Br. J. Pharmac. Chemother. (1967), **31**, 560–567.

THE EFFECT OF SODIUM MALEATE ON THE RENAL DEPOSITION AND EXCRETION OF MERCURY

BY

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(*Received August* 4, 1967)

Knowledge of the mechanism of renal deposition and excretion of metals provides a rational basis for dealing with two practical problems in heavy metal intoxication ; estimation of the degree of exposure from measurements of urinary excretion and the search for chemical agents capable of accelerating the excretion of the metal from the body. In the case of mercury the approach to these problems has been empirical because the mechanism of kidney deposition and of renal excretion of mercury is unknown. Recent work has cast doubt on the widely accepted theory (Staemmler, 1956) that mercury is first filtered by the glomerulus and then taken up by the tubular cells. This theory may hold in the case of acute mercury poisoning when the glomerular membrane may be damaged but it is probably not applicable otherwise. For example, the first deposits of mercury in the kidney are seen in the endothelial cells of the interstitial capillaries and only later in the glomerular tufts (Wockel, Stegnar & Jänisch, 1961). Mercury appears in the urine in advance of inulin, suggesting a transport of the metal directly across the tubular walls (Mambourg & Raynaud, 1965 ; Vostal, 1966). Furthermore, Berlin & Gibson (1963) have shown that more than 99% of the mercury in the plasma is not ultrafiltrable and that ligation of the ureter does not influence the rate of accumulation in the kidney. Since some glomerular filtration continues after ureteral ligation (Salomon & Lanza, 1962) a reduction in kidney uptake should occur if the glomerular filtration theory is true.

An alternative explanation is that the kidney preferentially takes up mercury because it possesses special binding sites of high affinity for mercury. This possibility was eliminated by Clarkson & Magos (1966) who found that liver and kidney homogenates have identical mercury binding properties.

The accumulation of mercury may be a more complicated process and coupled with the metabolism of the renal cells. Sodium maleate, which produces profound metabolic disturbances in renal cells (Rogulski & Angielski, 1963), also affects the accumulation of mercury. Experiments describing the effect of sodium maleate on the renal deposition and excretion of mercury are described here.

METHODS

White female (Porton strain) rats, 240±5 g were used unless otherwise stated. Urine and faeces were collected in metabolism cages. Food, M.R.C. Diet 41B, and water were available *ad libitum*.

Mercury, either as mercury cysteine complex or mercuric chloride, was injected intramuscularly into a hind leg. Mercury acetate was dissolved in 0.1 M cysteine solution buffered by 0.1 M sodium bicarbonate and 0.1 M sodium carbonate, to form the complex. Mercuric chloride was given in 0.9% NaCl. ^{203}Hg isotope was added to these solutions as mercuric acetate dissolved in 0.9% NaCl (Radiochemical Centre, Amersham) such that 0.2 ml. of the injection solution contained 100 μg Hg and 0.3 to 0.4 μc radioactivity.

Sodium maleate and fumarate (BDH) were injected under the skin of the neck in 0.5 to 1.5 ml. distilled water. Controls, unless otherwise stated, were given similar volumes of 0.9% NaCl.

The mercury content (measured as ^{203}Hg activity) of organs and excreta was determined by an automatic scintillation counter (Model 450A, Packard Inst., Co., La Grange, Ill., U.S.A.) having a counting efficiency of 40%. Volumes up to 4 ml. could be counted without loss in counting efficiency. The rats were killed under ether anaesthesia. Blood was collected directly from the aorta or from the chest cavity. Organs were removed, washed, blotted, weighed and counted. The two kidneys were counted in one test tube, but the liver and the faeces were distributed in two or more test tubes to keep the total volume in each counting tube to 4 ml. or less.

Protein-bound mercury in plasma and urine was determined after trichloracetic acid precipitation. Ice cold 7% trichloroacetic acid was added in 1:1 volume ratio to the plasma or urine. The sample was left at 4° C for 15 min and was centrifuged in the cold. The same volume of supernatant as the original volume of the sample (4 ml. or less) was removed and both this (S) and the remaining part containing the precipitate (P) were counted. The non-protein bound mercury was calculated from the equation: $\frac{200\ S}{S+P}$

