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Uptake and Distribution of Mercury in Mice from Ingesting Soluble and Insoluble Mercury Compounds

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Despite the high toxicity of mercury to man, mercury is still widely used in many types of insecticides, fungicides and paints. The Chinese frequently used cinnabar (crude mercuric sulphide) as one of the ingredients of mild laxative. Even up till now, many Chinese medicines were found to contain a variable proportion of cinnabar (KOH et al. 1977). According to the recommendation of FAO/WHO Expert Committee (WORLD HEALTH ORGANISATION 1972) on the safety limits for various form of mercury compounds, a weekly intake of 0.05 mg Hg/kg body weight is tolerable, that is about 0.3 mg per week for an adult. If this is the safety limit for humans, then the level of mercury in many Chinese medicines is too high to be safe for human consumption.

However, the toxicity of mercury to animals and man still depends greatly on the form of the compound. In general, organic mercury compounds are more poisonous than inorganic mercury (BIDSTRUP 1964). There have been many reports on the mercury uptake and distribution in different animal organs with soluble organic mercury (CEMBER 1962; MAGOS et al. 1980). But so far, very little work has been done by gavage on less soluble inorganic mercuric sulphide which is widely used in the Chinese medicine.

Therefore, it would be interesting to compare short term and long term uptake and tissue distribution of mercury in mice from ingesting pure and crude mercuric sulphide to a more soluble mercuric chloride.

MATERIALS AND METHODS

Animals: Unless otherwise stated, young adult male and female healthy mice of C3H strain of about 20 to 25 g were used. The mice were kept in well ventilated metal boxes. The water and mouse pellets were given ad libitum. The room used for keeping the mice was well-ventilated and kept at a constant temperature of 28°C.

Experimental designs: Mercuric chloride ($HgCl_2$) and mercuric sulphide (HgS) were purchased from Merck, West Germany. Stock solutions of these chemicals of 1000 ppm in distilled water were prepared. P.C. (Trade name) laxative pills manufactured in Hong Kong containing crude mercuric sulphide were used as a representative drug of Chinese medicine. The mercury content of the pills was analysed and was found to be about 3.09×10^3 ug of mercury (Hg^{2+})

per gm fresh weight. The pills were grounded into a powder form after which a required amount of distilled water was added into it so that 0.1cm^3 of the final mixture contained 100 ug of mercury.

Unless otherwise stated, mice were orally force-fed with 0.1cm^3 of the stock solution containing 100 ug of mercury. To introduce the mercury solution into the mouse's oesophagus, a small polyethylene tube attached to one end of the needle was used. The polyethylene tubing was inserted into the mouth of the anaesthetized mice and the tube was gently pushed downwards. It should easily slip into the oesophagus, after which it was then pushed onward into the stomach. If any obstruction was felt, no force was exerted, but another effort was made to find the oesophageal opening.

Determination of mercury: The control and test mice were killed by ether and their various tissues and organs were gently removed. Care was taken in order not to damage the organs such as liver, spleen, kidneys and brain. After the tissues and organs were trimmed into small pieces, they were separately put into conical flasks. Mercury was extracted according to the method of Agemian and Chau (1976) and was analysed by a Perkin-Elmer MAS 50A Mercury Analyzer System.

Histochemical and Histological methods: Internal organs of control and test animals, such as brain, liver, kidneys, lungs, spleen and intestine were fixed in 10% neutral formalin for 3-4 days. They were then dehydrated in the alcohol and cleared in xylene and impregnated in paraffin wax at 60°C. Sections were cut at 7,10 and 15 micron thickness. For histochemical test, sections were dewaxed in two changes of xylene. When almost dry, one drop of solution containing Stannous Chloride and Tantonic acid reagent was added. A coverslip was placed over it and then examined within ten minutes. As a confirmatory test, tincture of iodine was used to dissolve the mercury. Normal tissues were used as a control and subjected to above tests.

For histological studies, tissue sections were dewaxed in two changes of xylene. After which, they were stained with Mayer's Haemalum and subjected to dehydration and clearing and finally mounted at DPX.

RESULTS

Short and prolonged oral exposure to $HgCl_2$ or HgS. Seven groups (each consisting of 15 mice) of young adult mice were used. Mice of the first, second and third groups were treated with thrice weekly feeding of either $HgCl_2$, HgS or of laxative pills containing crude HgS for two weeks (short term exposure), while mice of the fourth, fifth and sixth groups were similarly treated as above but for eight weeks (long term exposure). The last group was used as a control.

