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THE MECHANISM OF ACTION OF MERCURIAL DIURETICS IN RATS; THE METABOLISM OF ^{203}Hg-LABELLED CHLORMERODRIN

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The mechanism by which mercurial diuretics produce diuresis has been the subject of continuing controversy. On the one hand it has been proposed that the diuretic acts as the intact molecule producing diuresis by a " lock and key " type interaction with the renal receptor sites (Kessler, Lozano & Pitts, 1957). In this mechanism considerable importance is attached to the structure of the organic moiety of the organic mercurial compound. On the other hand, it has been proposed that mercuric ion, liberated from the organic compound in the kidney, is the primary diuretic agent (Mudge & Weiner, 1958). Specifically, Weiner, Levy & Mudge (1962) from studies of over thirty different compounds of mercury have found that an organic mercurial will produce diuresis provided that the mercurial compound releases mercuric ion to a sulphydryl compound in acid solutions in the test-tube and that the mercurial is rapidly excreted by the kidneys. To explain these findings, it was proposed that a mercurial diuretic must first be excreted in such a way as to arrive in the vicinity of the diuretic receptor site and that in this area the carbon-mercury bond is broken, allowing mercuric ion to combine with the receptor site. Clearly a corollary of this theory is that mercuric ion must be present in kidney tissue during diuresis. As pointed out by others (Miller & Farah, 1962), this corollary has never been tested experimentally. Currently available methods of mercury analysis, in tissues, do not distinguish between the different chemical forms of the element.

This report describes a method capable of distinguishing between intact molecules of a mercurial diuretic and mercuric ion, in animal tissues, and of accurately measuring the amounts of each. The method has been applied to determine the amount of mercuric ion present in the kidneys and livers of experimental animals at various times after the administration of therapeutic doses of a mercurial diuretic.

METHODS

The method depends upon two properties of mercury; first, the speed with which isotopically labelled mercury exchanges with atoms of metallic mercury depends upon the state of chemical combination of the isotope (for a general review, see Basalo & Pearson, 1958) and, second, after the isotope has been converted to metallic mercury, it becomes volatile and can therefore pass from one liquid phase through a vapour phase to another liquid phase (Clarkson, Gatzy & Dalton, 1961). Thus in principle the chemical state of mercury

can be determined from its volatility in the presence of mercury vapour. Differences in volatility can be measured with a Conway microdiffusion unit (Conway, 1947).

The procedure was as follows: 1 ml. of a solution of the compound of mercury labelled with the γ-emitting isotope ^{203}Hg was placed in the outer vessel of a Conway No. 2 unit and 0.1 ml. of metallic mercury, triple-distilled, was added to the central well. To facilitate its removal from the unit, the metallic mercury was placed in a small cylindrical plastic vessel (polymethylmethacrylate) that could be easily placed in and removed from the central well. The lid was sealed to the diffusion unit with silicone stopcock grease, and the unit was allowed to stand at constant temperature (38° C). At intervals the metallic mercury was replaced with a fresh sample and its radioactivity was measured in a well-shaped NaI scintillation detector (Detector N664B and Scaler N610A, Ekco Electronics Ltd., Southend-on-Sea, Essex), having a counting efficiency for ^{203}Hg of 40% with a background of 160 counts/min. The activity remaining in the outer vessel of the diffusion cell was also counted after each run.

Correction factors had to be applied to counts made on the mercury droplets and on the activity remaining in the diffusion cell, because of self absorption and geometry. It was then possible to compare the radioactivity in the metallic phase and that remaining in the unit with the activity added at the start of the experiment. The recoveries of ^{203}Hg from the diffusion units were found to be 100% within statistical counting error.

Fig. 1 compares the rates of diffusion of ^{203}Hg from solutions of different compounds of mercury. The kinetics of the process are first order, the logarithm of the remaining diffusible activity giving a linear plot

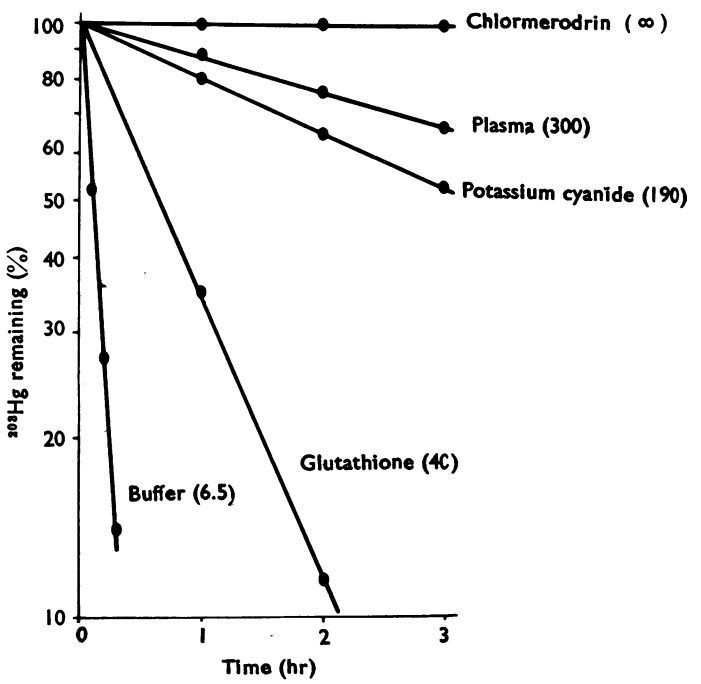

Fig. 1. The rates of diffusion of ^{203}Hg from solutions of different compounds. Mercuric chloride (10^{-5} M) labelled with ^{203}Hg was added to phosphate-buffer (*p*H 7.4; composition: $NaCl_2$ 137 mM, KCl 4.8 mM, Na_2HPO_4 2.5 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.2 mM and KH_2PO_4 0.6 mM) or to 10^{-3} M-reduced glutathione or 10^{-3} M-potassium cyanide in phosphate-buffer or added directly to heparinized human plasma. Chlormerodrin allowed no detectable diffusion of ^{203}Hg. Eventually all the radioactivity diffused into the metallic phase from the other compounds except from mercuric chloride where 90% diffused. The remaining 10% was bound to the glass of the diffusion unit, a common occurrence with such low concentrations of mercuric ion in solutions not containing strong complexing agents. Figures in parentheses give half-times in minutes.

