PLASMA REGRESSION, DISTRIBUTION AND EXCRETION OF RADIOMERCURY IN RELATION TO DIURESIS FOLLOWING THE INTRAVENOUS ADMINISTRATION OF Hg^{203} LABELLED CHLORMERODRIN TO THE DOG [1]

By R. R. M. BORGHGRAEF,[2] R. H. KESSLER,[3] and R. F. PITTS with the assistance of M. E. PARKS, W. VAN WOERT, and M. B. MacLEOD

(From The Department of Physiology, Cornell University Medical College, New York City, N. Y.)

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Radioactive mercury, administered intravenously in the form of an organic mercurial diuretic, Meralluride (Mercuhydrin®), leaves the plasma of man as a multiple exponential function of time. Threefoot, Ray, Burch, Cronvich, Milnor, Overman, and Gordon (1) demonstrated that no less than three major exponential rates are responsible for this regression of plasma mercury concentration. They postulated that the fastest rate represents mechanical mixing in the plasma compartment; that the intermediate rate represents rapid adsorptive, chemical and diffusion phenomena, including migration into tissues; and that the slowest rate represents mainly renal excretion.

Early in our work with mercurial diuretics labelled with radioactive mercury, we observed that Chlormerodrin (Neohydrin®), administered in a single intravenous dose, disappears from the plasma of the dog in a similar fashion. Indeed the time constants of the individual exponentials in the dog were comparable to those in man. Experiments described below were undertaken to test the postulates of Threefoot and his co-workers (1) concerning the nature of the processes involved in the clearing of mercury from the plasma and have served to verify and amplify their conclusions. Additional experiments describe the extraction, excretion and accumulation of mercury by the kidneys and relate these processes to the development of diuresis and to the disappearance of mercury from the plasma.

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[2] Fellow of the Belgian-American Educational Foundation.

[3] Fellow of the Life Insurance Medical Research Fund.

METHODS

Our experiments, some 34 in all, have been performed on female mongrel dogs lightly anesthetized with sodium pentobarbital. Arterial blood samples were drawn through an indwelling needle in the femoral artery. In certain experiments renal venous blood samples were drawn through a polyethylene catheter, introduced by way of the jugular vein and positioned in the right renal vein by manipulation through an abdominal incision. Urine samples were collected either through a Foley catheter, introduced into the bladder or through ureteral catheters, inserted by way of an abdominal incision.

The creatinine clearance has been employed as a measure of glomerular filtration rate and the creatinine clearance, divided by the plasma extraction ratio and corrected for urine flow (2, 3), has been employed as a measure of renal plasma flow. Chemical methods have been described in previous communications from this (4) and other (5) laboratories.

Chlormerodrin (3-chloromercuri-2-methoxy-propylurea, Neohydrin®) has been synthesized in our laboratory using radiomercury, Hg^{203}, according to directions supplied

TABLE I

An experiment on a normal dog illustrating the development of diuresis and the plasma regression, clearance and excretion of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin

ELAPSED TIME	URINE FLOW	CREATININE CLEARANCE	SODIUM			CHLORMERODRIN MERCURY			
			PLASMA	EXCRETED	REABSORBED	PLASMA	UV/P	C_{Hg}/C_{Cr}	EXCRETED
min.	ml./min.	ml./min.	mEq./L.	mEq./min.	% filtered	γgm./ml.	ml./min.		% dose administered
−90	Infuse 0.85% NaCl, 0.40% Creatinine at 5 ml./min., intravenously								
−30–15	1.53	46 7	152	0.33	95.1	—	—	—	—
−15–0	2.20	46.5	152	0.42	93.8	—	—	—	—
0	in 20 sec. 16.4 mg. (1.0 mg./kg.) Hg as Chlormerodrin, intravenously								
0–5	2 60	47 5	151	0.51	92.6	10.9	0.8	0.02	0.3
5–10	2.40	48.5	150	0.49	93 0	6.55	17.7	0.37	3.8
10–20	3 10	51 7	151	0 65	91.2	4.83	21.1	0.41	10.0
20–30	3 80	49.6	150	0 70	90.1	3.74	20.7	0.42	14.7
30–45	5.93	51.4	151	1 12	84.9	3.04	21.0	0.41	20.6
45–60	8.33	57 5	149	1.52	81.4	2.44	24.2	0.42	26.0
60–75	9.60	55 7	150	1 70	78.7	2.10	24.0	0.43	30.6
75–90	8.53	55.7	149	1.53	80.6	1.78	25.8	0.45	34.8
90–105	8.40	58.9	149	1.51	81 9	1.48	30.1	0.51	38.9
105–120	7.73	54.2	148	1.40	81.6	1.21	33.0	0.61	42.5
120–140	7.90	58.0	149	1.37	83.3	1.10	32.5	0.56	46.9
140–160	6.75	54.0	148	1 14	85.0	0.83	35.7	0.66	50.5
160–180	6.55	55.4	147	1.08	86.0	0.71	38.3	0.69	55.0