The results (Table 1) indicate that the kidneys and spleen of mice fed with $HgCl_2$ for two weeks had accumulated increased amounts of mercury which was significantly different from the rest of the organs ($P < 0.01$). Similar results were also seen in mice fed with $HgCl_2$ for eight weeks.

However, the kidneys and spleen obtained from mice of long term exposure showed three to five times more mercury than that of the kidneys of mice with short term exposure. The rest of the tissues and organs in both test groups showed a similar pattern of mercury accumulation i.e. the liver had the most, followed by brain and muscles. In general, there was a corresponding increase of mercury accumulation in various organs of mice with longer treatment of $HgCl_2$.

On the other hand, the results of the short term and prolonged oral force-feeding of HgS (Table 1) show that the amounts of mercury found in various tissues and organs were negligible and not significantly different from those of the control ($P > 0.01$). However, bones, intestines and lungs from the long term treated mice appeared to have slightly more mercury content than the rest of the studied organs.

No significant mercury concentration was also found in any of the four organs analysed in the two groups of crude HgS - (Chinese laxative pills) treated mice as compared to the control ($P > 0.01$). However, the spleen appeared to show relatively higher mercury concentration as compared to the other organs analysed.

Mercury retention after oral exposure to $HgCl_2$ or HgS. Three groups of young adult mice were used in this experiment. Each group consisted of 15 mice. The mice of the first group were given a total of 1000 ug Hg in the form of $HgCl_2$. The second group was treated with HgS over a period of 7 weeks, total 2100 ug Hg. The third group was used as a control. They were killed for analysis of total amount of mercury in their various tissues and organs on day 7, day 21 and day 35 after the last dose of the mercury was orally force-fed.

The results (Table 2) show that almost all the organs show a decline in the average mercury content. For instance it was found that there was a significant decrease of mercury in the kidneys ($P < 0.01$). In fact, 75% decrease was noted during the first three weeks, followed by a 60% decrease in the next two weeks. The others, e.g. the spleen also showed a decrease though the decrease was not as obvious as that found in the kidneys. The only tissue which showed a slight accumulation, instead of a reduction of mercury, was in the bones.

Mice of the second group fed with HgS showed that there was very little retention of mercury in their various organs. Most of the values were very low and insignificant ($P > 0.01$). The results, however, did show a significant decrease of mercury in the spleen ($P < 0.01$), from 1.18 ug Hg^{2+}/gFW to 0.37 ug Hg/gFW. It is interesting to point out that there was a similar increase of mercury in the bones three weeks after the last dose.

Histochemical and histological analysis. Histochemical studies were carried out in various organs of mice treated with $HgCl_2$, HgS or P.C.

Table 1. Concentrations of mercury in various tissues or organs of young adult mice after short and long term orally feeding of either $HgCl_2$, HgS or crude HgS in Chinese medicine.

Period of treatment (300ugHg^{2+} per week)	Mercury compounds	Amount of Hg^{2+}(ug/gFW)*							
		Muscle	Spleen	Brain	Liver	Kidney	Intestine	Bones	Lungs
2 weeks	Control (without treatment)	0.45±0.45	0.00±0.00	0.10±0.10	0.05±0.05	0.01±0.01	0.04±0.03	0.08±0.04	0.13±0.11
	$HgCl_2$	0.13±0.13	1.31±0.48	0.34±0.20	0.48±0.48	6.5±4.37	-	-	-
	HgS	0.00±0.00	0.45±0.45	0.36±0.28	0.00±0.00	0.22±0.09	0.22±0.22	0.31±0.16	0.06±0.06
	Crude HgS in laxative pills	-	1.41±0.97	0.16±0.12	0.15±0.05	0.79±0.21	-	-	-
8 weeks	Control (without treatment)	0.06±0.02	0.00±0.00	0.01±0.01	0.02±0.02	0.08±0.06	-	-	-
	$HgCl_2$	1.09±0.61	7.71±0.30	1.71±0.44	3.11±2.26	19.1±1.2	-	-	-
	HgS	0.06±0.04	0.26±0.12	0.08±0.03	0.01±0.01	0.34±0.19	0.88±0.38	0.92±0.23	0.72±0.33
10 weeks	Crude HgS in laxative pills	-	1.04±0.01	0.42±0.08	0.19±0.04	0.30±0.03	-	-	-

* Expressed as mean ± SE (Standard error)

FW = Fresh weight

Table 2. Mercury retention in various tissues and organs of mice at different time intervals after $HgCl_2$ (1000 ugHg^{2+}) or HgS (2100 ug Hg^{2+}) oral feeding.

