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Effect of Lead on Tissue Deposition of Mercury in Mice

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Lead and mercury are two common toxic industrial pollutants found in our living environment. The inorganic forms of these heavy metals enter food chains and end up in our food and drinks (Bryce-Smith and Waldron 1974; Koh et al. 1976). Many studies have been done on the uptake and toxicity of individual heavy metals in animal tissue (Berlin et al. 1966; Chisolm 1971; Sin et al. 1982). However, little is known about the interaction of lead with mercurial compounds in animal tissue. Congiu and his collaborators (1979) found that when lead nitrate was intravenously administered to mice followed by organic mercury via gavage, the accumulation of mercury in the kidneys was enhanced.

In view of this, it would therefore be interesting to examine the deposition of inorganic mercury in animal body when lead and the inorganic mercury were both orally co-administered or administered separately.

MATERIALS AND METHODS

Young adult male C3H mice weighing 20 to 25g were used throughout the experiment. Six animals were used per group and each experiment was repeated 5 times.

Lead nitrate and inorganic mercuric chloride from Merck, West Germany were used. Stock solutions of these chemicals of 2000 ppm in distilled water were prepared. Unless otherwise stated, mice were orally force-fed with 0.1 cm^3 of water diluted stock solution containing 25 µg, 50 µg and 100 µg of lead, followed by 0.1 cm^3 (200 μgHg^{2+}) of mercury chloride.

All mice were killed 24 hours after the mercury treatment. This was done by anesthetizing them with ether and then bled to death by draining their blood from jugular vein. Three visceral organs (kidneys, liver and spleen) were removed. Mercury was extracted by the method of Agemian and Chau (1976) and quantitatively determined with a Perkin-Elmer MAS 50A Mercury Analyser System. All the results are expressed as mean ± standard error and then analysed by Student's t-test for their significance.

The three visceral organs of these animals were also fixed in 10% neutral formalin for 3-4 days. They were then dehydrated and impregnated in paraffin wax. Sections were cut at 10 micron thickness and dewaxed. The unstained slides were examined for mercury deposits with stannous chloride and tartaric acid reagent. The presence of the mercury particles were further confirmed by iodine solution as previously described (Sin et al. 1983). These slides were then stained with Mayer's Haemalum.

Occasionally, free hand sections of these organs were prepared. After 3 days in formalin fixative, they were frozen overnight. The next day, the samples were thawed and cut with razor blade. The sections were then washed and mounted onto the slides using glycine jelly.

RESULTS AND DISCUSSION

In the present study three different series of treatments were performed. In the first series of treatment, the mice were pretreated with $Pb(NO_3)_2$ followed with $HgCl_2$. In this series four groups of young adult mice were used. Group I was used as a control and without lead treatments. Group II, III and IV were fed with 25 μg, 50 μg and 100 μg Pb^{2+} respectively by gavage. The mice in each group were then administered with mercury by gavage 24 hours after the lead treatment.

The results (Table 1) showed that mercury accumulated most in the kidneys followed by the liver and spleen in all the test and control mice. In the kidneys of mice treated with 25 μg lead, the Hg^{2+} concentration did not differ significantly from that of the control. However, kidneys from mice treated with 50 μg and 100 μg Pb^{2+} showed significantly lower ($P<0.01$) Hg^{2+} concentration than that of the control and the 25 μg Pb^{2+} treated animals. There was a slight decrease of Hg^{2+} concentration in the liver of all lead treated animals but this decrease was not significant ($P>0.01$) when compared with that from the control group. In general, the amount of the Hg^{2+} in spleen of the treated mice was found to be slightly higher than that of the control animals.