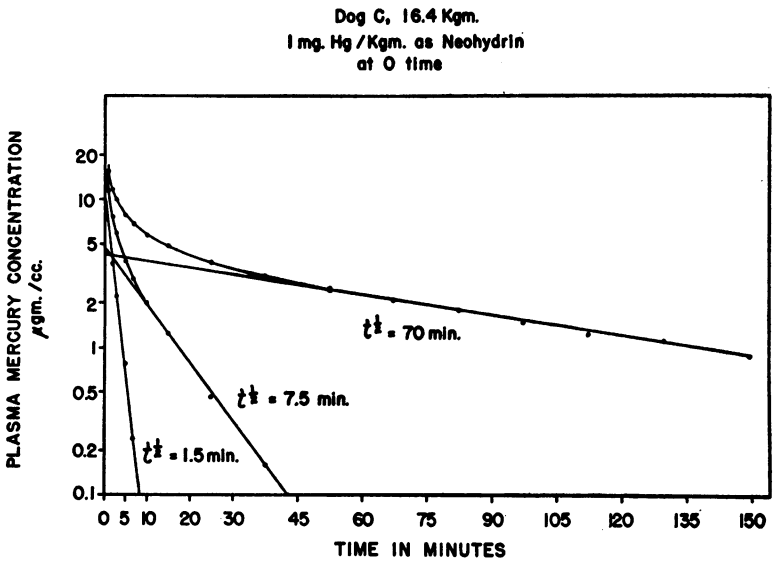

FIG. 1. AN ANALYSIS OF THE CURVE DESCRIBING THE REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN

The upper original or mother curve is a semilogarithmic plot of data obtained in the experiment presented in Table I. The three straight lines representing the dominant exponentials making up the mother curve were derived graphically as described in Footnote 6.

by Dr. H. L. Friedman of the Lakeside Laboratories, Inc. In all experiments, 1.0 mg. of mercury as Chlormerodrin per kg. of body weight has been administered intravenously into a foreleg vein in 20 seconds. This dose is equivalent to the human therapeutic dose of most mercurial diuretics. Plasma and urine samples were counted without preparation other than dilution, in a well-type scintillation counter; sufficient counts were accumulated to provide an accuracy of 1 to 5 per cent.[4] In experiments in which the uptake of mercury by the kidney was measured directly, the left kidney was exteriorized in the flank and a shielded Geiger tube applied directly to its surface, separated only by a single layer of Saran wrap. A sheet of dental dam, perforated to permit passage of the renal pedicle, served to hold the kidney in close approximation to the Geiger tube.

RESULTS

In Table I are summarized data obtained in a representative experiment in which 1.0 mg. of mercury, Hg^{203}, per kg. body weight was administered as Chlormerodrin. Following two 15-minute control periods, the diuretic was injected rapidly into a foreleg vein in 20 seconds. It is evident that the diuresis began rather slowly, either within the 10 to 20-minute period or perhaps the 20 to 30-minute period following the injection. It reached its peak in terms of urine flow, sodium excretion and depression of sodium reabsorption during the 60 to 75-minute period, although for the full three-hour duration of the experiment depression of salt and water reabsorption was clearly evident.

During the first five minutes after injection of Chlormerodrin, the excretion of mercury was negligible. Thereafter, the clearance [5] of mercury rose to a level some 40 to 60 per cent of filtration rate, and by the end of three hours, 55 per cent of the

[4] Activity of Chlormerodrin has ranged between 4,000 and 800 counts per minute per microgram of mercury when counted in a well-type scintillation counter.

[5] The term clearance has certain unique connotations when applied to mercurial diuretics. Chlormerodrin is excreted in the urine as a cysteine or acetyl-cysteine complex. The former is presumably formed in the kidney in the process of tubular transport; the latter is formed in the liver (6). In the plasma it is in large part bound to SH groups of proteins. Thus the form of the compound in plasma differs from that in urine, a fact which necessitates the broadest possible interpretation of clearance. Of even more import is the fact that large amounts of the diuretic are stored in the tubules in the course of renal excretion. An undetermined time lag is introduced between removal of the drug from the blood plasma and its delivery into the urine.

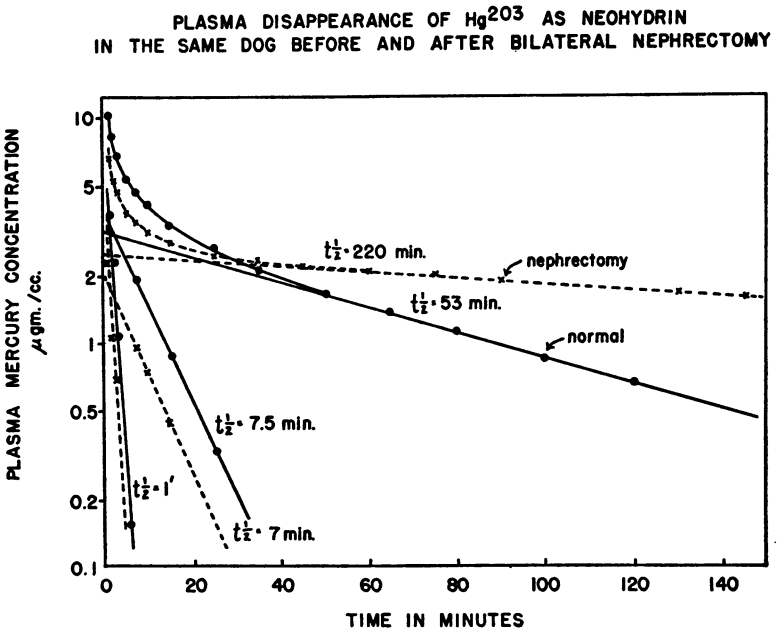

FIG. 2. A COMPARISON OF CURVES OF REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN IN THE SAME DOG IN A CONTROL EXPERIMENT AND FOLLOWING BILATERAL NEPHRECTOMY

dose administered had been excreted in the urine. Comparable clearances and rates of mercury excretion have been previously noted in man (7, 8) following the administration of Meralluride. Such rapid rates of excretion are the more surprising in view of the high degree of binding of mercurial diuretics to plasma protein (9, 10). At the lower plasma mercury concentrations, one per cent or less of Chlormerodrin is filterable, hence free to enter the urine in the glomerular filtrate. Obviously tubular secretion must play an important role in the urinary excretion of this agent.