Weeks after the last dose of oral feeding	Mercury Compounds	Amount of Hg^{2+}(ug/gFW)*								
		Blood	Muscle	Bones	Brain	Lungs	Spleen	Intestine	Liver	Kidney
1	Control (without treatment)	0.05±0.03	0.08±0.02	0.10±0.05	0.03±0.03	0.17±0.15	0.00±0.00	0.05±0.05	0.05±0.03	0.09±0.04
	$HgCl_2$	0.28±0.15	0.36±0.30	0.88±0.76	1.37±0.36	3.52±0.09	3.60±1.08	1.41±0.55	2.50±1.00	16.7±5.9
	HgS	0.00±0.00	6.00±0.00	0.19±0.19	0.00±0.00	-	1.18±0.24	-	0.00±0.00	0.01±0.01
3	$HgCl_2$	0.00±0.00	0.19±0.19	1.37±0.89	0.19±0.10	2.08±0.67	0.82±0.44	1.32±0.38	0.16±0.10	4.12±0.25
	HgS	0.11±0.11	0.04±0.04	1.13±0.33	0.02±0.02	-	0.37±0.07	-	0.00±0.00	0.06±0.06
5	$HgCl_2$	0.00±0.00	0.05±0.05	2.36±0.80	0.13±0.17	0.38±0.17	0.36±0.19	0.44±0.22	0.01±0.01	1.67±0.42
	HgS	-	-	-	-	-	-	-	-	-

*Expressed as mean ± SE (Standard error)

laxative pills.

Using histochemical tests, brown to brownish black metallic deposits were noted in various organs, indicating the presence of mercuric particles (Fig. 1). Upon addition of tincture of iodine, these dark particles disappeared, thus confirming the presence of mercury deposits. On the other hand, various organs from controls showed negative results.

In kidney sections (Fig. 1a & 1b), clumps of mercury particles were seen in the veins of the cortical regions, especially in their endothelial cells. Some mercury particles were found scattered in tubule cells and interstitially. Mercury particles were also found in the cells of alveolar walls of the lungs and in the red pulp of the spleen. In the liver (Figs. 1c & 1d), Kupffer cells and endothelial cells of the veins were found to accumulate more mercury particles than elsewhere.

Histological study of the various organs from treated animals showed that there were no histopathological changes in all the studied soft tissues.

DISCUSSION

The results demonstrate that distribution patterns of mercury after administration of various mercuric compounds are similar, irrespective of the degree of solubility. The highest concentration of mercury was found in the kidneys and the least amount was seen in the muscle, blood, skin and brain. This is well illustrated in Table 1. Using $HgCl_2$, it is found that amounts of mercury in kidneys were always significantly higher with respect to all the other studied organs.

The higher concentration of mercury in kidneys was explained by Wisniewska et al. (1970) that kidneys might be an organ to detoxify mercury by binding it to metallothionein, a low molecular-weight protein. Histochemical studies showed that many mercury particles were mostly found in the cortical vein and the cells of the convoluted tubules. In fact, Friberg (1956) showed that after repeated injections of ^{203}Hg, at least a part of mercury excreted in urine was derived from the mercury accumulation in kidney tubules. The results in Table 2 showed that in kidneys there was a significant decrease of about 75% of mercury three weeks after the last feed. This at least indicates the body particularly the kidneys can efficiently excrete mercury from the body provided its various tissues and organs are not being overloaded with the heavy metal.