against time. Mercuric ion, present as mercuric chloride or complexed to various organic ligands, for example thiol, cyanide and those groups present in plasma, allowed diffusion of ^{203}Hg with half-times ranging from 6.5 min (mercuric chloride) to 300 min (plasma). In contrast, ^{203}Hg was completely indiffusible when bound to the carbon atom in an organo-mercurial compound such as the diuretic chlormerodrin.

The mechanism must involve an exchange of the radioactive mercuric ion with an atom of metallic mercury since, when the diffusion unit did not contain any metallic mercury, the radioactive mercury did not volatilize from any of the compounds described in Fig. 1. That diffusion of ^{203}Hg from the aqueous solution into the metallic phase is exponentially related to time is to be expected. Two processes may be rate-limiting; either the rate of exchange of radioactive mercury with elemental mercury or the subsequent diffusion of the labelled mercury. The first process involves the movement of the isotope down its specific activity gradient and necessarily follows exponential or first order kinetics (Solomon, 1960). The construction of the microdiffusion unit is such that the diffusion process is also an exponential function of time (Conway, 1947).

Male Wistar rats, weights from 140 to 160 g, were injected intramuscularly with either mercuric ion complexed with cysteine (mercuric-cysteine) or with chlormerodrin, each labelled with ^{203}Hg. The chemical dosage was 2.5 mg of mercury per kg of body weight, and the radioactivity dosage of ^{203}Hg was approximately 1 μC per animal. The mercuric ion solution was prepared by dissolving mercuric acetate in 0.1 M-cysteine solution buffered by 0.1 M-sodium bicarbonate and 0.1 M-sodium carbonate solutions, such that 0.1 ml. of the final solution contained the chemical dosage for the rat. ^{203}Hg was added to the above solution as mercuric acetate solution, *p*H 2.8 and specific activity 1 mC/mg of mercury in 0.5 ml. of solution, as supplied by the Radiochemical Centre (Amersham). Chlormerodrin (Mercloran, Parke Davis & Co.) was dissolved in 0.1 N-sodium hydroxide solution to give the chemical dosage in 0.1 ml. Labelled chlormerodrin (Radiochemical Centre) was added to this solution, specific activity approximately 2 mC in 29 mg of mercury in 3.2 ml. of 0.9% saline. Cysteine concentrations were measured by the method of Ellman (1959).

At periods of time varying from 3 to 90 hr after receiving the mercury, the rats were killed by intraperitoneal injection of pentobarbitone sodium, the kidneys and livers were excised and homogenized in phosphate buffer, *p*H 7.4 at 2 to 4° C, using a ground-glass homogenizer. The homogenate was diluted in the same buffer to give a 5% suspension which was immediately frozen and stored overnight at −20° C. Samples of this suspension were used the following day for diffusional analysis, after a further dilution in buffer to give a final 1% homogenate.

The diuretic activity of chlormerodrin in rats was measured as follows: male Wistar rats weighing from 175 to 200 g were placed in six metabolism chambers (two rats per chamber) essentially similar to those described by Gage (1961). Food and water were given *ad libitum*. After 24 hr in the chamber, a urine and faecal separator and collector was positioned beneath the chamber (Gage, 1961) and 24-hr urine samples were collected for 3 days. The rats in three chambers (experimentals) were injected with chlormerodrin (2.5 mg of mercury per kg body weight) as described previously and the remaining rats (controls) were injected with a salt solution identical to that in the chlormerodrin injection solution. 24-hr urine samples were collected for the 9 days following injection.

RESULTS

Diffusion of ^{203}Hg from Robinson-Ringer phosphate suspensions

The diffusion of ^{203}Hg from a kidney homogenate from a rat injected with mercuric cysteine or chlormerodrin 18 hr before the removal of the kidneys is shown in Fig. 2. After 60 hr equilibration, approximately 96% of the radioactivity had diffused from the mercuric ion homogenate compared to only 50% from the chlormerodrin homogenate. The exact size of the diffusible component and its half-time of diffusion can be calculated from Fig. 3. The half-time for the homogenate containing mercuric ion was 13 hr; that for chlormerodrin was 14.5 hr.

The size of the diffusible component and its half-time are given in Fig. 4 for a series of rats killed at various times up to 100 hr after administration of mercuric cysteine or chlormerodrin. Following injection of mercuric cysteine, virtually all the ^{203}Hg was diffusible with

a half-time of approximately 13 hr. With chlormerodrin, the amount of diffusible ^{203}Hg increased with time until 40 hr after injection, and all of it was diffusible. The fact that the half-time of the diffusible component from chlormerodrin was identical to that of the mercuric ion strongly suggested that the mercurial was breaking down in the tissues to liberate mercuric ion.