Figure 1 continues the analysis of the data presented in Table I. In the upper curve, the logarithm of the plasma concentration of mercury has been plotted as a function of time. This curve has been graphically analyzed into its three exponential components by the method of successive subtraction.[6] The processes which determine these exponentials have half-times of 70, 7.5 and 1.5 minutes,[7] respectively, values comparable to those observed for the processes involved in the removal of Meralluride from the plasma of man.

Origin of the slowest exponential

As was mentioned above, Threefoot and his associates (1) postulated that the slowest exponential is dominated by renal excretion of the diuretic. If so, bilateral nephrectomy should markedly alter the later time course of the plasma concentration curve. In Figure 2, the upper two curves describe the disappearance of mercury

[6] The upper or so-called mother curve is described by an equation of the following form: $P = Ae^{-at} + Be^{-bt} + Ce^{-ct}$, in which P is the plasma concentration at any time, t; a, b, and c are the slopes of the three straight lines on semi-logarithmic coordinates; and A, B and C are the extrapolated intercepts of these straight lines on the X axis. To analyze the mother curve into its exponential components, the best straight line is fitted by inspection to the points from 50 to 150 minutes. The differences between the mother curve and this straight line, extrapolated to the X axis, are plotted as the first daughter curve. This curve analyzed in the same fashion yields the third straight line. In essence, this means that at any moment at least three separate and independent groups of processes are operating to clear mercury from the plasma. Each group operates at a rate that is proportional at any moment to the existing plasma mercury concentration.

[7] In 14 similar experiments the half-times of these 3 exponentials have ranged between 50 and 80, 6 and 9, and 0.5 and 1.5 minutes, respectively.

from the plasma in two experiments on the same dog; the first, a control experiment; the second, immediately following bilateral nephrectomy. The half-time of the slow exponential in the control experiment was 53 minutes; following bilateral nephrectomy, it was greatly prolonged, namely to 220 minutes. Although the slow exponential was markedly altered by nephrectomy, its slope did not approach zero. Accordingly extrarenal clearance mechanisms must remove mercury from the plasma at an appreciable rate; hence renal excretory mechanisms do not alone determine the later time course of plasma clearance. Graphic analysis indicated that the other two exponentials were not appreciably modified by nephrectomy. Similar findings were noted in two additional pairs of experiments performed prior to and after bilateral nephrectomy.

Origin of the intermediate exponential

According to Threefoot and his associates (1), migration of mercury from the vascular compartment into the tissues is the major determinant of the intermediate exponential. Several lines of evidence indicate that BAL, dithiopropanol, increases the diffusibility of Chlormerodrin. Administration of BAL plus Chlormerodrin increases the mercury content of all tissues other than the kidney (11). Chlormerodrin enters red cells but slightly. Addition of BAL to whole blood containing Chlormerodrin causes rapid penetration of cells by mercury (12). Administration of BAL to an animal in which plasma Chlormerodrin has reached a plateau causes a sharp drop in plasma concentration (*cf.* Table III). It is evident that the half-time of the intermediate exponential of plasma regression of the BAL-Chlormerodrin complex should be significantly shorter than that for Chlormerodrin alone. In four experiments, one of which is summarized in Table III and Figure 3, this has been found to be true. However, since BAL has effects other than that on diffusibility, results of these experiments are more complex than one would wish.

In the experiment summarized in Table II and Figure 3, Chlormerodrin was complexed with dithiopropanol in saline in a 1 : 4 molar ratio, neutralized to phenol red and immediately injected intravenously. A dose of 2 ml. of 10 per cent BAL in oil was given intramuscularly 30 minutes before the start of the experiment. Excess BAL was deemed necessary because of its known rapid rate of oxidation.

It is evident from Table II that no true diuresis resulted from administration of the BAL-Chlor-

TABLE II

An experiment on a BAL treated dog illustrating the plasma regression, clearance and excretion of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as the Chlormerodrin-BAL complex

ELAPSED TIME	URINE FLOW	CREATININE CLEARANCE	SODIUM			CHLORMERODRIN MERCURY			
			PLASMA	EXCRETED	REABSORBED	PLASMA	UV/P	C_{Hg}/C_{Cr}	EXCRETED
min.	ml./min.	ml./min.	mEq./L.	mEq./min.	% filtered	μgm./ml.	ml./min.		% dose
−150	Infuse 0.85% NaCl, 0.40% Creatinine at 5 ml./min., intravenously								
−60	2 ml. 10% BAL in oil, intramuscularly								
−30–15	2.27	51.2	144	0.15	98.0	—	—	—	—
−15–0	3.00	54.3	144	0.20	97.5	—	—	—	—
0	in 20 sec. 14.0 mg. (1.0 mg./kg.) Hg as Chlormerodrin-BAL complex, I.v.								
0–5	3.00	50.8	143	0.20	97.5	0.85	89.0	1.75	2.7
5–10	2.43	55.9	144	0.18	97.8	0.48	136.0	2.44	6.5
10–20	3.00	55.5	143	0.24	97.0	0.42	78.7	1.42	8.4
20–30	3.30	54.8	143	0.25	96.8	0.40	73.5	1.34	10.6
30–45	4.10	54.4	143	0.26	96.8	0.35	58.5	1.08	12.8
45–60	4.40	54.9	145	0.24	97.0	0.46	32.8	0.60	14.4
60–75	4.60	52.3	145	0.22	97.1	0.38	28.7	0.55	15.6
75–90	5.47	51.9	144	0.23	97.0	0.34	30.5	0.59	16.8
90–105	6.40	50.3	144	0.25	96.6	0.27	33.5	0.67	17.7
105–120	6.60	50.5	145	0.25	96.8	0.35	25.6	0.51	18.7
120–135	7.12	51.8	147	0.26	96.7	0.28	32.9	0.62	19.7
135–150	6.97	51.2	148	0.32	96.1	0.30	30.0	0.59	20.6