A female dog, body weight 13 kg, was prepared with exteriorized kidneys according to Rhoads (1934). The animal was given penicillin (0.5×10^6 units) and allowed to recover. Three weeks later (the creatinine clearance, measured according to Smith (1962), was found to be in the normal range), the animal was given intravenously 0.1 mg/kg radioactive mercury as the cysteine complex. One week later the dog was anaesthetized with pentobarbitone (30 mg/kg) and creatinine clearance measurements started during an infusion of 0.9% NaCl at 4 ml./min. The infusion lasted 6 hr. At the fifth hour sodium maleate (1.3 g in 50 ml. of solution) was infused intravenously over a period of about 1 min. At the end of the infusion, the dog was placed in a metabolism cage and urine was collected overnight. The radioactivity in the exteriorized kidneys was measured throughout the infusion period, and on the next morning by the following procedure. A shielded sodium iodide crystal, diameter 1 in., was placed immediately adajacent to the kidney. The efficiency of the scintillation probe was 1% and was determined by placing standard solutions of radioactive mercury at known distances from the crystal.

Bicarbonate was measured according to Conway (1950), creatinine as described by Brod & Sirota (1948), chloride by the method of Cotlove, Trantham & Bowman (1958).

RESULTS

Sodium maleate, given 90 min before the administration of 100 μg Hg as the mercury-cysteine complex, altered the distribution of mercury in the body so that, with increasing doses of maleate, the amount of mercury in the kidneys decreased whereas the mercury in liver and whole blood increased (Fig. 1). Mercury in the spleen (not recorded in Fig. 1) changed in parallel with the changes in the liver. Fumarate, a structural isomer of maleate, at a dose of 500 mg/kg body weight, had no effect on the distribution of mercury as compared to control animals treated with 0.9% NaCl.

A dose of 200 mg/kg sodium maleate, besides decreasing kidney levels of mercury, produced a large increase in the urinary excretion of the metal (Table 1). This increase in urinary excretion was approximately 15-fold but was only partly responsible for the

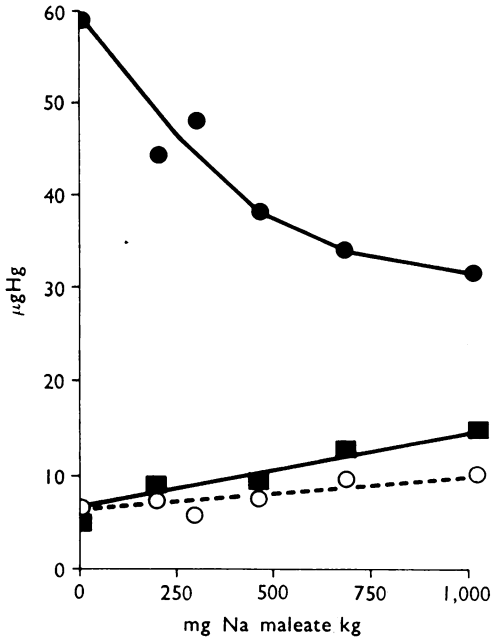

Fig. 1. The effect of increasing doses of sodium maleate on the distribution of mercury in rats. Sodium maleate was injected 90 min before the administration of 100 μg mercury complexed with cysteine and the animals killed 4 hr later. The μg of mercury in whole blood was calculated on the basis that the blood volume is 7% of the body weight. Kidney, ●——●; liver, ■——■; blood, ○——○.

TABLE 1

EFFECT OF PRETREATMENT WITH SODIUM MALEATE (200 MG/KG) ON THE BLOOD AND KIDNEY LEVELS AND URINARY EXCRETION OF MERCURY AFTER ADMINISTRATION OF MERCURY COMPLEXED WITH CYSTEINE

The animals were divided into five metabolic cages, three per cage. All animals received 125 μg mercury complexed with cysteine. Ninety minutes before dosing with mercury, animals in two cages were injected subcutaneously with 0·5 ml. 0·4 M $NaHCO_3$ (controls) and the other animals received maleate. All animals were killed 4 hr after the injection of mercury. Figures for blood level of mercury are recorded as described in the legend of Fig. 1. Numbers in parentheses after the kidney values are the standard errors of the means.

Treatment	Animals (no.)	Mercury contents (μg)			Kidney loss of mercury due to maleate (μg)
		Blood	Kidney	4 hr urinary excretion	
Controls	6	3·95	67·15 (±0·84)	0·33	
Sodium maleate	9	7·77	51·40 (±2·35)	5·90	15·75±2·50 $P \ll 0.0005$

fall in the kidney level. The increase in the concentration of mercury in blood suggests that sodium maleate also induced a transfer of mercury from kidney to blood and subsequently to other tissues. In the same experiments, maleate increased urine flow and bicarbonate excretion and decreased chloride excretion but had no effect on the creatinine clearance.