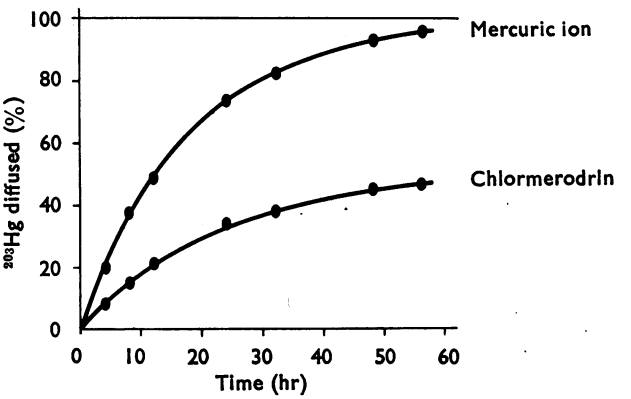

Fig. 2. The diffusion of ^{203}Hg from a 1% kidney homogenate from a rat injected with labelled mercuric-cysteine or with labelled chlormerodrin 18 hr before removal of the kidney. 96% of the original activity from the mercuric ion homogenate and 50% of activity of the chlormerodrin homogenate diffused into the metallic phase in 60 hr.

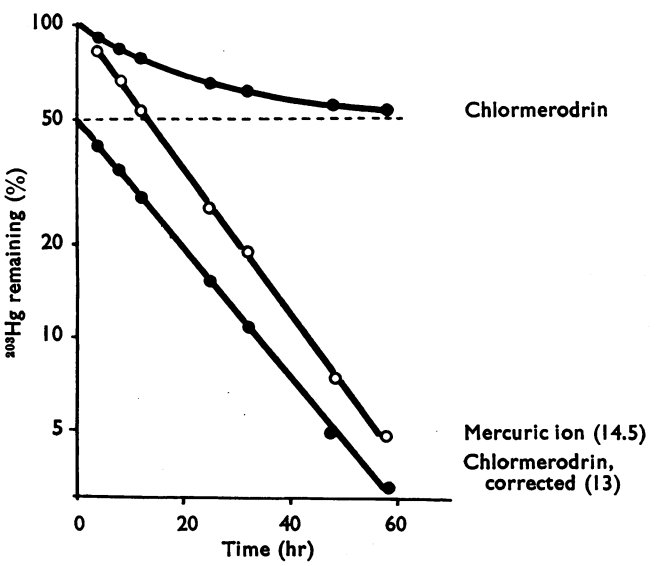

Fig. 3. The diffusion of ^{203}Hg from kidney homogenates (values from Fig. 2) plotted as the log of the percentage remaining activity against time. The corrected chlormerodrin line is the original curve minus the asymptote (interrupted line). The exact value of the latter is determined by successive approximates since only the correct value will give a straight line. Figures in parentheses give the half-times in hours.

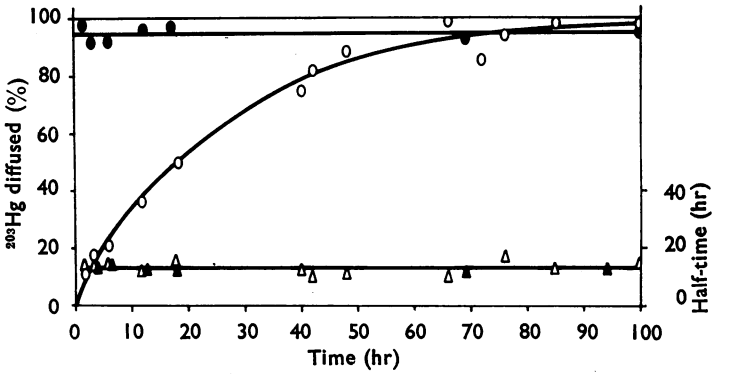

Fig. 4. The size of the diffusible component (circles, left-hand ordinate) and its half-time (triangles, right-hand ordinate) for a series of rats killed at various times up to 100 hr after administration of labelled mercuric-cysteine or chlormerodrin, are plotted against the times at which the rats were killed. Filled symbols, mercuric ion; empty symbols, chlormerodrin.

To see whether the rate of diffusion of the volatile component from homogenates containing chlormerodrin changed in a similar fashion to that of mercuric ion, the following experiments were performed.

Diffusion of ^{203}Hg from 0.1 M-*cysteine in saturated sodium sulphite solution*

The method was modified by adding cysteine (final concentration 0.1 M) and sodium sulphite (saturated) to prevent oxidation (Cecil & McPhee, 1955). In the presence of cysteine, ^{203}Hg diffuses over with a half-time of about 40 min from mercuric ion in buffer or in homogenate and with a half-time of 500 min from chlormerodrin. Volatilization from chlormerodrin is due to the liberation of mercuric ion from this mercurial by cysteine (Weiner *et al.*, 1962). These half-times are sufficiently different to allow accurate graphical analysis of the diffusion of ^{203}Hg from mixtures of mercuric ion and the mercurial (Solomon, 1960). Table 1 gives the results of such an analysis on known mixtures of mercuric

TABLE 1

THE DIFFUSION OF ^{203}Hg FROM KIDNEY HOMOGENATES IN 0·1 M-CYSTEINE AND SATURATED SODIUM SULPHITE SOLUTION CONTAINING KNOWN MIXTURES OF MERCURIC ION AND CHLORMERODRIN

Mercuric ion in added mixture (%)	Rapid component		Slow component		Non-diffusible ^{203}Hg (%)
	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	
100	44	96	—	—	4
80	40	80	460	14	6
69	44	69	500	28	3
42	50	45	500	44	11
0	50	5	500	93	2

cysteine and chlormerodrin added to the buffer in which the kidney was homogenized. The rapidly diffusing ^{203}Hg corresponds closely to the amount of added mercuric ion and its half-time is the same as that observed from mercuric ion. The slow component similarly corresponds with chlormerodrin. The small amount (5%) of rapidly diffusing ^{203}Hg

observed when chlormerodrin only was added is probably due to breakdown of the mercurial during and after homogenization.