TABLE III

An experiment on a unilaterally nephrectomized dog illustrating the development of diuresis in relation to plasma regression, excretion and renal extraction and accumulation of mercury following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin

ELAPSED TIME	URINE FLOW	GLOMERULAR FILTRATION RATE	RENAL PLASMA FLOW	CHLORMERODRIN MERCURY				
				ARTERIAL PLASMA	VENOUS PLASMA	EXTRACTION	EXCRETION	ACCUMULATION	UV/P
min.	ml./min.	ml./min.	ml./min.	μgm./ml.		μgm. cumulative			ml./min.
−150	Infuse 0.85% NaCl, 0.4% Creatinine at 5 ml./min., intravenously								
−30–15	1.7	26.1	111	—	—	—	—	—	—
−15–0	2.0	27.3	101	—	—	—	—	—	—
0	in 20 sec. 13.0 mg. (1.0 mg./kg.) Hg as Chlormerodrin, intravenously								
1	—	—	—	11.05	8.65	279	—	—	—
2	—	—	—	7.98	5.80	529	—	—	—
3	—	—	—	6.52	4.45	764	—	—	—
4	—	—	—	5.83	4.13	958	—	—	—
0–5	2.0	26.5	109	5.15	3.82	1112	230	892	6.4
5–10	2.2	22.9	90	4.60	3.29	1737	498	1239	11.7
10–20	5.35	30.3	145	3.30	2.59	2877	980	1897	14.6
20–30	5.50	31.0	180	2.73	2.23	3897	1319	2578	12.4
30–45	5.67	26.8	149	2.11	1.70	4959	1774	3185	14.3
45–60	6.00	24.8	105	1.70	1.32	5725	2187	3538	16.2
60–77	6.06	25.1	110	1.36	1.12	6273	2609	3664	18.2
77–90	5.85	24.9	108	1.175	0.965	6642	2917	3725	20.2
90–105	4.60	24.9	96	0.992	0.733	7067	3245	3822	22.1
105–120	3.80	22.6	75	0.869	0.604	7400	3517	3883	21.0
120	2 ml. 10% BAL in oil, intramuscularly								
120–135	1.33	13.7	37.2	0.176	0.290	7342	4025	3317	192.0
135–150	0.20	19.6	62.8	0.175	0.398	7144	5395	1749	522.0

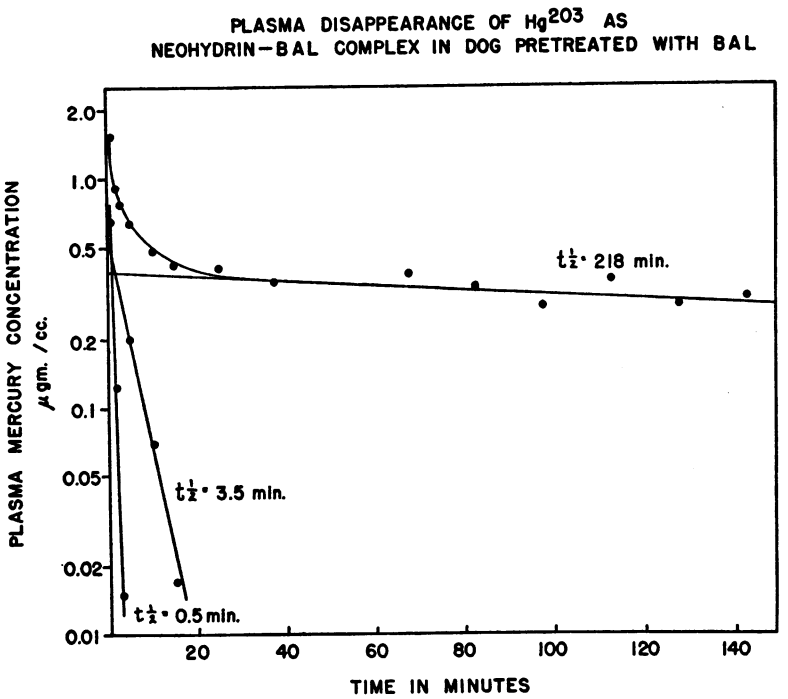

FIG. 3. AN ANALYSIS OF THE CURVE DESCRIBING THE REGRESSION OF PLASMA CONCENTRATION FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS THE CHLORMERODRIN-BAL COMPLEX TO A BAL TREATED DOG

merodrin complex in a BAL treated dog (13, 14). Since saline was administered intravenously at a rate of 5 ml. per minute for the duration of the experiment as well as for 90 minutes preceding the start of urine collection, the eventual increase of urine flow to 6 and 7 ml. per minute was not unexpected. However, not less than 96 per cent of the filtered sodium was reabsorbed during any period, a value incompatible with significant mercurial diuresis.

The clearance of mercury was very high during the first five minutes following the administration of the BAL-Chlormerodrin complex, *i.e.*, 89 ml. per minute in contrast to 0.8 ml. per minute in the experiment presented in Table I. For the first 30 minutes the clearance remained considerably higher than in the control experiment. However, except for the first 10 minutes, the absolute rate of excretion and hence the per cent of the dose excreted was lower in the BAL than in the control experiment. This ambiguity of high clearance, yet low rate of excretion finds explanation in the extremely low plasma concentrations of mercury in the BAL experiment. It is evident from these findings, namely slow excretion (only 20.6 per cent of the dose in 150 minutes) and low plasma concentration (roughly 1/10th of the values obtained in the initial periods of the experiment in Table I), that the half-time of the slow exponential must be increased, whereas that of either the intermediate or fast exponential or both must be reduced.