Sodium maleate may cause the release of mercury from the kidneys even when given 6 days after the administration of mercury (Table 2). Maleate was effective at a dose of

TABLE 2

EFFECT OF SODIUM MALEATE ON THE DISTRIBUTION AND EXCRETION OF MERCURY WHEN GIVEN 6 DAYS AFTER THE ADMINISTRATION OF 100 μG $HgCl_2$

The animals were killed 48 hr after administration of sodium maleate. The figure for urinary and faecal excretion represent total excretion for the 48 hr period following maleate injection. Each figure is the mean of observations on two animals

Treatment		Mercury contents (μg)					Volume of 48 hr urine (ml.)	Weight of 48 hr faeces (g)
Hg (μg)	Maleate (mg/kg)	Blood	Liver	Kidneys	48 hr urine	48 hr faeces		
Nil	Nil	—	—	—	—	—	21·5	11·96
100	Nil	0·19	0·60	55·15	1·32	0·76	24·5	11·35
100	50	0·25	0·66	56·75	1·00	0·98	20·3	11·28
100	100	0·30	0·65	43·90	4·30	1·61	20·5	10·20
100	200	0·40	0·95	39·45	10·30	1·35	31·0	11·42
100	400	1·74	4·07	25·05	8·64	1·17	30·30	3·21
Nil	400	—	—	—	—	—	30·0	6·65

100 mg/kg but release of mercury from the kidney increased with increasing doses of maleate, until, at 400 mg/kg, over 50% of the mercury was released from the kidney. At doses of maleate up to 200 mg/kg the loss of mercury from the kidney was accompanied by an increased urinary excretion. Higher doses of maleate (400 mg/kg) caused a major redistribution to other tissues. The diuretic effect of maleate was evident at a dose of 200 mg/kg. The weight of the daily faeces was reduced at 400 mg/kg indicating a reduced food uptake.

In a similar experiment, using 4 treated and 4 control animals, a seven-fold increase in urinary excretion was observed when maleate was given 5 days after the mercury but, again, this increase did not account for the loss of mercury from the kidney. Sodium fumarate, 200 mg/kg under the same conditions, produced no effect on the kidney levels of mercury.

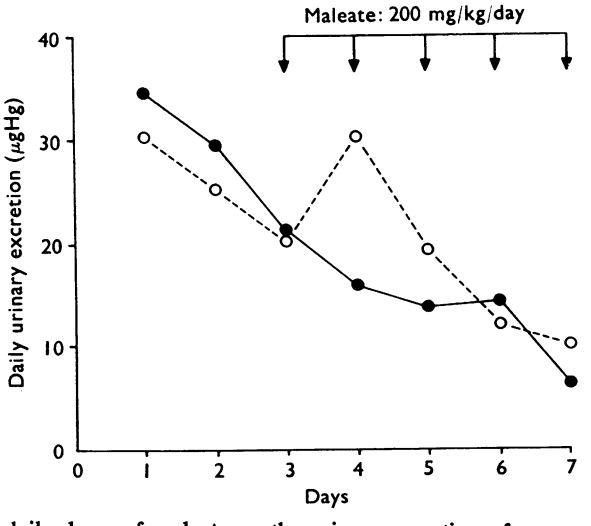

Fig. 2. The effect of daily doses of maleate on the urinary excretion of mercury. Mercuric chloride 2.1 mg/kg) was given to four rats. Two of these animals were treated daily with 200 mg/kg sodium maleate (○——○) from the third day. The other two animals (controls) were given saline ●——●. The animals were killed on the 7th day.

When sodium maleate was given daily to rats previously treated with $HgCl_2$ (Fig. 2) the first dose produced a sudden increase in the urinary excretion of mercury but on subsequent days the mercury excretion fell back to the control level. After the fourth dose of maleate the kidney levels of mercury were very much lower (26.4 and 13.9 μg) than in the controls (55.4 and 50.4 μg), yet on days 5, 6 and 7 (Fig. 2) the excretion of mercury from maleate treated animals was higher or equal to the control rate despite a reduced kidney level.