Table 2 summarizes the results of the graphical analysis of the diffusion of ^{203}Hg from kidneys of rats killed at various times after injection of mercuric cysteine or chlormerodrin. The amount of rapidly diffusible ^{203}Hg increased with time after injection of the mercurial

TABLE 2

THE DIFFUSION OF ^{203}Hg FROM MERCURIC-CYSTEINE AND CHLORMERODRIN IN SOLUTIONS OF, AND IN HOMOGENATES SUSPENDED IN, 0·1 M-CYSTEINE AND SATURATED SODIUM SULPHITE SOLUTION

Labelled mercuric ion complexed to cysteine or labelled chlormerodrin was added to Ringer solution (Ringer, column 1), to the Ringer-buffer in which the kidneys were homogenized (*in vitro*) or *in vivo* to kidney by intramuscular injection

Time in rat (hr)	Mercuric ion		Chlormerodrin				Total diffusion of ^{203}Hg (%)
			Rapid component		Slow component		
	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	
0 (Ringer)	42	100	60	8	550	93	101
0 (Ringer)	35	97	40	8	460	91	99
0 (*in vitro*)	44	96	50	6	500	93	99
0 (*in vitro*)	53	88	45	4	380	97	101
1	45	95	42	16	440	86	102
2	46	95	38	11	450	89	100
3	48	96	47	4	470	88	92
4	43	93	32	16	450	83	99
5	50	89	32	16	500	84	100
6	50	93	35	10	350	93	103
40	64	88	40	96	—	—	96

to read approximately 100% after 40 hr. The half-time was the same as for the injected mercuric ion, approximately 40 min. The slow component had a half-time similar to that of chlormerodrin given *in vitro* (see also Table 1).

Diffusion of ^{203}Hg from 0.1 M-*cysteine in* 0.2 N-*sodium hydroxide solution*

In the experiments described in this section, sodium sulphite was replaced by sodium hydroxide to encourage the breakdown of cysteine at such a rate that it would be completely destroyed by the time the total amount of inorganic mercury had diffused into the pool of mercury. With 0.1 M-cysteine and 0.2 M-sodium hydroxide solution, about half of the cysteine was destroyed in 150 min and its concentration was less than 0.01 M in 300 min. Under these conditions the total release of mercuric ion from chlormerodrin was less than 10% and with a half-time of about 300 min. Mercuric ion was completely diffusible, with a half-time of about 25 min. Thus the diffusion kinetics from mixtures of mercuric cysteine and chlormerodrin approximated those of a two-component system (diffusible—mercuric cysteine and indiffusible—chlormerodrin).

Tables 3 and 4 summarize experiments with the cysteine–sodium hydroxide system similar to those described for the cysteine–sodium sulphite system in Tables 1 and 2. Table 3 also includes results on liver homogenates. Since the conclusions are exactly the same as those deduced from Tables 1 and 2, no further discussion is required.

Diffusional analysis by the cysteine–sodium hydroxide system was greatly simplified by two changes in the experimental procedure. First, more concentrated homogenates could

TABLE 3

THE DIFFUSION OF ^{203}Hg FROM KIDNEY AND LIVER HOMOGENATES IN 0·1 M-CYSTEINE AND 0·2 N-SODIUM HYDROXIDE SOLUTION CONTAINING KNOWN AMOUNTS OF MERCURIC ION AND CHLORMERODRIN

The weights of the liver samples were similar to the total wet weight of both kidneys of the rat, approximately 1·3 g

Mercuric ion in added mixture (%)	Kidney				Liver			
	Rapid component		Slow component		Rapid component		Slow component	
	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)
100	25	98	—	—	26	99	—	—
80	29	80	260	6·5	24	81	270	6·4
69	27	69	280	7·5	26	65	280	7·4
42	24	50	270	7·3	22	47	270	6·7
0	26	4	300	7·7	25	3·8	290	8·0

TABLE 4

DIFFUSION OF ^{203}Hg FROM 0·1 M-CYSTEINE IN 0·2 N-SODIUM HYDROXIDE SOLUTION

Labelled mercuric ion complexed to cysteine or labelled chlormerodrin was added to phosphate-buffer solutions (Ringer, column 1) or to the buffer in which kidneys were homogenized (*in vitro*) or *in vivo* to kidney by intramuscular injection

Time in rat (hr)	Mercuric ion		Chlormerodrin				
			Rapid component		Slow component		Calculated rapid component (%)
	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	Half-time (min)	Amount (%)	
0 (Ringer)	21	94	?	<1	320	9·5	0·5
0 (Ringer)	15	93	?	<1	350	9·7	0·1
0 (*in vitro*)	24	97	21	4·7	360	7·7	4·5
0 (*in vitro*)	30	91	32	3·4	350	8·0	3·7
1	27	98	24	13	350	10	12·7
2	23	98	28	10·5	240	9	12
3	25	99	21	4·6	340	8·2	4·8
4	22	93	24	9·8	320	6·2	9·8
5	25	93	25	10·9	320	6·7	10·8
6	26	98	28	11	350	7·7	10·1
40	24	96	25	95	—	—	96

be used (1 : 25 instead of 1 : 100) without adversely affecting the half-times of diffusion. Second, the amount of mercuric ion in any homogenate may be calculated by removing only two samples of the metallic mercury, one at 300 min, the other at 1,500 min. Thus at 300 min one half of the slow component and all of the mercuric ion will have diffused over. After 1,500 min, both components will have completely diffused. It may be readily demonstrated that fluctuations in the half-time of the slow component from 240 to 300 min have little effect on the final answer, since this component is relatively small (less than 10%). Fluctuations in the fast component will not affect the 300-min values for half-times below 40 min.

