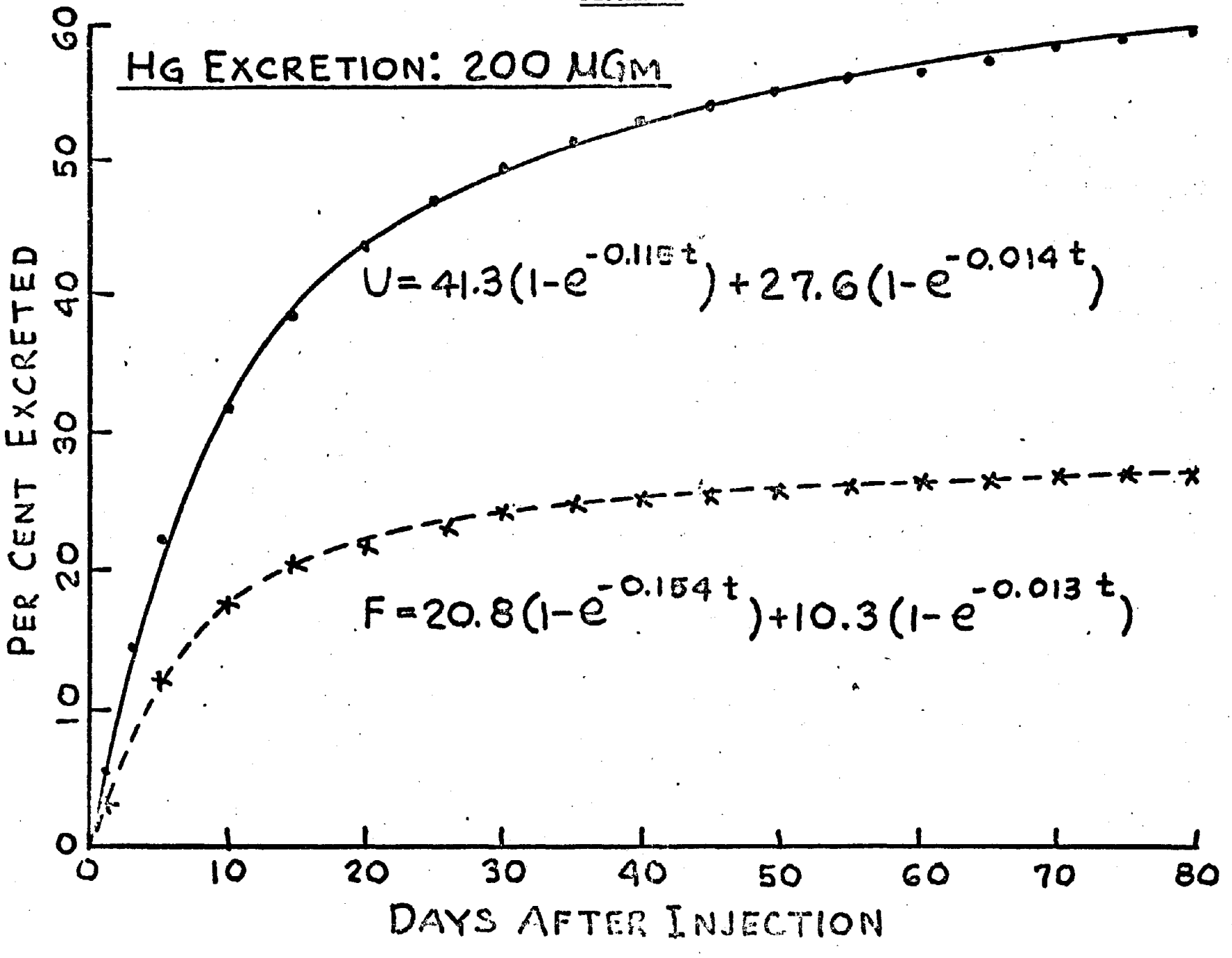