There is no relationship between increased urine flow induced by sodium maleate and increased excretion of mercury (Table 3). Diuresis was maximal in the first hour, and fell in the second and third hour collection periods. Mercury excretion was lowest in the first hour and highest in the fourth hour. The first and second hour urine samples were clear but subsequent samples were turbid. Analysis showed that $70.1 \pm 10.4\%$ of the

TABLE 3

THE RELATIONSHIP BETWEEN URINE VOLUME FLOW AND MERCURY EXCRETION FOLLOWING INJECTION OF MALEATE

Ten rats were given 100 μg $HgCl_2$ and 4 to 6 days later injected with sodium maleate (400 mg/kg). Urine samples were collected at hourly intervals for the next 5 hr. Figures following the $\pm$ sign are standard deviations.

Collection period (hr)	Urine volume (ml.)	Urinary excretion of Hg (μg)
First	$4{\cdot}57 \pm 2{\cdot}38$	$0{\cdot}027 \pm 0{\cdot}014$
Second	$2{\cdot}89 \pm 0{\cdot}90$	$0{\cdot}10 \pm 0{\cdot}15$
Third	$0{\cdot}97 \pm 0{\cdot}32$	$0{\cdot}98 \pm 0{\cdot}74$
Fourth	$0{\cdot}89 \pm 0{\cdot}43$	$1{\cdot}72 \pm 1{\cdot}41$

TABLE 4

EFFECT OF SODIUM MALEATE ON THE KIDNEY LEVELS AND URINARY EXCRETION OF MERCURY IN A DOG WITH EXTERIORIZED KIDNEYS

It was given approximately 0·1 mg Hg/kg as the cysteine complex. The radioactivity dose was $15{\cdot}6 \times 10^6$ counts/min. One week later the dog was anaesthetized and routine creatinine clearance measurements started. Sodium maleate was infused intravenously as indicated in the Table. Radioactive mercury in the urine samples was counted in the well crystal (efficiency 50%) and in the left kidney by an exteriorized scintillation probe (efficiency 1%). Counts recorded in the Table were not corrected for the mean background count of 1560 counts/5 min from the well crystal and 300 counts/min from the probe. Further details of this experiment are given in the methods section.

Collection period	Urine sample		Left kidney (counts/min)
	Counts/5 min/ml.	Volume (ml.)	
January 4–5			
4.00 p.m.–7.30 a.m.	2533	317	42,000
January 5			
10.30 a.m.	Dog anaesthetized and saline infusion started at 4 ml./min.		
11.30 a.m.–12.30 p.m.	1711	234	41,000
12.30 p.m.– 1.30 p.m.	1993	237	40,000
1.30 p.m.– 2.30 p.m.	1785	340	39,600
2.30 p.m.– 3.30 p.m.	1685	303	39,200
3.30 p.m.– 4.30 p.m.	1·3 g sodium maleate given in 50 ml. at pH 7·35		
3.30 p.m.– 4.00 p.m.	1722	100	
4.00 p.m.– 4.30 p.m.	1715	140	
4.30 p.m.– 5.00 p.m.	1692	173	
5.00 p.m.– 5.30 p.m.	1727	91·5	
5.45 p.m.	Dog returned to metabolism cage. Overnight urine collection started.		
January 5–6			
5.45 p.m.–7.30 a.m.	5124	730	30,000

mercury in the urine excreted during the third and fourth hours was not bound to protein. The mercury released to the blood was mainly bound to protein but in plasma samples collected 4 hr after injection of 400 mg sodium maleate the proportion of non-protein bound mercury increased from less than 1% to $5.3 \pm 2.7\%$.

Sodium maleate exhibited the same effect on mercury distribution and excretion in the dog as in rats. After intravenous administration of the mercury cysteine complex, the level of mercury in the kidney rose rapidly to a steady value and remained at this level for the next 6 days. Maleate produced no change in kidney levels or urinary excretion of mercury up to three hours after injection but, thereafter, urinary excretion of mercury was increased (Table 4). For example, 81,000 counts/min were excreted in the overnight period before maleate injection as compared with 520,000 counts/min in the subsequent overnight sample.

DISCUSSION

Prior to these findings, 2,3-dimercaptopropanol (BAL, dimercaprol) was the only chemical agent known to release mercury from the kidneys (Adam, 1951). Dimercaprol appears to act by complexing the metal with its thiol groups. That sodium maleate, a dicarboxylic acid containing no thiol groups, is effective in removing mercury from kidney, is therefore of some interest.