The accuracy of the two-sample techniques applied to the 1 : 25 homogenate was checked by adding known mixtures of mercuric cysteine and chlormerodrin to Robinson–Ringer solutions and to kidney and liver samples just before homogenization (Table 5). To simulate *in vivo* conditions as closely as possible, the counts added to the kidney and liver were adjusted to be within the range of counts found in injected animals, namely 20,000 counts/min/ml. of final homogenate of kidney and 700 counts/min/ml. of liver. In buffer solutions in

TABLE 5

RECOVERIES OF ADDED MERCURIC ION FROM 1 : 25 HOMOGENATES

Known mixtures of mercuric ion and chlormerodrin were added to phosphate-buffer solutions or to the buffer solution in which the kidneys and livers were homogenized. Figures in the first row are means and standard deviations (single observations) of four experiments. The recoveries from the kidney and liver homogenates were calculated by subtracting from the observed figures the amount of mercuric ion found in experiments where only diuretic was added (first row)

	Mercuric ion						
		In buffer solution		Kidney		Liver	
Expt. no.	Added (%)	Found (%)	Recovery (%)	Found (%)	Recovery (%)	Found (%)	Recovery (%)
1, 2, 3, 4	0	<1	—	2·4 (0·4)	—	11 (3)	—
5	23	22	96	22	85	28·5	76
6	23	—	—	22	85	34	100
7	43	45	96	40	80	50	83
8	47	—	—	43	86	51	85
9	73	73	100	66	87	69·5	80
10	—	—	—	67	88	67·5	77
11	100	99	99	89	87	96	85
12	100	—	—	88	86	94	83
Mean recovery			98		85		84
Standard deviation (single observations)			(2)		(3)		(7)

the absence of homogenate the agreement between the fraction of mercuric ion bound (found) and that added was excellent, an average of 98%. The recoveries from the kidney and liver homogenates were calculated by subtracting from the observed figures the amount of mercuric ion found in experiments where only chlormerodrin was added (first horizontal run). In both liver and kidney homogenates, the recoveries averaged approximately 85%. The reproducibility of the recoveries, standard deviation=3% for kidney and 7% for liver, was considered satisfactory for routine measurements with this method. The greater variability found for liver homogenates is in part due to the statistical errors in the lower counting rates, which amount to 3%.

Table 6 presents measurements of metabolism of chlormerodrin in kidneys and liver of three animal species using the two-sample method and the 1 : 25 homogenate. The metabolism of chlormerodrin in the rat kidney is summarized in Fig. 5.

Fig. 6 shows the change in urine volume of 24-hr samples following injection of chlormerodrin into rats (2.5 mg of mercury per kg of body weight, intramuscularly). The urine volume was increased on the first day after injection, reached a maximum value on the third day and had returned to nearly normal values on the fifth day. To allow statistical analysis, additional experiments were made measuring the diuretic effect on the first and second day after injection. The non-parametric statistic of Mann-Whitney (Auble, 1953) was used by expressing the results as the ratio " 24-hr urine volume after diuresis to the urine volume on the day before injection " and allocating ranks to the experimental and control values. The diuretic effect on the first and second day after injection was significant at the 98% confidence level.

In order to correlate the metabolism of chlormerodrin with the time course of diureses in Fig. 6 additional experiments were made which are not included in Fig. 5. Six rats were injected with chlormerodrin and killed 9 days later and the kidneys were analysed. All the

TABLE 6

THE METABOLISM OF LABELLED CHLORMERODRIN IN KIDNEYS AND LIVERS OF RATS, RABBITS AND CHICKENS

Mercuric ion in the homogenates was measured by the two-sample method using 1 : 25 homogenates. The mercuric ion figures have been corrected for mercurial breakdown during homogenization (2·4% for kidney, 11% for liver, Table 5) and for low recovery (85% in kidney, 84% in liver, Table 5). Columns headed "Dose" and "Mercuric ion" refer respectively to the percentage of injected dose found in the organ and to the fraction of total mercury present in the organ as mercuric ion. Numbers in parentheses are standard deviations (single observations)

			Kidney		Liver	
Species	No. of animals	Time after injection (hr)	Dose (%)	Mercuric ion (%)	Dose (%)	Mercuric ion (%)
Rat	6	2	68 7)	8·4	2·6 (1·0)	4·5 (3·4)
Rat	6	25	12·5 (2)	93 (6·0)	1·1 (0·4)	72 (7·0)
Rabbit	1	2	21	10	0·9	37
Rabbit	1	4	33	17	3·2	13
Rabbit	1	16	30	44	1·0	33
Rabbit	1	21	8	91	1·4	30
Chicken	1	2·5	43	15	3·0	35
Chicken	1	4·5	11	30	1·2	49
Chicken	1	6·5	18	38	3·5	20
Chicken	1	23·5	18	70	1·7	58

