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Combined effect of sodium maleate and some thiol compounds on mercury excretion and redistribution in rats

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1. (+)-Penicillamine in a dose of 193 μmoles/kg given subcutaneously twice a day on the sixth and seventh days after the administration of 100 μg mercury increased the urinary excretion of rats more than the equimolar dose of N-acetyl-(+)-penicillamine but less than 2,3-dimercaptopropanol 48.3 μmoles/kg.

2. Sodium maleate in a dose of 156 μmoles/kg given on the sixth and seventh days after the mercury did not influence mercury excretion or redistribution. Sodium maleate in the same dose increased considerably the effect of (+)-penicillamine on the urinary excretion and redistribution of mercury. It increased the effect of N-acetyl-(+)-penicillamine only slightly. There was a tendency to decrease the effect of 2,3-dimercaptopropanol.

3. All the complexing agents decreased the kidney content of mercury and increased the liver and blood concentration of mercury. These changes were highest with 2,3-dimercaptopropanol. The combination of sodium maleate with (+)-pencillamine caused higher mercury excretion and lower kidney content but a smaller increase in the liver and blood mercury contents than 2,3-dimercaptopropanol.

It was shown by Clarkson & Magos (1967) that sodium maleate in a dose of 200 mg/kg, which is near to the toxic level, brought about a ten-fold increase in the urinary excretion of mercury. They suggested that the metabolic disturbances caused by maleate might liberate an agent from the cells capable of forming a strong complex with mercury and thereby remove the metal from the kidney tissues. Sodium maleate, however, might also affect the transport of the mercury complex. If the lowest dose for the facilitation of transport is less than that required for the hypothetical mercury complexing agent, sodium maleate might increase the urinary excretion of mercury in the presence of an exogenous complexing agent.

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In this paper we record the effect of a dose of 25 mg/kg (196 μmoles/kg) on the urinary excretion of mercury in rats treated with (+)-penicillamine, N-acetyl-(+)-penicillamine or 2,3-dimercaptopropanol (BAL).

Methods

Animals. Albino rats, Porton Wistar strain, 200–250 g body weight were used.

Injection solutions. Mercuric acetate (BDH Chemicals Ltd., Poole, Dorset) was dissolved in 0.9% NaCl and labelled with ^{203}Hg isotope (Radichemical Centre, Amersham), to give a solution containing 100 μg Hg and 0.2 to 0.5 μc radioactivity in 0.2 ml.; sodium maleate (BDH) 1.6% solution in distilled water; sodium fumarate (Hopkin & Williams Ltd., Chadwell Heath, Essex), 1.6% solution in distilled water; sodium chloride (BDH), 0.9% solution in distilled water; (+)-penicillamine hydrochloride (Dista Products Ltd., Liverpool), 3.58% solution in