In the second series of treatments, the effect of lead on mercury uptake in mice when co-administered by gavage was studied. The mice were divided into 5 groups. Group I was used as a control and orally fed with a dosage of 0.05 cm^3 of distilled water and 0.05 cm^3 $HgCl_2$ (200 μg Hg^{2+}). Groups II, III, IV and V were also orally fed with 0.05 cm^3 of $HgCl_2$ (200 μg Hg^{2+}) but co-administered with 0.05 cm^3 of $Pb(NO_3)_2$ of various concentrations, 25 μg, 50 μg, 100 μg and 200 μg respectively. The results (Table 2) showed that the kidneys accumulated Hg^{2+} most followed by lower amounts of mercury in the liver and spleen. However, there was no significant difference ($P > 0.01$) in the mercury concentrations in the kidneys or liver with increasing lead concentrations. This was in contrast to the spleen where there was a significant increase ($P < 0.01$) in the mercury concentrations

Table 1. Mercury content in three visceral organs of mice orally fed with various dosages of Pb^{2+} followed by $HgCl_2$ (200 μg Hg^{2+})

Group No. (Lead treatment)		Amount of Hg^{2+} (μg/g wet wt)		
		Kidneys	Liver	Spleen
I	(Control)	21.09 ± 1.44	4.29 ± 0.41	1.13 ± 0.43
II	(25 μg)	20.46 ± 1.87	3.26 ± 0.59	3.28 ± 0.50
III	(50 μg)	14.28 ± 1.42*	3.34 ± 0.57	3.14 ± 0.34
IV	(100 μg)	15.23 ± 1.48*	3.99 ± 0.37	2.25 ± 0.14

* Significantly different from control ($P < 0.01$)

with the increase in lead concentrations, especially when the lead concentrations were 100 μg and 200 μg Pb^{2+}.

In the third series of treatments, the effect of lead administered by intravenous injection on mercury retention in mice was examined. Three groups of mice were used in this series. Group I was used as a control and without any treatments. Group II was given 400 μg Pb^{2+} via intravenous injection followed by 200 μg Hg^{2+} by gavage. Group III was subjected to 0.9% physiological saline via intravenous injection followed by oral-feeding of 200 μg Hg^{2+}.

The results (Table 3) showed that the kidneys of group II had significantly lower mercury content ($P < 0.01$) than those of group III. On the contrary, the spleen of group II showed significant higher mercury content ($P < 0.01$) than that of group III. However, no significant difference was found in the total amount of mercury from the four tissue samples between group II and III.

In our previous work (Sin et al. 1983) we have demonstrated that there is a consistent pattern of mercury distribution in the various tissues and organs of mice treated with different mercuric compounds irrespective of the degree of their solubility. The highest concentration of mercury was found in the kidneys followed by a relatively lower amount of Hg^{2+} in the liver and spleen. In the present study, it was further shown that this distribution pattern of mercury was not altered when the animals were pretreated or co-administered with different dosages of lead.

However, the present results indicate that some interactions occured between the two studied elements after they were introduced into the animal body. Table 1 showed that when mice were pretreated with 50 μg and 100 μg of lead followed by gavage introduction of 200 μg Hg^{2+}, the total concentration of mercury

Table 2. Amount of Hg^{2+} (μg/g wet wt) in different organs of mice when $HgCl_2$ (200 μg Hg^{2+}) was orally co-administered with various $Pb(NO_3)_2$ concentrations.

Organs	Pb Dosage (μg Pb^{2+})				
	Group I (0)	Group II (25)	Group III (50)	Group IV (100)	Group V (200)
Kidneys	26.30 ± 1.10	27.23 ± 1.00	25.04 ± 3.18	23.67 ± 2.18	23.83 ± 0.86
Liver	3.73 ± 0.53	5.13 ± 0.90	4.19 ± 0.44	5.13 ± 0.65	4.75 ± 0.35
Spleen	1.31 ± 0.12	3.72 ± 1.17	5.81 ± 0.43*	9.08 ± 1.86*	10.24 ± 1.04*

* Significantly different from control ($P < 0.01$)

Table 3. Mercury content in the kidneys, liver, spleen and blood of mice intravenously given a dosage of either $Pb(NO_3)_2$ (400 μg Pb^{2+}) or physiological saline followed by $HgCl_2$ (200 μg Hg^{2+}) by Gavage 24 hours later.