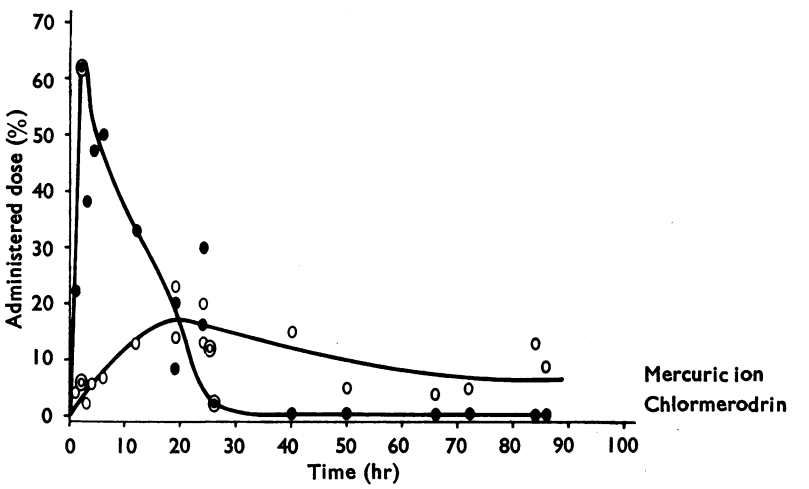

Fig. 5. The amounts of chlormerodrin and mercuric ion in rat kidney at various times after injection of chlormerodrin. Mercuric ion levels are maximal approximately 20 hr after injection and intact chlormerodrin has disappeared after 30 hr. The points at 2 and 25 hr are taken from Table 6 and are the means of six experiments.

mercury present was in the form of mercuric ion and amounted to a mean of 2.3% (standard deviation=0.3) of the injected dose.

DISCUSSION

The old hypothesis, that organic mercurials act after releasing inorganic mercury, has recently been reaffirmed by Mudge & Weiner (1958). As yet, however, there has been no experimental analysis of the form of mercury within the kidney.

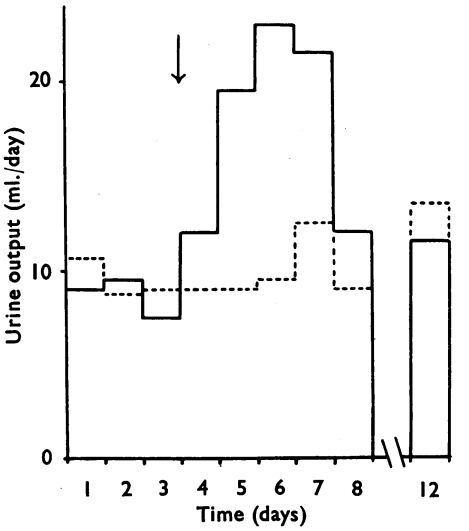

Fig. 6. The diuretic effect of chlormerodrin in rats. Six rats, two per metabolism chamber, were injected with chlormerodrin (2.5 mg of mercury per kg of body weight, intramuscularly; continuous line) at the end of the third day (at the arrow). At the same time six control rats were injected with a saline solution similar to that in which the chlormerodrin was dissolved (interrupted line; see Methods).

A new method of analysis has been developed which has clearly established the presence of mercuric ion in kidneys of experimental animals given a therapeutic dosage of an organomercurial diuretic. Specifically the half-times of diffusion of ^{203}Hg from mercuric ion added to kidneys *in vitro* or *in vivo* correspond closely with the rapidly diffusible component observed in kidneys of rats injected with chlormerodrin when the homogenates were suspended in three different solutions—phosphate buffer (half-time 13 hr), cysteine–sodium sulphite (half-time 40 min) and cysteine–sodium hydroxide (half-time 25 min).

Clearly, for these findings to be of significance to the mechanism of action of mercury diuretics, chlormerodrin must be shown firstly to be capable of producing diuresis in the animal species studied in this report and, secondly, to do so by direct action on the kidney. The first requirement is well supported in the literature for chickens (Campbell, 1960), rabbits (Farah & Maresh, 1947) and rats (Brunner, 1959a,b). The question of the potency of mercurial diuretics in the rat deserves some discussion. As indicated by Modi, Vartak & Sheth (1963) mercurial diuretics are potent in rats but have a slow onset of action. The sluggish action of the mercurials probably accounts for the view expressed by some workers (Farah & Miller, 1962) that these compounds produce little if any diuresis in the rat. Furthermore, sodium mersalyl was used in many of the early experiments and is perhaps the least effective of the mercurials in rats (Dicker, 1948; Brunner, 1959a). Recent work, however, indicates that other mercurial diuretics, including chlormerodrin, are effective in a dosage range of 2 to 4 mg of mercury per kg, similar to that used in man, dog and other species (Brunner, 1959a; Light, 1959; Sisson, Haynes, Lipchuck & Cummings, 1959; Bondini & Rapisard, 1961). The second requirement, that mercurial diuretics act directly on kidney tissue, has been conclusively demonstrated in dogs (Govaerts, 1928; Bartram, 1932; Borghgraef & Pitts, 1956) and in chickens (Campbell, 1960). There is no relevant

evidence for the rabbit. In the case of the rat, the following observations support a renal site of action: chlormerodrin is highly concentrated by rat kidney, up to 80% of the injected dose appearing in kidney 6 hr after injection (Borghgraef & Pitts, 1956; see also Fig. 5); chlormerodrin at a dose of 4 mg of mercury per kg produces changes in the protein bound sulphydryl groups in kidney (Farah & Kruse, 1960) and other mercurials produce damage to the proximal tubular cells as evidenced by electronmicroscopic (Sanabria, 1963) and histochemical (Bickers, Bresler & Weinberger, 1960) observations.