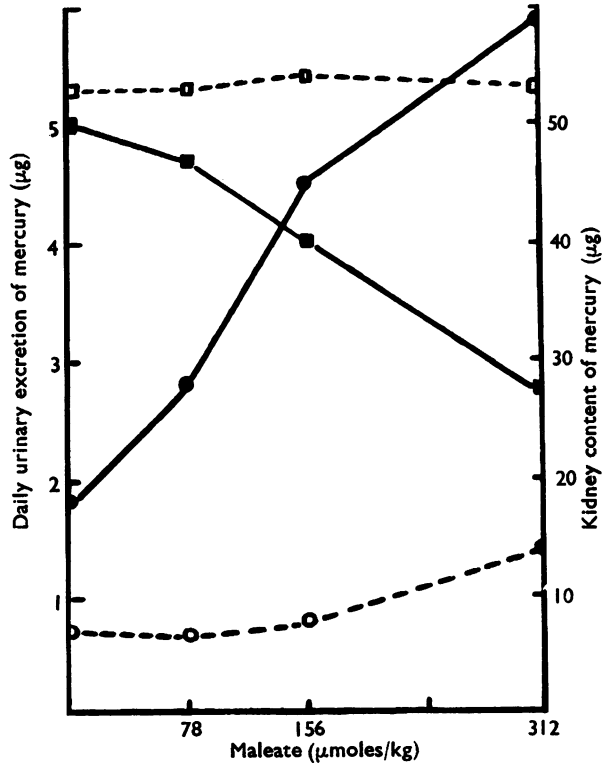

FIG. 1. Effect of maleate given alone or in combination with (+)-penicillamine on the urinary excretion and kidney content of mercury. Thirty-two animals were given 100 μg Hg intramuscularly as mercuric salt labelled with ^{203}Hg. Five days later the animals were divided into four groups and groups of two were placed in metabolism cages. On the sixth and seventh days after treatment the groups were given subcutaneously once a day maleate in the following doses: none; 78 μmoles/kg; 156 μmoles/kg and 312 μmoles/kg. Four of every group were given (+)-penicillamine 193 μmoles/kg intramuscularly twice a day. Maleate was given with the first dose of (+)-penicillamine. During the 2 days of treatment urine was collected for the estimation of ^{203}Hg. At the end of the 2 days animals were killed, kidneys were removed and kidney content of ^{203}Hg was estimated. Kidney content of mercury in (+)-penicillamine-treated (■——■) and in control animals (□ - - □); daily urinary mercury excretion in (+)-penicillamine-treated (●——●) and in control animals (○ - - ○).

0.1M carbonate buffer (pH 9.0); N-acetyl-(+)-penicillamine (Dista Products) 3.7% solution in 0.1M carbonate buffer; 2,3-dimercaptopropanol (BAL) (Boots Pure Drug Co. Ltd.), 1.2% solution in arachis oil. All the solutions were prepared immediately before injection.

Experiments. Each rat was injected intramuscularly into one thigh with mercuric acetate (100 μg/Hg) and 96 hr later groups of two animals of the same sex were placed in metabolism cages. The total weight of the rats was about the same in each cage. Food (M.R.C. diet 41B) and water were available *ad libitum*. Faeces and urine were collected for each 24 hr period on the fifth, sixth and seventh days. At this time the total body burden was 64 to 74% of the dose given and the distribution of mercury in the body was nearly complete and consequently any change in the kidney level and in the amount excreted was easily detected. The animals were killed under ether anaesthesia 168 hr after the mercury injection. Blood was taken from the chest cavity, kidneys and liver were removed, washed, blotted and weighed.

The LD50 of sodium maleate given subcutaneously to male rats was estimated according to Weil (1952).

The mercury content (measured as ^{203}Hg) of organs and excreta was estimated by an automatic scintillation counter (Model 50A, Packard Instrument Co., La Grange, Illinois, U.S.A.) having a counting efficiency of 40%. The body burden was estimated in twelve animals on days 5–7 after the mercury injection with a scintillation counter (Detector N664B, scaler N610A, Ekco Electronics, Ltd., Southend-on-Sea) while the rats were placed in a holder over the crystal. Efficiency was 1.2%.

In vitro *experiments.* Millilitres of phosphate buffer, pH 7.4, M/15, was mixed with 1 ml. 35 mM sodium maleate and later with 1 ml. 3.5 μM (+)-penicillamine or N-acetyl-(+)-penicillamine or 1.75 μM BAL (prepared by Boots without arachis oil and antioxidant). In the controls the thiols were mixed with 9 ml. phosphate buffer. Before and after adding the thiols, the samples were gassed with nitrogen. The thiol concentrations were estimated with DTNB reagent (5,5'dithiobis-2-nitrobenzoic acid) (Aldrich Chemical Co., Milwaukee, Wisconsin) using the extinction coefficient given by Beutler, Duron & Kelly (1963) before and after incubation for 3 hr at 4° C.