It is unlikely that maleate acts like dimercaprol in forming an undissociable complex with mercury since carboxyl groups form only weak complexes with this metal (Gurd & Wilcox, 1956). It is equally unlikely that maleate can compete with mercury for binding to the thiol groups in tissue proteins (Morgan & Friedman, 1938). Rogulski (1960) found a considerable reduction in the protein and non-protein thiol groups of the kidney after administration of maleate, but this finding was not confirmed by Worthen (1963).

The process of mercury accumulation by the kidney may be coupled to metabolism, and the inhibition of mercury uptake might be the result of depressed metabolism. However, such mechanisms of action would not explain the ability of maleate to release mercury from kidney several days after the administration of the metal and when all renal uptake has ceased. Sodium maleate interferes with oxidation of Krebs cycle intermediates by kidney, enhances amino acid synthesis and keto acid accumlation in kidneys (Rogulski & Angielski, 1963 ; Schubert & Barritt, 1966), activates the phosphate-independent glutaminase (Berliner, Kennedy & Hilton, 1950) and increases urinary phosphate, glucose, amino acid (Harrison & Harrison, 1954) and ammonia excretion (Katunuma, Tomino & Nishino, 1966). At present, it is impossible to say which specific metabolic defect is responsible for the action of maleate on the storage of mercury in the kidneys. Nevertheless, it seems likely that, as a consequence of one or more of the metabolic disturbances, an agent is liberated from the cells capable of forming a strong complex with mercury and thereby removing the metal from kidney tissue. The findings reported here lend some support to his idea. Thus:

1. Virtually all the mercury in the plasma is protein bound but after the administration of maleate, a significant fraction becomes non-protein bound.

2. Most of the mercury present in urine samples is not bound to protein despite the presence of large amounts of protein in the urine of animals treated with maleate.

3. The pattern of redistribution of mercury following maleate resembles the action of dimercaprol (Adam, 1951) in that kidney levels are reduced and urinary excretion increased. Both sodium maleate and dimercaprol (Fitzsimmons & Kozelka, 1950) cause a redistribution of mercury from the kidney to other organs. The principal difference lies in the times of action, dimercaprol producing nearly an immediate effect (Weiner, Garlid, Sapir & Mudge, 1959) whereas the action of sodium maleate is apparent only after 1 to 2 hr. This suggests that it takes time for the release of sufficient quantities of endogenous complexing agent. It is interesting that Fried, Rosenthal & Schubert (1956) have reported that low doses of fluoroacetate protect against the lethal effect of lead salts by blocking the Krebs cycle and causing an accumulation of citric acid which complexes with the lead.

The present findings with sodium maleate may suggest a new therapeutic approach to mercury poisoning. However, this work has shown two limiting factors in the action of sodium maleate. The response is not maintained with repeated dosing (Fig. 2) and there is only a small margin between effective and toxic doses. Thus the lowest therapeutic dose was found to be 100 mg/kg of body weight and the 400 mg/kg dose produced signs of toxicity. The 10 days LD_{50} of female rats of 240 g weight was found to be 813 mg/kg. However, it may be encouraging that the renal excretion of mercury is not related to the toxic effects of sodium maleate—for example, diuresis and proteinurea—and that other compounds may be found with a wider therapeutic ratio.

SUMMARY

1. Sodium maleate given before sublethal doses of mercury increases the urinary mercury excretion and decreases the mercury level in kidney.

2. Given days after the mercury, sodium maleate releases a part of the mercury accumulated in the kidney and increases the urinary excretion.

3. The raised urinary excretion is only partly responsible for the decrease in kidney level. Some of the mercury released from the kidney is taken up by the blood and by other organs.

4. There is no time relationship between the diuretic and mercury excretory effect of sodium maleate. Maximum diuresis appeared in the first hour of a 4 hr experiment and fell in the second and third hour collection period. Mercury excretion was lowest in the first hour and highest in the fourth hour.

5. Only one third of the mercury excreted during the third and fourth hour, when proteinurea was apparent, was bound to protein. In the plasma sodium maleate increased the proportion of non-protein bound mercury.

We wish to thank Mr. L. Ferraio and Mr. R. Lock for valuable technical assistance, and Mr. G. Whitford for help in preparing the exteriorized kidneys.

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