Tissues and Organs	Amount of Hg^{2+} (μg/g wet wt)		
	Group I	Group II*	Group III*
Kidneys	0.14 ± 0.02	15.27 ± 1.51	21.19 ± 0.94
Liver	0.01 ± 0.01	4.04 ± 0.56	3.88 ± 0.56
Spleen	0.32 ± 0.08	4.37 ± 0.50	0.78 ± 0.12
Blood	0.13 ± 0.07	0.66 ± 0.12	0.70 ± 0.09

* No significant difference in total Hg^{2+} from the four tissue samples between group II and III.

from the three organs studied was much lower than that of the control. This decrease was obviously due to the significant decrease of Hg^{2+} deposition in their kidneys. However, no such effect was observed when the 50 μg and 100 μg of lead and 200 μg mercury were co-administered by gavage (Table 2). There are two possible ways to explain this phenomenon. It has been shown that kidneys, bones and liver accumulated the highest lead concentration after artificial feeding of mice (Fick et al. 1976) and fish (Sin et al. 1982) with lead salts. Therefore, it is reasonable to assume that many tissue sites in those organs for binding heavy metals were probably occupied by the Pb^{2+} which was administered 24 hours before the Hg^{2+} treatment. Thus, this might result in the decrease of the Hg^{2+} uptake in these organs, particularly the kidneys. Another explanation is that a dosage of over 50 μg lead administered 24 hours earlier than the Hg^{2+} treatment might affect the permeability of the epithelial tissues of gastrointestinal tract towards the mercury. But how this lead affects the absorption rate of the mercury in the gastrointestinal tract remains to be further investigated. The toxicity of lead is very complex. It is known to exert its toxic effect in part through interference with copper, zinc and iron metabolsim (Niklowitz and Yeager 1973). But so far, very little work has been reported of its effect on mercury. Since lead (Danielson 1970) and mercury (Clarkson 1971) are both known to complex with SH groups and other ligands in the tissues of the body, therefore, one could assume the decrease of total mercury uptake in the organs of mice might be due to the competition between the two metals for similar transport binding sites. It is possible that the cross-linkage between lead and the substances of the gastrointestinal tract might interfere with the absorption rate of Hg^{2+} by the tissue. This view was further supported by the results of Table 3 when 400 μg Pb^{2+} was administered to mice by

intravenous injection, followed by 200 μg Hg^{2+} via gavage. The findings showed that there was no significant difference in the total amount of mercury from the organs between the two studied groups. This experiment clearly demonstrated that the presence of lead in the blood did not affect the absorption rate of the mercury through the gastrointestinal tract. On the other hand, the findings showed that the presence of lead in the blood circulation would decrease the Hg^{2+} uptake of the kidneys as seen in those mice pretreated with lead. Therefore, it is reasonable to conclude that lead not only affects the kidney's uptake of Hg^{2+} but also influences the absorption rate of Hg^{2+} in the epithelial tissues of the gastrointestinal tract.

The increase of Hg^{2+} in the spleen was encountered in the lead-treated mice throughout the present studies. Histological and histochemical studies of the spleen showed that the mercury was mainly found in the lumen of veins and the phagocytic cells in the red pulp of this organ (Fig. 1). These findings thus indicated that phagocytic activity of the reticulo-endothelial system of the spleen was enhanced. On the contrary, the decrease of Hg^{2+} deposition was encountered in the kidneys of those lead-treated animals, particularly those which had intravenous injection of lead (Table 3). It is possible that the lead might interfere the mercury transfer from the kidneys to the spleen, resulting in relatively lower kidney concentrations.

Since co-administration of the two elements caused a significant increase in mercury concentration in the spleen with increasing lead concentrations, the interaction between these two elements might be responsible for the increase of Hg^{2+} uptake in the spleen. The chemical formation of aggregates between lead and mercury is unlikely. However, the presence of considerable amount of Hg^{2+} in the packed blood cells of the veins in these visceral organs suggested that the two elements interacted with the blood cells. Therefore, it is possible that both lead and mercury when administered at short intervals or combined together are both attracted to the SH groups of the circulating erythrocytes and thus damaged the erythrocytes more rapidly than the individual element alone. As a result, these chemically altered erythrocytes would behave like old worn-out cells which were phagocytised and rapidly removed from the blood circulation by the phagocytic cells in the reticulo-endothelial system of the spleen. This might account for the increase in mercury in the spleen during co-administration of the two elements. It is interesting to point out that similar increased accumulation of mercury was also found in the spleen of animals after simultaneous administration of both mercury and selenium (Hansen and Kristensen 1979).