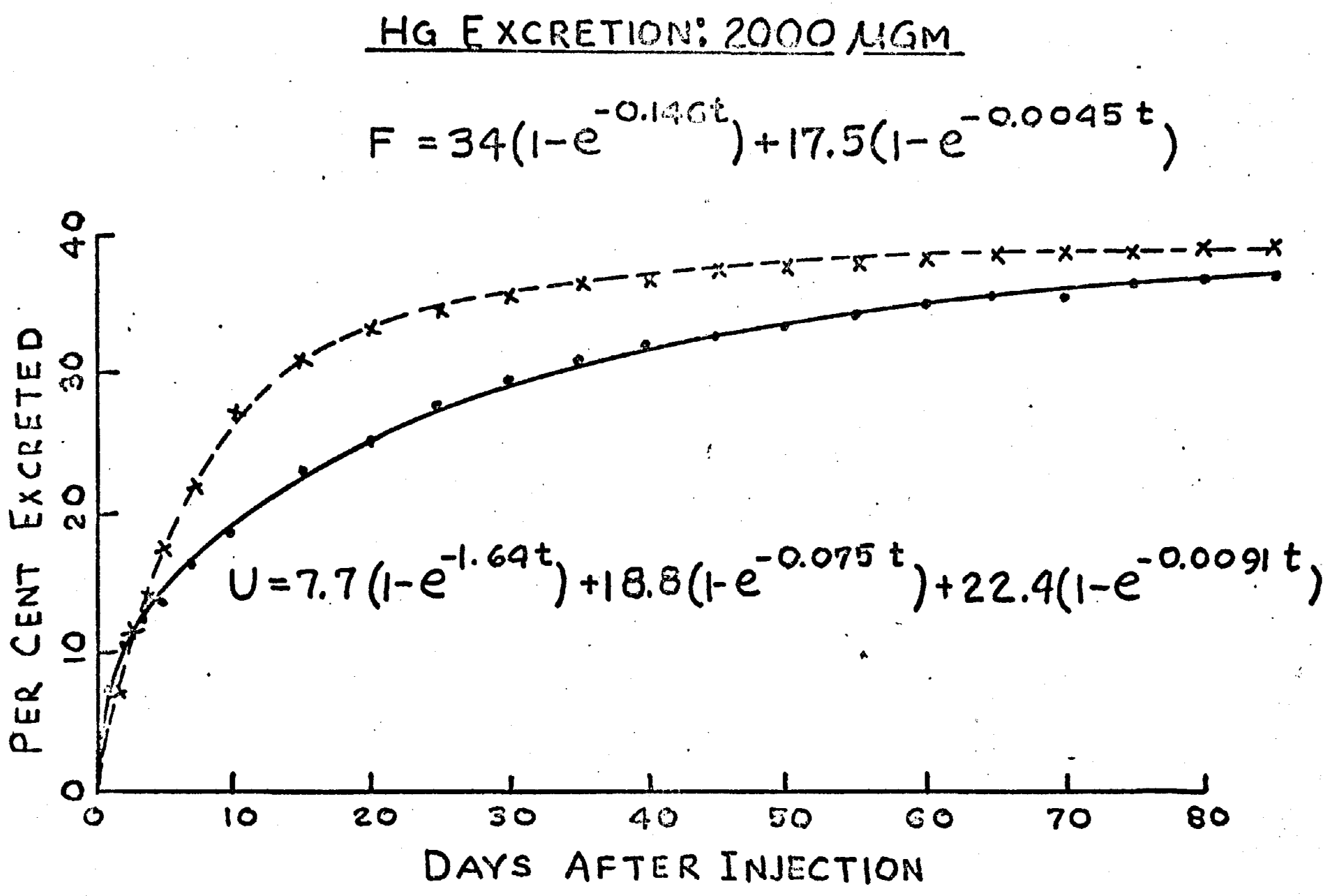