In Figure 3 is presented a graphic analysis of the experiment presented in Table II. It is evident that the slow exponential had a time course of the same order of magnitude as is observed after bilateral nephrectomy, a reflection of the very low rate of excretion of the BAL complex. In contrast, the intermediate exponential was definitely shortened, a function, we assume, of increased diffusibility. In this and in the 3 other experiments of a similar nature, the half-times of the intermediate exponentials have been reduced by roughly 50 per cent. In contrast the fast exponentials did not differ significantly from those observed in certain experiments with Chlormero-

drin alone. However, the graphic construction of this fast exponential is a bit uncertain at best and rather more uncertain following BAL, because of low and variable plasma mercury concentration.

Origin of the fast exponential

The fast exponential has been interpreted by Threefoot and his associates (1) as due to the mechanical mixing of the diuretic in the circulating plasma volume. The initial rapid decline of plasma concentration of Evans Blue has been interpreted in similar fashion by a number of investigators (15–17). We have made a series of simultaneous comparisons of the disappearance of Evans Blue and either Chlormerodrin (2 experiments) or the Chlormerodrin-BAL complex (4 experiments). An example of one of these latter experiments is summarized in Figure 4.

On the right are shown the simultaneously measured plasma concentration curves for Evans Blue and for the Chlormerodrin-BAL complex, and on the left, their fast exponential components. The range of uncertainty in determining half-times of these fast processes is large, ranging from 0.5 to 1.5 minutes. However, in this series fair agreement has been observed, a fact favoring the mixing hypothesis.

Renal extraction, excretion, and accumulation of Chlormerodrin

According to Greif, Sullivan, Jacobs, and Pitts (18), Chlormerodrin is accumulated in highest concentration in the cortex of the kidney of the dog, in lesser amounts in the outer medulla and scarcely at all in the papillary medulla. As a first approximation, it is reasonable to assume that the drug exerts its characteristic diuretic action where it is specifically and most highly concentrated.[8] Accordingly we have attempted to correlate diuresis with the accumulation of radiomercury in the renal cortex, measured qualitatively in terms of counts per minute by a Geiger tube in contact with the kidney surface. Because of the relatively low energy of the beta emission of Hg^{203} (0.205 MEV), beta activity measured at the surface is derived almost exclusively from the outermost few millimeters of the cortex. To some extent, of course, the Geiger tube responds to the gamma emission from the entire kidney, although its efficiency is very low.

In Figure 5 are summarized data obtained in an experiment in which at 30 minutes, 1.0 mg. of mercury per kg. was administered intravenously as Chlormerodrin and at 150 minutes, 2.0 ml. of 10 per cent BAL in oil were given intramuscularly. The upper curve describes the plasma concentration of radiomercury, plotted semilogarithmically as a function of time. Note the sharp drop which occurred immediately following the administration of BAL, an indication of increased diffusibility and rapid exit of the diuretic from the plasma. In the second curve, the urinary excretion of mercury is plotted cumulatively in terms of per cent of the dose administered. It is evident that rate of excretion was highest immediately following injection of the diuretic, diminished gradually as the plasma concentration fell and increased sharply following BAL. The third curve is that of radioactivity recorded at the surface of the kidney and represents qualitatively the accumulation of mercury in the outermost layers of the cortex. It is evident that accumulation was most rapid during the first 10 to 20 minutes following the diuretic, *i.e.*, during the time that the plasma concentration was highest and also falling most rapidly. Thereafter, accumulation proceeded more slowly, approaching a plateau of 2250 to

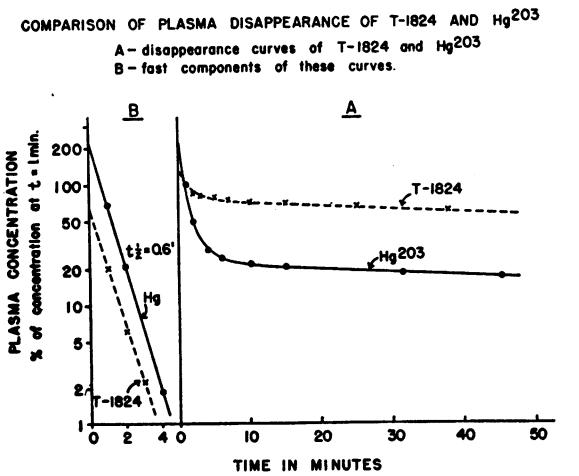

FIG. 4. A SIMULTANEOUS COMPARISON OF THE FAST COMPONENTS OF THE PLASMA REGRESSION CURVES FOR EVANS BLUE AND CHLORMERODRIN-BAL COMPLEX

[8] It is possible that accumulation in the renal cortex is concerned with renal tubular secretion, that secretion is unrelated to diuresis, and that diuresis results from a lesser accumulation in another part of the kidney.