Results

Figure 1 shows that when sodium maleate was given to rats on the sixth and seventh days after the administration of 100 μg Hg, doses of 78, 156 and 312 μmoles/kg had no effect on the kidney content of mercury and only 312 μmoles/kg had a marginal effect on the urinary excretion of mercury. There was, however, a dose-dependent increase in the urinary excretion of mercury and a decrease in the kidney content of mercury when the same doses were given in combination with (+)-penicillamine. Table 1 shows that maleate 156 μmoles/kg significantly enhanced the effect of (+)-penicillamine on the urinary excretion of mercury in both sexes but the increase was higher in females. Maleate increased the effect of N-acetyl-(+)-penicillamine only marginally while the effect of BAL was decreased though not significantly. Table 1 also shows that sodium fumarate (the *trans* isomer of maleate) had no effect on the urinary excretion of mercury either with or without

TABLE 1. *Effect of maleate on the urinary excretion of mercury in rats treated with different thiol complexing agents*

Groups	Daily dose of thiol complexing agents (μmoles/kg)	Sex	No. of animals	Daily urinary mercury excretion (μg): Last day before treatment (96–120 hr)	During treatment (120–168 hr): With saline	With fumarate	With maleate
Controls with no complexing agent	—	F	30	1·02(±0·101)	0·69(±0·039)	0·73(±0·083)	0·76(±0·114)
(+)-penicillamine	2×193	F	36	1·06(±0·083)	1·89(±0·127)	1·85(±0·217)	4·20(±0·461)*
(+)-penicillamine	,,	M	24	1·06(±0·041)	—	1·82(±0·115)	2·52(±0·168)†
N-acetyl-(+)-penicillamine	,,	F	28	0·94(±0·065)	—	1·31(±0·106)	1·54(±0·103)
BAL	2×48·3	F	24	1·10(±0·040)	—	3·29(±0·524)	2·86(±0·394)

* $P<0·0025$; † $P<0·01$.

The animals were given 100 μg Hg intramuscularly as mercuric salt labelled with ^{203}Hg. Ninety-six hours later each group was divided into two and groups of two were placed in metabolism cages. Urine and faeces were collected at 24 hr intervals. 120 hr after the mercury the rats were given subcutaneous injections of sodium maleate or fumarate 156 μmoles/kg (groups 1–5) or sodium chloride 156 μmoles/kg (groups 1–2). Groups 2–5 were given one of the thiol complexing agents intramuscularly at 120 hr and again 6 hr later. The next day the whole treatment was repeated. The numbers in parenthesis are the standard errors.

TABLE 2. *Effect of maleate on the kidney, liver and blood contents of mercury in rats treated with different thiol complexing agents*

Groups	Sex	Mercury content (μg): Kidneys: Control	Kidneys: Maleate	Liver: Control	Liver: Maleate	Blood: Control	Blood: Maleate
No complexing agent	F	53·0(±1·15)	52·3(±1·13)	0·84(±0·029)	0·88(±0·060)	0·56(±0·097)	0·60(±0·157)
(+)-penicillamine	F	52·3(±0·61)	43·2(±1·79)*	1·05(±0·068)	1·25(±0·089)†	0·77(±0·018)	0·91(±0·064)†
(+)-penicillamine	M	43·5(±1·10)	40·4(±1·59)†	1·18(±0·098)	1·39(±0·116)	0·71(±0·034)	0·72(±0·034)
N-acetyl-(+)-penicillamine	F	45·9(±1·31)	47·4(±1·43)	0·96(±0·043)	0·95(±0·063)	0·90(±0·073)	0·75(±0·055)
BAL	F	49·1(±1·42)	49·5(±1·63)	1·19(±0·047)	1·23(±0·060)	1·16(±0·036)	1·18(±0·048)

* $P<0·0025$; † $P<0·05$.

Groups and treatments were the same as in Table 1. Rats were killed 7 days after the mercury—that is, at the end of the 2 days of treatment. In the first two groups controls include both the fumarate and saline-treated animals. Mercury content in the whole blood was calculated assuming a blood volume of 7 ml./100 g body weight. The numbers in brackets are the standard errors.