HG EXCRETION: 200 MGM
U = 41.3(1-e^-0.118t) + 27.6(1-e^-0.014t)
F = 20.8(1-e^-0.154t) + 10.3(1-e^-0.013t)
PER CENT EXCRETED
0
10
20
30
40
50
60
70
80
DAYS AFTER INJECTION

FIGURE 28


HG EXCRETION: 2000 µGM
F = 34(1-e^-0.146t) + 17.5(1-e^-0.0045t)
U = 7.7(1-e^-1.64t) + 18.8(1-e^-0.075t) + 22.4(1-e^-0.0091t)
PER CENT EXCRETED
0
10
20
30
40
0
10
20
30
40
50
60
70
80
DAYS AFTER INJECTION

previous studies, the highest dose level shows three component curves for the urinary clearance and for the whole body retention. These rabbit data, however, differ from the rat data. In rats we found an increasing fraction of mercury eliminated in the urine as the size of the dose increased. This was not observed in the case of the rabbits, where the fraction of injected mercury eliminated in the urine did not seem to be related to the size of the dose. The highest dose level, however, resulted in a three component urinary collection curve. The additional compartment being characterized by a very rapid clearance; the additional compartment could be explained either by assuming the additional mercury to be due to glomerular filtration of the excess mercury (either as a free ion or bound to a small, filterable molecule) and its elimination into the urine, or by assuming the mercury in the proximal convoluted tubules, after becoming concentrated due to resorption of water, to have been bound to the protein in the proximal tubules in acutely cytocidal amounts and immediately killing the cells. The dead cells are then sloughed off, carrying the bound mercury into the urine. We believe that second mechanism to be the one operative. Although no histological studies were done on the kidneys from these rabbits, examination of kidney sections from earlier studies, in which rats treated with high doses of mercury and which showed a similar very shortlived kidney component showed desquamation of the epithelium in the proximal convoluted tubules. It is reasonable to expect a similar phenomenon in the case of rabbits.

SUMMARY AND CONCLUSION

The results of these studies show the kinetics of mercury elimination to be influenced by the size of the dose. Whole body counting of rats following injection with either 10, 50, 100, or 500 μgm Hg as ^{203}Hg tagged $HgCl_2$ showed the mercury retention to be simulated by a three compartment model in which each compartment empties exponentially:

$$Y = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} .$$

For the 50, 100, and 500 μgm doses, the parameters A and α were found to be functionally related to the dose, d, by the equations

$$A = 108\ d^{-0.227}$$

$$\alpha = 0.0315 - (0.515/d).$$

The other parameters were not found to be functionally related to the dose.

Whole body counting of rabbits injected with either 20, 200, or 2000 μgm ^{203}Hg tagged $HgCl_2$ showed the percent of the injected dose remaining t days after injection to be given by the following equations

$$B(20) = 21e^{-0.17t} + 79e^{-0.045t}$$

$$B(200) = 44e^{-0.13t} + 56e^{-0.039t}$$

$$B(2000) = 8e^{-0.9t} + 62e^{-0.11t} + 30e^{-0.015t} ;$$

the two lower dose groups are seen to be rather similar, but the highest dose group is very different from the others.

In the case of the rabbits, the systematic dependence of mercury elimination in the feces and urine can be seen in the equations that relate the cumulative percent of the injected dose eliminated through each pathway with time after injection. The following equations for the feces,

$$F(20) = 27(1-e^{-0.14t}) + 14.5(1-e^{-0.023t})$$

$$F(200) = 20.8(1-e^{-0.159t}) + 10.3(1-e^{-0.013t})$$

$$F(2000) = 34(1-e^{-0.146t}) + 17.5(1-e^{-0.0045t})$$

all show two compartments which empty into the fecal reservoir, with the more rapidly clearing compartment containing about twice as much mercury as the slower clearing compartment. Furthermore, the clearance rate of this rapidly

clearing compartment is independent of the size of the dose; this compartment empties at a rate of about 15% per day, corresponding to a half life of about 4½ days. Clearance from the other compartment is highly dose dependent. The rate from the 20 μgm rabbits is 2.3% per day, corresponding to a half life of 30 days. For the 200 μgm treatment level, the clearance rate is about halved to 1.3% per day. Another increase of an order of magnitude in dose to 2000 μgm Hg leads to another reduction in clearance rate, somewhat more than a factor of two, to 0.45% per day.