This view is further supported by the findings in mice which were orally-fed with pure or crude HgS of very low solubility. In the experiment (Table 1) the kidneys of the HgS treated mice showed no significant increase in the level of mercury despite prolonged treatment of the heavy metal. This result further demonstrates the excretion efficiency of kidney if the amount of absorption of Hg^{2+} in the intestine is low. In order to prove this point (unpublished data), a group of young adult mice were fed with more soluble $HgCl_2$ of low concentration in their drinking water (25 ul/ml) for 9 months. It was found that there were no significant difference in the amount of mercury accumulated in kidneys, liver and spleen. On the other hand, the amount of mercury in testis was about twice as

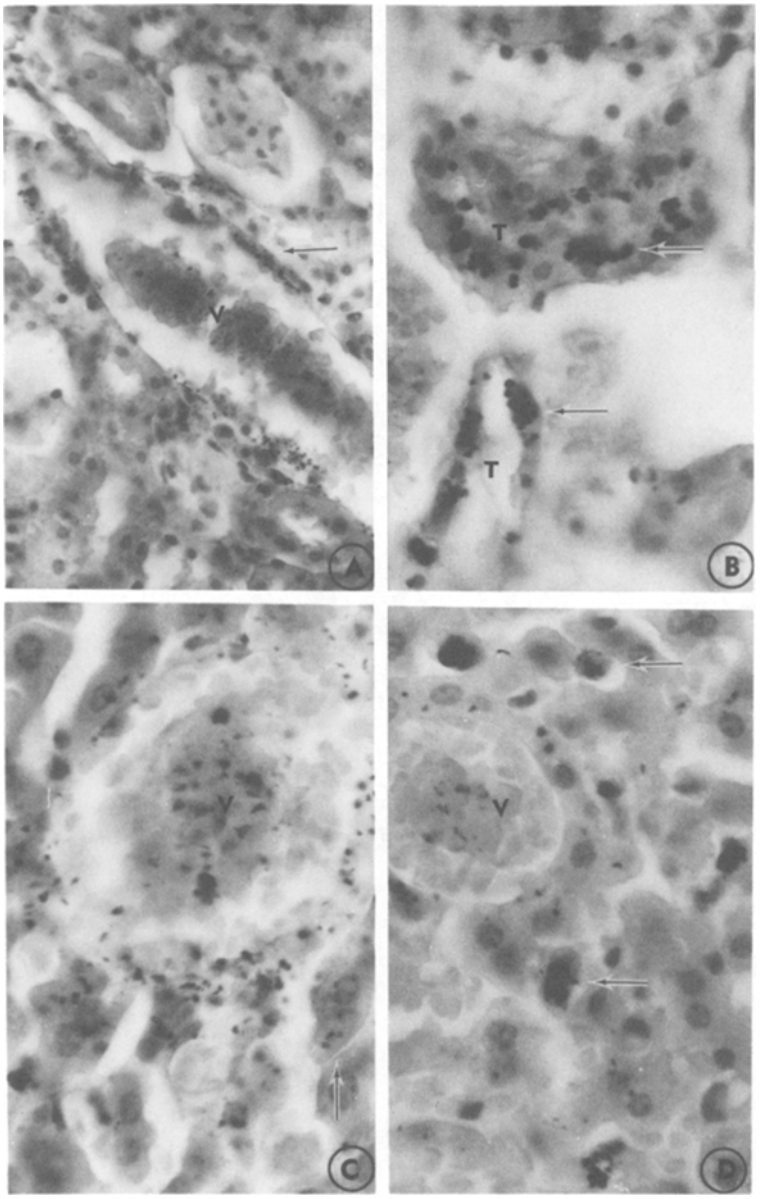

Figure 1. Organs from a mercury-treated mouse. A : Kidney. The dark granules are the mercury granules found in the lumen of vein (V) and its endothelial cells (arrow pointed). B: Kidney. Mercury granules within the tubule cells (arrows pointed). C : Liver. Vein (V) and its endothelial cells contained abundant mercury granules. The arrow points to a liver cell with mercury granules. D : Liver. Cells loaded with abundant dark granules (mercury) are Kupffer cells. There are fewer granules in the lumen of the vein (V).

much as the kidneys.

In our experiments, $HgCl_2$ and HgS were used and their solubilities are 6.9 g/100 cm^3 and 1 x 10^{-6} g/100 cm^3 respectively. Data obtained from Table 1 showed a lower mercury absorption rate was found in mice fed with a less soluble compound - HgS. Even though one group of mice had a prolonged oral exposure to P.C. laxative pills (Table 1), there was no significant retention of mercury in any of the studied organs. This indicates that the nature of mercury sulphide had not been affected during the preparation of these pills at least in terms of its solubility and toxicity.

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