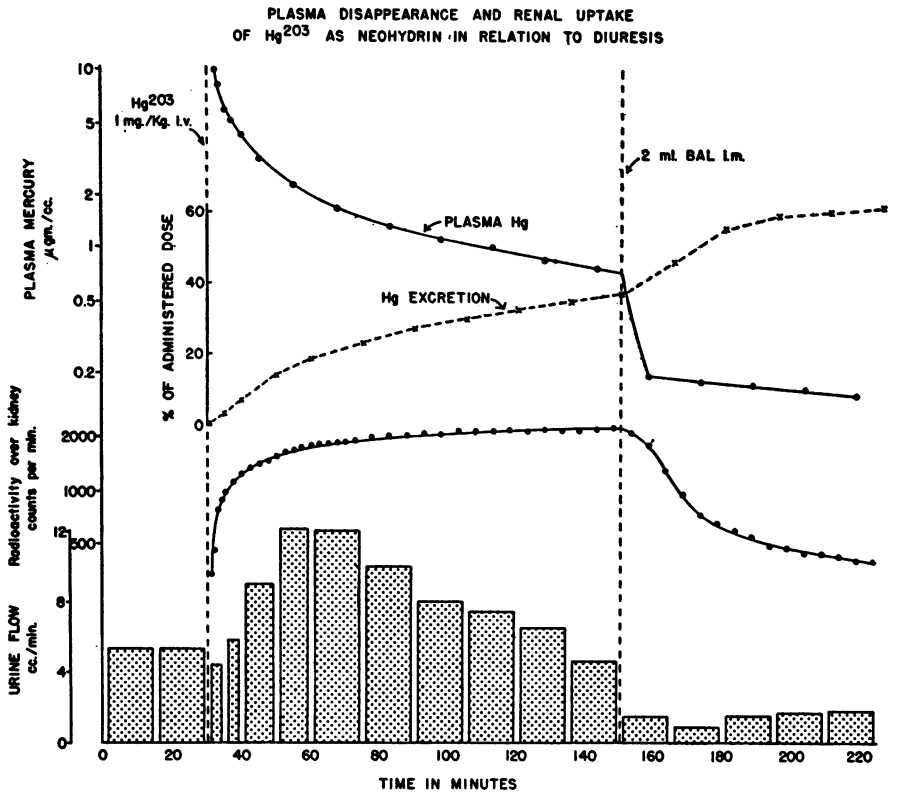

FIG. 5. AN EXPERIMENT ILLUSTRATING REGRESSION OF PLASMA CONCENTRATION OF MERCURY, EXCRETION OF MERCURY, ACCUMULATION OF MERCURY IN THE KIDNEY AND DIURESIS IN A DOG GIVEN 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN INTRAVENOUSLY

2300 counts per minute after an hour and a half. When BAL was given, the renal mercury was promptly discharged, in major part into the urine as shown by the excretion curve, in small part into the renal venous blood, as demonstrated in subsequent experiments. Urine flow, as shown at the bottom of the figure, dropped slightly in the first five minutes after the intravenous injection of Chlormerodrin, rose to a peak in 20 to 40 minutes and then diminished slowly over the subsequent hour and a half. With the administration of BAL, urine flow dropped sharply. It is evident that the onset of diuresis correlates well with the rapid uptake of mercury by the kidney during the first 30 minutes after the injection of the diuretic. The sharp drop in urine flow after BAL likewise correlates reasonably well with the loss of accumulated mercury, although it is known that increased secretion of Pitressin® contributes to the antidiuresis (19). However, it is evident that urine flow was not maintained at the peak diuretic value despite maintenance of a high level of accumulated mercury in the renal cortex. How well the diuresis is sustained depends in part on the magnitude of positive fluid and salt balance developed prior to administering the diuretic.

The accumulation of mercury by the kidney has been further analyzed in the manner shown in Figure 6. As is evident in the upper curve of Figure 6A, radioactivity, recorded over the kidney, increased rapidly for an hour or so after injection and then reached a plateau of roughly 2275 counts per minute, a fact apparent in Figure 5. In the upper curve of Figure 6B, the differences between this plateau and the observed counts are plotted semilogarithmically as a function of time. This curve, which expresses the rate at which the plateau is approached, may be analyzed graphically into two exponentials with half-times of 16.0 and 1.3 minutes, respectively. The two components of

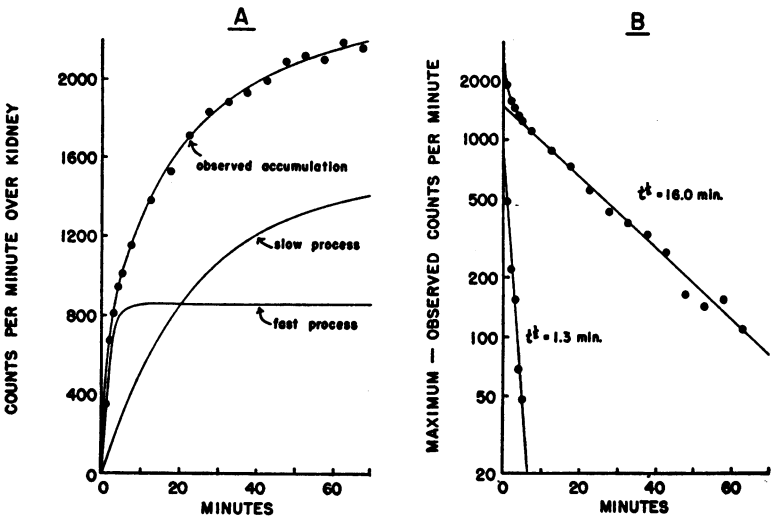

FIG. 6. AN ANALYSIS OF THE UPTAKE OF MERCURY BY THE KIDNEY IN THE EXPERIMENT SUMMARIZED IN FIGURE 5

In the upper curve on the left, the observed rate of accumulation of mercury is plotted as a function of time on an arithmetic scale. Assuming a maximum value of 2275 counts per minute (cf. Figure 5, 1.5 to 2 hours after injection), the difference between this asymptote and the observed rate is plotted logarithmically as the upper curve on the right. This may be analyzed into two exponentials having half-times of 1.3 and 16 minutes, respectively. These constitute the fast and slow processes of accumulation shown on the left, which summate to give the observed accumulation.

accumulation, one saturating rapidly, the other slowly, are shown as the lower two curves of Figure 6A. One might interpret these two exponentials in terms of two systems or mechanisms of binding. On the other hand only one mechanism might be operative and the two exponentials result from two components of plasma regression.