The results of the present studies, however, do not agree with the findings of Congiu et al. (1979) who had shown that lead nitrate treatment via intravenous injection followed with MeHgCl via gavage increased the mercury content in the kidneys. These workers concluded that the increased mercury deposition in the

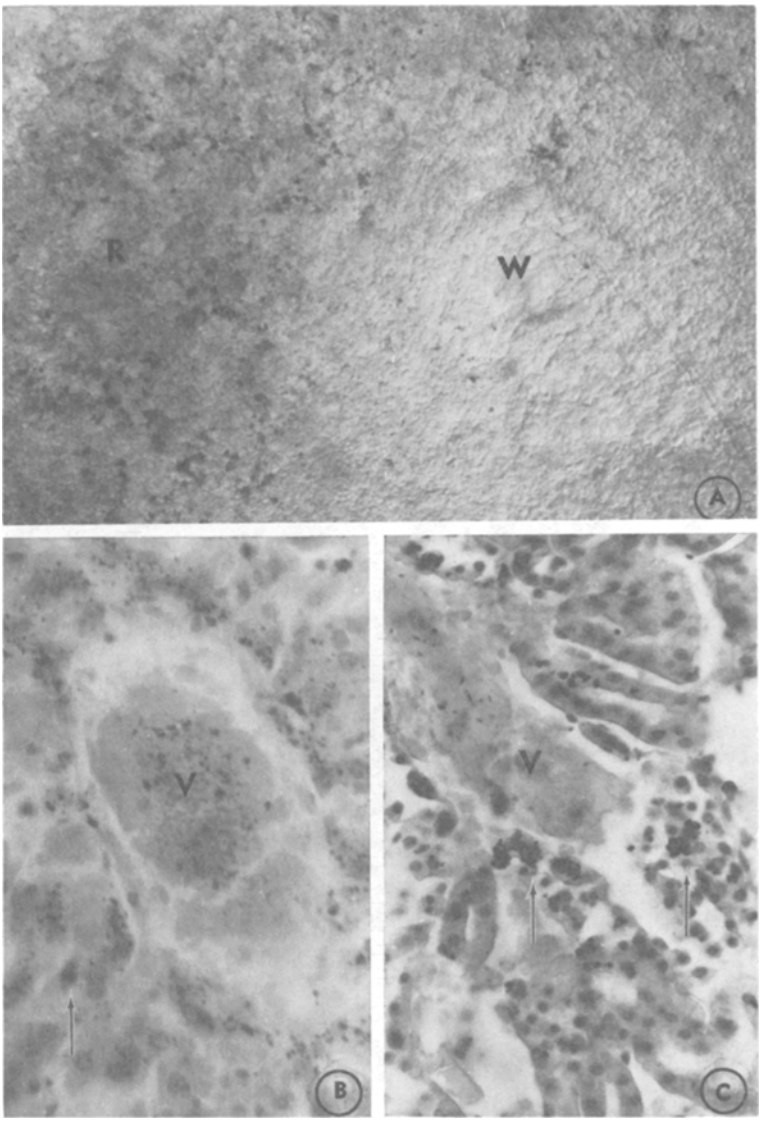

Fig 1. Sections of visceral organs from mice orally fed with lead nitrate and followed by mercury chloride. (A) spleen (unstained free hand section). The dark granules (mercury deposits) were found in the red pulp(R) but rarely seen in the white pulp(W). (B) Liver. Lumen of a vein(V) and the adjacent liver cells contained mercury granules. The arrow points to a Kupffer cell containing mercury granules. (C) Kidney. The tubule cells next to a vein(V) were loaded with mercury deposits (arrows pointed).

kidneys was due to the increase of tissue glutathione level in the kidneys. If that is the case, then the present experiments strongly suggest that unlike methylmercury the inorganic mercury does not complex with glutathione. This accounts for the relatively similar levels of mercury in the kidneys in spite of varying concentrations of lead being given.

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