(+)-penicillamine as the urinary excretion was the same in the fumarate and in the saline controls. The faecal excretion of mercury after the complexing agents ranged from 1.04 μg to 2.10 μg Hg per day, but treatment with fumarate or maleate had no effect on this.

All the complexing agents reduced the kidney mercury content (Table 2) and some further decrease was brought about when maleate was given to (+)-penicillamine-treated animals, though the decrease was significant only in the females ($P<0.0025$). Table 2 also shows that not all the mercury removed from the kidneys by the complexing agents was excreted but some was redistributed, resulting in an increase in the blood and liver contents of mercury. Sodium maleate affected the liver and blood content of mercury only in the group treated with (+)-penicillamine.

When the complexing agents used in these experiments were incubated with sodium maleate, it was found that BAL reacted with it but (+)-penicillamine and N-acetyl-(+)-penicillamine did not (Table 3).

Discussion

The results show that maleate in a dose which by itself had no effect on mercury excretion enhanced the effect of one complexing agent ((+)-penicillamine) on the urinary excretion of mercury. During the 2 days of treatment female rats excreted 8.4 μg Hg in urine. This amount equalled 12% of the body burden. Maleate slightly decreased the effect of BAL, however, and had only a slight effect on N-acetyl-(+)-penicillamine. The fact that maleate reacts *in vitro* with BAL but not with (+)-penicillamine may in part account for these findings. Any reaction between maleate and BAL *in vivo* could decrease their concentration and consequently their effect on mercury excretion. N-acetyl-(+)-penicillamine alone was less effective than (+)-penicillamine in increasing the urinary excretion of mercury; maleate enhanced this effect only slightly.

The increase in the urinary excretion of mercury is always associated with a redistribution demonstrated by a change in the kidney, liver and blood contents. Excretion and redistribution do not, however, run parallel. In rats treated with maleate and penicillamine the excretion was higher but the redistribution within the animals was less than in animals treated with BAL, either alone or with maleate. The observed difference in the excretion: redistribution ratio should give a positive advantage to the maleate-penicillamine treatment over BAL when the main aim of the treatment is to reduce the body burden.

TABLE 3. *Effect of maleate on the thiol groups of (+)-penicillamine, N-acetyl-(+)-penicillamine and BAL*

Thiol compound	Thiol concentration (mM)	Decrease in thiol groups (% of initial concentration)	
		Without maleate	With maleate
(+)-penicillamine	0·35	3·2	1·5
N-acetyl-(+)-penicillamine	0·35	5·2	2·0
BAL	0·35	14·2	35·8

Eight millilitres of phosphate buffer (pH 7·4, M/15)+1 ml. 35 mM sodium maleate or 9 ml. phosphate buffer was mixed with 1 ml. 3·5 mM (+)-penicillamine or N-acetyl-(+)-penicillamine or 1·75 mM BAL. The samples were gassed with nitrogen and incubated at 4° C for 3 hr. The numbers are the average of three separate experiments.

(+)-Penicillamine (Aposhian & Aposhian, 1959) is much less toxic than BAL (Fitzhugh, Woodard, Braun, Luskey & Calvery, 1946), but on the molar bases BAL increases the urinary excretion of mercury more effectively than (+)-penicillamine. The experiments reported here, however, have shown that the effect of (+)-penicillamine can be potentiated by a non-toxic dose of sodium maleate. In these experiments the dose of sodium maleate was 30–40 times less than the LD50 for female (5,070 μmoles/kg) or for male (6,400 μmoles/kg) rats (Clarkson & Magos, 1967). The slight difference in the acute toxicity of maleate might explain why it was less effective in male than in female rats in potentiating the action of (+)-penicillamine. The possibility cannot be excluded, however, that this sex difference was at least partly associated with a difference in sensitivity to mercury (Harber & Jennings, 1965) reflected also in the lower kidney content of mercury in male animals treated only with (+)-penicillamine (Table 2).

The present findings may suggest a new therapeutic approach to increase the effect of some metal complexing agents, especially if the decrease of the body burden is the main aim of the treatment.

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