Renal extraction, excretion and accumulation of mercury were measured in absolute units in two dogs following the intravenous administration of 1.0 mg. of mercury per kg. as Chlormerodrin. These experiments are very similar in design to those of Weston and his co-workers (20) in man and may be considered as basically confirming their findings. One of the experiments, in which the animal was unilaterally nephrectomized immediately preceding the experiment, is summarized in Table III and Figure 7. Renal venous blood was obtained through a polyethylene catheter introduced through a jugular vein and positioned in the renal vein by manipulation through an abdominal incision. Extraction, excretion and accumulation are presented cumulatively in terms of micrograms of mercury. Renal plasma flow was calculated from rate of excretion and arterio-venous difference for creatinine, corrected for urine flow (2, 3). Extraction and accumulation of mercury by the kidney have been calculated as follows:

Renal Extraction (μgm./min.) = R.P.F.$\cdot$($A_{Hg} - R_{Hg}$) + V$\cdot R_{Hg}$.

Renal Accumulation (μgm./min.) = Renal extraction $- U_{Hg}\cdot V$.

R.P.F. = Renal plasma flow in ml./min.

A_{Hg} = Arterial plasma mercury concentration in μgm./ml.

R_{Hg} = Renal venous plasma mercury concentration in μgm./ml.

U_{Hg} = Urinary mercury concentration in μgm./ml.

V = Urine flow in ml./min.

It is evident from Table III and Figure 7 that mercury was much more rapidly extracted from the plasma than excreted during the interval immediately following injection of Chlormerodrin,

i.e., for the first 30 to 60 minutes. Hence the quantity of mercury accumulated in the kidney increased rapidly, a finding in the dog much like that of Weston and his associates in man (20). From 60 to 120 minutes, extraction and accumulation tended to reach a plateau. Following the administration of BAL, extraction became slightly negative, *i.e.*, the concentration of mercury was higher in the venous than in the arterial blood. However, the marked decrease in renal accumulation was due mainly to increased excretion and only in small part to negative extraction. It is interesting to note that the clearance of mercury in the two periods after BAL was far in excess of the renal plasma flow, a result explainable only in terms of the discharge of mercury accumulated in tubular cells into the urine. The quantity stored in the kidney decreased by more than 50 per cent in the course of half an hour.

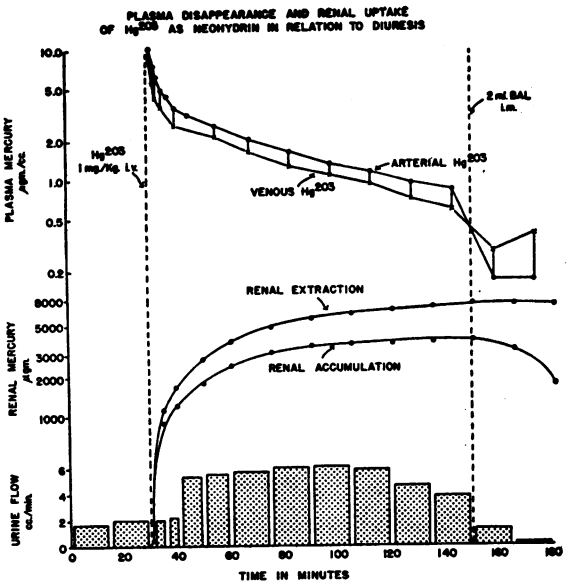

FIG. 7. AN EXPERIMENT ILLUSTRATING REGRESSION OF PLASMA CONCENTRATION, RENAL EXTRACTION AND RENAL ACCUMULATION OF MERCURY IN RELATION TO DIURESIS IN A UNILATERALLY NEPHRECTOMIZED DOG GIVEN 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN INTRAVENOUSLY

Data from the experiment presented in Table III.

DISCUSSION

Three major exponential rates determine the regression of plasma mercury concentration following the intravenous injection of Chlormerodrin in the dog. Similar observations were made following Meralluride in man (1). Each of these major rates is no doubt the resultant of the activity of a number of independent processes and only in a limited sense can one consider that any one process is the major determinant of any one rate.

The fast exponential with a half-time of 0.5 to 1.5 minutes would seem most clearly dominated by the physical process of mixing within the plasma compartment. The fact that we have observed good agreement between these components of the regression curves of Evans Blue and Chlormerodrin argues in favor of this hypothesis.

The intermediate exponential with a half-time of 6 to 9 minutes is obviously of complex origin. No doubt, as Threefoot and his co-workers (1) postulated, it represents diffusion of mercury into tissues and binding to tissue proteins. The kidney cortex binds mercury in highest concentration, namely 100 to 150 μgm. per gm.; liver and spleen bind the metal in lower concentration but in appreciable total amounts; skeletal muscle binds but little (11). Thus, penetration of a variety of tissues, perfused at different rates and exhibiting differing affinities for the heavy metal summate to yield the intermediate exponential of the plasma regression curve. BAL, which greatly increases the diffusibility of Chlormerodrin and increases the mercury content of all tissues and organs except the kidney, reduces the half-time of this intermediate exponential.

The slow exponential with a half-time of 50 to 80 minutes represents, in large part, activity of renal excretory mechanisms. This is most clearly shown by the fact that bilateral nephrectomy markedly decreases the rate of fall of plasma mercury from 30 minutes on. However, following nephrectomy, the slope of this portion of the plasma regression curve does not become zero. We assume that excretion into the gut and continuing penetration of tissues account for the residual slope of the slow component of the plasma regression curve.