The cumulative urinary mercury data were found to fit the following equations

$$U(20) = 29.3(1-e^{-0.077t}) + 29.3(1-e^{-0.027t})$$

$$U(200) = 41.3(1-e^{-0.115t}) + 27.6(1-e^{-0.014t})$$

$$U(2000) = 7.7(1-e^{-1.64t}) + 18.8(1-e^{-0.075t}) + 22.4(1-e^{-0.0091t})$$

The immediately apparent dose effect is the appearance of a third compartment that empties into the urinary reservoir in the case of the 2000 μgm dose, while the urinary mercury data from the two lower dose groups were found to fit two component curves. The first compartment of the two lower dose animals and the corresponding compartment of the 2000 μgm group (the second term in the equation) show about the same clearance rates, and thus, as in the case of the feces accumulation curves, seem to be independent of the dose. The last compartment, however, which empties more slowly, does seem to be dose dependent. As in the case of the feces, we see a reduction in clearance rate of about one-half (somewhat less than that for the 2000 μgm dose) for every ten-fold increase in dose.

The accumulation of mercury in the urinary and fecal reservoirs of the rats differs from that of the rabbits. The rats tend towards an increasing proportion of mercury in the urine and a decreasing proportion in the feces with increasing dose. This trend was not seen in the rabbits. Except for the lowest (10 μg) dose level to the rats, the fecal excretion data are very similar - no real dose effect is seen. The data imply that the fecal mercury comes from two compartments, one that empties at a rate of about 36% per day (this compartment is seen in the 10 μgm group, too), and a second compartment that empties at about 4½% per day. The urinary excretion, on the other hand does show a tendency towards a dose effect.

$$U(500) = 25.6(1-e^{-0.43t}) + 25.3(1-e^{-0.048t})$$

$$U(100) = 32.5(1-e^{-0.10t}) + 13.4(1-e^{-0.025t})$$

$$U(50) = 21.4(1-e^{-0.11t}) + 23.3(1-e^{-0.018t})$$

$$U(10) = 7(1-e^{-0.10t}) + 29.4(1-e^{-0.03t})$$

$$F(500) = 24.2(1-e^{-0.31t}) + 10.7(1-e^{-0.041t})$$

$$F(100) = 25.9(1-e^{-0.34t}) + 19.4(1-e^{-0.048t})$$

$$F(50) = 21.8(1-e^{-0.37t}) + 23.6(1-e^{-0.048t})$$

$$F(10) = 25.7(1-e^{-0.41t}) + 25.8(1-e^{-0.073t}) + 15.1(1-e^{-0.018t})$$

The curves from the four treatment levels are all two compartment exponential curves. In the three lowest dose levels, the clearance rates from the more rapidly emptying compartment are the same, 10 percent per day. Among the highest dose level, this compartment empties at a rate of 43 percent per day. This finding is consistant with our other findings of a very rapid initial clearance in high dose animals, presumable because of the cytotoxic effect of the mercury on the epithelial cells of the renal tubules. The slower emptying compartments, on the other hand, do show a dose effect. If we look at the clearance rates, we find 4.8, 2.5, and 1.8 percent per day respectively for the 500, 100, and 50 microgram rats. These parameters are quantitatively related for $50 \leq D \leq 500$, by the equation.

$$\log k = 0.426 \log D - 2.469,$$

where k is the clearance rate, in units of per day, and D is the dose in micrograms. The lowest dose level, 10 μg, did not follow the functionality.

Both groups of animals - the rats and rabbits, showed a systematic dependence of elimination rate on dose. However, the two groups of animals differed qualitatively in their response to differences in dose. In the case of the rats, the clearance rate of the long component increased with increasing dose, whereas in the case of the rabbits, the clearance rate of the longest lived component decreased with increasing dose. The reason for this difference is not immediately apparent. Anatomically, the two species are similar, except for the fact that the rabbit has a gall bladder, while the rat does not. How this difference affects the kinetics of clearance remains to be learned from further research into the detailed biochemical pathways of mercury within the animals and on the mechanism of elimination in the urine.