Granted that chlormerodrin acts directly on rat kidney, it is of interest to compare the metabolism of chlormerodrin in rat kidney (Fig. 5) with the time course of diuresis (Fig. 6). The levels of chlormerodrin in kidney do not correlate with the magnitude of the diuretic effect. Maximum diuresis was observed on the third and fourth days after injection (Fig. 6) when no detectable amounts of chlormerodrin remained in the kidney (Fig. 5). Greatest concentrations of chlormerodrin were observed on the first day when diuresis was small. More specifically, Brunner (1959a) has reported no diuresis in the first 4 hr after injection of chlormerodrin into rats. According to Fig. 5 the concentration of chlormerodrin in kidney reached a maximum value during this time.

In distinction to chlormerodrin, the levels of mercuric ion correlate well with diuresis. Assuming that the minimum level of mercuric ion required to produce diuresis is approximately 7% of the injected dose (2.5 mg of mercury/kg), the levels of mercuric ion in Fig. 5 would predict that diuresis should start approximately 10 hr and continue for at least 70 to 90 hr after injection. This corresponds well with the observed diuretic period in Fig. 6 and also with Brunner's (1959a,b) observations that diuresis did not commence until later than 4 hr after injection and continued for approximately 3 days. At 9 days after injection, when urine excretion had returned to normal (Fig. 6), the levels of mercuric ion in kidney had fallen to 2.3% (mean of six rats) of the dose. In short, correlation of the levels of chlormerodrin and mercuric ion in kidney with the changes in water excretion strongly suggest that inorganic mercury is the primary diuretic agent.

A threshold level of mercuric ion in kidney equivalent to between 5 to 10% of the injected dose implies that only a small amount of mercury need be removed from the kidney to reduce levels below the threshold and thereby to abolish diuresis. This conclusion agrees with that of Weiner, Garlid, Sapir & Mudge (1959) as well as with that of Miller & Farah (1962).

In the scheme put forward by Weiner *et al.* (1962) the breakdown of the mercurial to release mercuric ion takes place in kidney tissue. The finding of mercuric ion in kidney as reported here does not necessarily establish this organ as the site of release of mercuric ion from the mercurial diuretic. Mercuric ion may be released in other organs and carried by the blood stream to the kidneys. The fact that the mercuric ion was found in the livers of animals given chlormerodrin (Table 6) and the mercuric ion injected into animals is preferentially accumulated by kidney (Rothstein & Hayes, 1960) lends support to this possibility. However, the quantitative results argue against this idea. For example, 2 hr after injection of chlormerodrin into rats, an average of 68% of the injected dose was recovered in the kidney (Table 6), compared to 2.6% in liver. The quantity of mercuric ion in kidney at this time was 6.2% of the injected dose, more than double the total amount of mercury both as chlormerodrin and mercuric ion found in the liver. If liver or other tissues were the primary source of renal mercuric ion, it would be necessary to postulate a

very rapid breakdown of the mercurial in nonrenal tissue, an equally rapid removal by the blood stream, and a practically complete clearance of mercuric ion from the blood by the kidney. Evidence from experiments in which mercuric ion was injected intravenously into rats indicates that, after an initially rapid binding to liver, kidney and other tissues, the build-up in kidney required several days (Rothstein & Hayes, 1960).

The slow response in rats contrasts with the rapid onset of diuresis (within 60 min of injection of chlormerodrin) observed with chickens (Campbell, 1960). If, in view of the above discussion, the levels of mercuric ion in kidney determine the extent of diuresis, it might be anticipated that diuretic breakdown in the chicken would be much more rapid than in the rat. The results of Table 6 indicate that this is probably not so. For example, 2.5 hr after injection into the chicken, the mercuric ion level in kidney was 6.7% of the injected dose. This lies in the range of levels found in the rat (Fig. 5). However, the more rapid onset of diuresis in the chicken may reflect an increased sensitivity to mercuric ion, and is not incompatible with mercuric ion being the diuretic agent in both species. Clearly the quantitative aspects of this report (for example the threshold levels mercuric ion in kidney) based mainly on rats should not be glibly applied to other species.

SUMMARY

1. Hitherto no method has been available to test the old hypothesis that mercurial diuretics break down in kidney tissue to release mercuric ion; the principle behind the method described is that the speed with which isotopically labelled mercury exchanges depends upon its state of chemical combination.

2. The exchange half-times in homogenates of kidneys from animals given chlormerodrin were compared with the half-times from animals injected with mercuric cysteine.

3. The results indicated that chlormerodrin released mercuric ion to the kidney tissue of rats, rabbits and chickens, and that approximately 1 day after injection most of the mercury was present as mercuric ion.

4. Studies on the rat indicated that kidney levels of mercuric ion correlated well with the onset and duration of diuresis. Assuming that a threshold level of mercuric ion was required to initiate diuresis, this level was approximately 7% of the injected dose (2.5 mg of mercury per kg body weight) of chlormerodrin.

5. These results are discussed in relation to the " intact molecule " and " mercuric ion " hypotheses on the mechanism of action of mercurial diuretics.

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REFERENCES

AUBLE, D. (1953). Extended tables for the Mann–Whitney statistic. *Bull. Inst. educ. Res. Indiana Univ.*, **1**, 1.

BARTRAM, E. A. (1932). Experimental observations on the effects of various diuretics when injected directly into one renal artery of the dog. *J. clin. Invest.*, **11**, 1197.

BASALO, F. & PEARSON, R. G. (1958). *Mechanisms of Inorganic Reactions.* London : John Wiley.