The distribution of mercury in the body and its excretion in the urine following the intravenous administration of 1.0 mg. Hg per kg. as Chlormerodrin are summarized in Figure 8. The data upon which the graph is based were derived from the experiment presented in Table III and Figure 7. The per cent of the dose in the plasma compart-

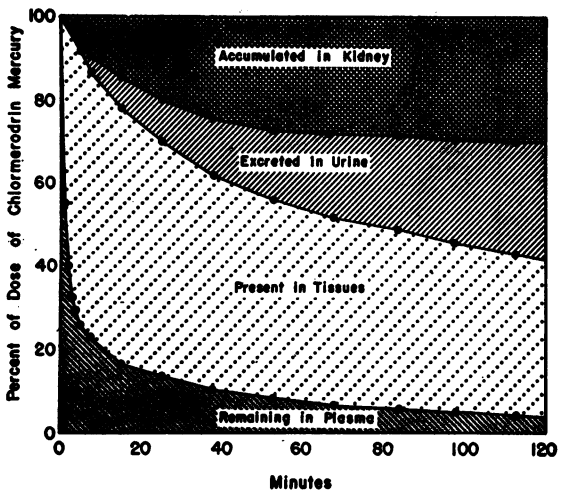

FIG. 8. SUMMARY OF THE DISTRIBUTION OF MERCURY IN THE BODY AS A FUNCTION OF TIME FOLLOWING THE INTRAVENOUS ADMINISTRATION OF 1.0 MG. OF MERCURY PER KG. AS CHLORMERODRIN

Data from the experiment presented in Table III and Figure 7.

ment was calculated as the product of the plasma concentration and the plasma volume, the latter estimated as 5 per cent of body weight. The per cent of the dose accumulated in the kidney and the per cent excreted in the urine were calculated as previously described. Since the animal was unilaterally nephrectomized, both excreted and accumulated moieties are no doubt less than would be found in the intact animal. The per cent taken up by the tissues was the difference between 100 and the sum of the three values for plasma content, kidney accumulation and excretion. This graph is rather inaccurate during the first few minutes but is reasonably valid thereafter. It resembles very closely one presented by Milnor, Burch, Ray, Threefoot, and Berenson (21), describing the distribution of Meralluride in man. Its significant features are the following: the mercury, initially delivered into the plasma, is rapidly distributed into the tissues, which thereafter serve as a reservoir to replenish the plasma store as renal accumulation and urinary excretion account for a progressively increasing fraction of the total.

In the past some have speculated that the mercury which accumulates in the tubular cells and serves to inhibit reabsorption, is that which has entered the glomerular filtrate. That this view is incorrect is evident from the data of Table III. The extraction of mercury from the renal arterial plasma averaged 22 per cent in this experiment. Since the filtration fraction averaged 0.23 and the filtrable moiety ranged between 1 and 5 per cent, it is obvious that not more than 1.5 per cent of the mercury delivered to the kidney in the arterial blood could have found its way into the filtrate. Therefore most and probably essentially all of the mercury must have entered the tubular cells from the peritubular fluid.

Although binding by plasma proteins restricts the entry of Chlormerodrin into the glomerular filtrate, it does not equally restrict distribution into tissues. A minute fraction of the compound is free and in equilibrium with the bound moiety. If tissue proteins, *e.g.*, renal tubular cells, have a higher affinity for Chlormerodrin than do the plasma proteins, the drug can be rapidly transferred from plasma to kidney, dissociating from plasma as taken up by kidney. The kinetics of this process in a variety of tissues, determines the time course of the intermediate exponential.

Tubular secretion of Chlormerodrin involves at least two processes, binding by and concentration in renal tubular cells and combination with some sulfhydril containing substance. According to Müller (6), essentially all of the excreted diuretic is eliminated in combination with either cysteine or acetyl cysteine. Binding might well be a *passive* process, depending on high affinity of cells of the renal cortex for the free valence of mercury in the mercurial. The *active* step in tubular secretion could then be assigned to the splitting of the cell complex and to the formation of the cysteine derivative. Transfer into tubular urine might depend on diffusion forces. This view is suggested by the observation that the administration of the sulfhydril compound, BAL, causes the rapid discharge of Chlormerodrin from the kidney, for the most part into the urine, but also into the renal venous blood. This last observation could mean that diffusion plays a role.

The action of Chlormerodrin as a diuretic must be related to its specific accumulation within renal tubular cells where it serves to block reversibly certain enzyme systems concerned with the reabsorption of salt. Delay in onset of diuresis following intravenous administration is no doubt re-

lated to the time required to accumulate some critical concentration of drug in the renal cortex. Cessation of diuretic activity is of course ultimately associated with complete elimination of drug in the urine. However, a maintained high renal concentration of the diuretic does not ensure a maintained high output of urinary salt and water. Magnitude of diuretic response depends in addition upon the volume of extracellular fluid, its ionic pattern and concentration, the adequacy of glomerular filtration and the degree of stimulation of tubular reabsorption (22).

SUMMARY

Following the intravenous injection in the dog of 1.0 mg. per kg. of Hg^{203} as Chlormerodrin, plasma concentration regresses as a multiple exponential function of time. Three exponentials have been identified with half-times of 0.5 to 1.5, 6 to 9 and 50 to 80 minutes, respectively. The first of these exponentials agrees reasonably well with that describing the initial regression of the plasma concentration of Evans Blue when the dye and diuretic are administered simultaneously. Thus, it must be dependent to a considerable degree on the physical process of mixing within the circulating volume of plasma. The second exponential represents an average of the rates of transfer of mercury from the plasma to a variety of tissues and organs perfused at varying rates and exhibiting different affinities for the diuretic. The administration of Chlormerodrin complexed with BAL greatly increases diffusibility of the mercury and its uptake by all tissues other than the kidney. Uptake by the kidney is reduced. The net effect of BAL is to speed significantly this second component of regression of plasma concentration. The third exponential is dominated by renal excretion as has been clearly demonstrated by bilateral nephrectomy. However, it represents to an appreciable degree extrarenal clearance, including gastro-intestinal secretion and a slow component of penetration of tissues.

Chlormerodrin is rapidly taken up by the renal cortex, fixed within the tubular cells, and excreted into the urine. Delay in the onset of diuresis following intravenous administration is related to the time required to accumulate a critical concentration of the drug within the tubular cells. Due to high plasma binding, most of the mercury excreted in the urine is eliminated by tubular secretion.

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