BICKERS, J. N., BRESLER, E. H. & WEINBERGER, R. (1960). The acute effect of organic mercurial diuretics on the rat kidney; a histochemical study. *J. Pharmacol. exp. Ther.*, **128**, 283–288.

BONDINI, M. & RAPISARD, C. (1961). Effect of adiuretine on diuresis in rats treated with mercaptomerin *Boll. Soc. ital. Biol. sper.*, **37**, 1114–1115.

BORGHGRAEF, R. R. M. & PITTS, R. F. (1956). The distribution of chlormerodrin (Neohydrin) in tissues of the rat and dog. *J. clin. Invest.*, **35**, 31–37.

BRUNNER, H. (1959a). Renale Wasser- und Elektrolytausscheidung durstender Ratten innerhalb von 32 Stunden nach einmaliger intravenöser Gäbe von Chlormerodrin oder Mersalyl. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **236**, 521–539.

BRUNNER, H. (1959b). Vëranderungen der Wasser- und Salzausscheidung sowie der Nierenstruktur während des 2–10 Tages nach einmaliger intravenöser Zufuhr von Chlormerodrin. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*, **236**, 540–548.

CAMPBELL, D. E. S. (1960). Modification by bromcresol green or probenecid of the excretion and diuretic effect of three mercurial diuretics, diurgin, chlormerodrin and mercumatilin. *Acta pharmacol. (Kbh.)*, **17**, 213–232.

CECIL, R. & MCPHEE, J. R. (1955). A kinetic study of the reactions of some disulphides with sodium sulphite. *Biochem. J.*, **60**, 496–506.

CLARKSON, T. W., GATZY, J. & DALTON, C. (1961). Studies of the equilibrations of mercury vapour with blood. *Univ. of Rochester AEP Report*, No. 582.

CONWAY, E. J. (1947). *Microdiffusion Analysis and Volumetric Error*, revised ed., p. 10. London : Crosby Lockwood & Son.

DICKER, S. E. (1948). The action of mersalyl, calomel and theophylline sodium acetate on the kidney of the rat. *Brit. J. Pharmacol.*, **1**, 194–209.

ELLMAN, G. L. (1959). Tissue sulphydryl groups. *Arch. Biochem.*, **82**, 70–77.

FARAH, A. & KRUSE, R. (1960). The relation of mercurial diuresis to cellular protein-bound sulphydryl changes in renal cells. *J. Pharmacol. exp. Ther.*, **130**, 13–19.

FARAH, A. & MARESH, G. (1947). The influence of sulphydryl compounds on diuresis and renal and cardiac circulatory changes caused by mersalyl. *J. Pharmacol. exp. Ther.*, **92**, 73–82.

FARAH, A. E. & MILLER, T. B. (1962). Renal pharmacology. *Ann. Rev. Pharmacol.*, **2**, 269–312.

GAGE, J. C. (1961). The distribution and excretion of inhaled mercury vapour. *Brit. J. industr. Med.*, **18**, 287–294.

GOVAERTS, P. (1928). Origine rénale ou tissulaire de la diurèse par un composé mercurial organique. *C.R. Soc. Biol. (Paris)*, **99**, 647–649.

KESSLER, R. H., LOZANO, R. & PITTS, R. F. (1957). Studies on structure diuretic activity relationships of organic compounds of mercury. *J. clin. Invest.*, **36**, 656–688.

LIGHT, A. E. (1959). Diuretic activity of various compounds as determined by urinary excretion studies in rats. *J. Amer. pharm. Ass., sci. Ed.*, **48**, 335–345.

MILLER, T. B. & FARAH, A. E. (1962). On the mechanism of inhibition of mercurial diuresis by *p*-chloromercuribenzoic acid. *J. Pharmacol. exp. Ther.*, **136**, 10–19.

MODI, K. N., VARTAK, M. N. & SHETH, U. K. (1963). Evaluation of 5 hr. and 24 hr. duration of urine collection for assessing diuretic activity in rats. *Arch. int. Pharmacodyn.*, **142**, 539–549.

MUDGE, G. H. & WEINER, I. M. (1958). The mechanism of action of mercurial and zanthine diuretics. *Ann. N.Y. Acad. Sci.*, **71**, 344–354.

ROTHSTEIN, A. & HAYES, A. D. (1960). The metabolism of mercury in the rat studied by isotope techniques. *J. Pharmacol. exp. Ther.*, **130**, 166–176.

SANABRIA, A. (1963). Ultrastructural changes produced in the rat kidney by a mercurial diuretic (meralluride). *Brit. J. Pharmacol.*, **20**, 352–361.

SISSON, G. M., HAYNES, J. D., LIPCHUCK, L. M. & CUMMINGS, J. R. (1959). Body weight, fluid load and diuretic drugs and their relation to five-hour urine volumes in normal and fasted dehydrated rats. *Physiologist*, **2**, 109.

SOLOMON, A. K. (1960). In *Mineral Metabolism*, ed. COMAR, C. L. & BRONNER, F., vol. 1, part A, chapter 5. New York : Academic Press.

WEINER, I. M., GARLID, K., SAPIR, D. & MUDGE, G. H. (1959). The effect of dimercaprol (BAL) on the renal excretion of mercurials. *J. Pharmacol. exp. Ther.*, **127**, 325–331.

WEINER, I. M., LEVY, R. I. & MUDGE, G. H. (1962). Studies on mercurial diuresis; renal excretion, acid stability and structive-activity relationships of organic mercurials. *J. Pharmacol. exp. Ther.*, **138**, 96–112.