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Abstract

To clarify the relationship between the catalase activity in mouse organs and the amounts of metallic mercury exhaled, normal, homozygous hypocatalasemic and acatalasemic mice were injected with mercuric chloride. The cumulative amount of metallic mercury exhaled by mice was evidently expressed in the descending order of acatalasemic, hypocatalasemic, and normal mice. Statistically significant differences in the cumulative exhaled metallic mercury levels were observed between acatalasemic and hypocatalasemic mice, between normal and hypocatalasemic mice, and between acatalasemic and normal mice using the method of one way analysis of variance (ANOVA). A linear relationship was obtained through logarithm of catalase activity in the lungs or the blood, and logarithm of the cumulative amount of the exhaled mercury.

KEYWORDS: catalase, metallic mercury, acatalasemic mice, hypocatalasemic mice, exhalation

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Exhaled Metallic Mercury in Acatalasemic, Hypocatalasemic and Normal Mice Injected with Mercury (II) Chloride

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To clarify the relationship between the catalase activity in mouse organs and the amounts of metallic mercury exhaled, normal, homozygous hypocatalasemic and acatalasemic mice were injected with mercuric chloride. The cumulative amount of metallic mercury exhaled by mice was evidently expressed in the descending order of acatalasemic, hypocatalasemic, and normal mice. Statistically significant differences in the cumulative exhaled metallic mercury levels were observed between acatalasemic and hypocatalasemic mice, between normal and hypocatalasemic mice, and between acatalasemic and normal mice using the method of one way analysis of variance (ANOVA). A linear relationship was obtained through logarithm of catalase activity in the lungs or the blood, and logarithm of the cumulative amount of the exhaled mercury.

Key words : **catalase, metallic mercury, acatalasemic mice, hypocatalasemic mice, exhalation**

There have been many reports describing that the metallic mercury is oxidized to Hg^{2+} by catalase *in vitro* and *in vivo*. Magos *et al.* (1) estimated that metallic mercury would be oxidized to Hg^{2+}, by catalase complex I ($Fe^{3+}OOH$) using the reaction formula described by Deisseroth and Dounce (2).

An excretion of mercury by laboratory animals in urine and feces does not account for 100 % of the body-burden loss (3, 4). Clarkson *et al.* (5) reported the exhalation of small amounts of mercury from anesthetized rats injected with mercury (II) chloride and has suggested that mercuric ion was reduced to metallic mercury in the blood and excreted through the lungs (6). Dunn *et al.* (7) reported that the rate of mercury exhalation from mice injected with $^{203}HgCl_2$ increased dramatically after administration of ethanol compared with control mice.

An oxidation of metallic mercury by catalase *in vitro* and *in vivo* was demonstrated by using the blood of acatalasemic Japanese patients (8) and acatalasemic mice (9).

The exhalation of metallic mercury by animals exposed to mercury vapor (10) or by acatalasemic mice injected with metallic mercury (11) was demonstrated. An inverse relationship between catalase activity and the amount of the exhaled mercury has been observed. This report concerns the difference in the amount of metallic

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mercury exhaled by acatalasemic, hypocatalasemic and normal mice injected with $^{203}HgCl_2$.

Materials and Methods

Mice. Acatalasemic ($C^3H/AnLC_s^b$) (12), hypocatalasemic ($C^3H/AnLC_s^c$) and normal mice ($C^3H/AnLC_s^a$) of an inbred strain, weighing 20–25 g, were used in the present study. Radiation-induced hypocatalasemic and acatalasemic mice were obtained through the courtesy of R. N. Feinstein. Groups of acatalasemic, hypocatalasemic and normal male mice (four mice in each group) were injected intraperitoneally with 10 μCi of radioactive mercury (II) chloride solution diluted in 0.9 % of saline solution.

Reagents. $^{203}HgCl_2$ solution was used with a specific activity of 0.52 mCi/mg (New England Nuclear, Boston, MA, USA). Hopcalite (I) (MnO_2 50 %, CuO 30 %, Co_2O_3 15 %, and Ag_2O 5 % mesh 10–24) was purchased from Nakarai Chemicals Ltd., Kyoto, Japan and used as an absorbent for exhaled metallic mercury vapor. All other reagents used were of analytical grade.

Injection and measurement of mercury. Immediately after intraperitoneal injection of $^{203}HgCl_2$ into acatalasemic, hypocatalasemic and normal mice, each mouse was placed in an exhalation chamber at room temperature (20 °C). A tube (6 mm $\varnothing \times$ 4 cm) was packed with 1.0 g of Hopcalite (I) and both ends of the tube were plugged with cotton wool. The metallic mercury exhaled from mouse in the exhalation chamber was trapped in the Hopcalite (I) tube. Air in the exhalation chamber was aspirated at a rate of 1.5 L/min using a air pump (AP-032Z, Iwaki Co. Ltd., Tokyo, Japan). The radioactivity of metallic mercury exhaled from acatalasemic, hypocatalasemic and normal mice in the Hopcalite (I) in the absorbent tube was determined with a Multi-Mode Scaler scintillation counter (Aloka Co. Ltd., Tokyo, Japan TDC-601).

Assay of Catalase activity. Catalase activity in the lungs and venous blood of the mice was determined by Feinstein's method (13).

Statistical analysis. One-way analysis of variance (ANOVA) was used for the statistical procedure, primarily because the numbers of mice in each group were limited to 4. The timed (*i.e.* by the hour) amounts of exhaled metallic mercury and the cumulative amounts of exhaled metallic mercury among the normal, hypocatalasemic and acatalasemic mice were analysed by the use of one-way ANOVA. After the analysis, the differences in the mean values for the timed (hourly) exhaled metallic mercury and those for the cumulative one were analysed between each pair among the normal, hypocatalasemic and acatalasemic mice by using the multiple comparison of Tukey's mthod (14).

Results

Catalase activity. The mean values and standard error of means in the catalase activity in the lungs seen in experimental acatalasemic, hypocatalasemic mice and normal mice were (7.6 ± 0.7), (14.7 ± 1.2), and (42.3 ± 4.1) (PU/g wet weight), respectively, while those in blood, found in acatalasemic, hypocatalasemic and normal mice were (36.8 ± 4.5), (164.8 ± 27.9), and (825.9 ± 80.5) (PU/g Hb), respectively.

Time course of exhalation of metallic mercury. The hourly exhaled metallic mercury levels over a period of time for acatalasemic, hypocatalasemic and normal mice are shown in Table 1. The hourly exhaled metallic mercury levels increased gradually over a time and presented a descending order in acatalasemic, hypocatalasemic and normal mice at all times after each injection. The results of one way ANOVA of the hourly exhaled metallic mercury among normal, hypocatalasemic and acatalasemic mice injected with $^{203}HgCl_2$ are shown in Table 1. Moreover, significant diffrences were seen over the entire time course ($p < 0.05$). The differences among the mean values for the hourly exhaled metallic mercury levels in a span of time for each group were analysed by Tukey's method for purposes of multiple comparison (14) (Table 1). Significantly, this method has demonstrated that the hourly exhaled metallic mercury levels differed (or varied) substantially ($p < 0.05$) throughout the time duration after injection of $^{203}HgCl_2$ between acatalasemic, and hypocatalasemic, between acatalasemic and normal, and between normal and hypocatalasemic mice.

Cumulative levels of metallic mercury in the

Table 1 Statistic analysis of the data for the amout of hourly exhaled metallic mercury and cumulative levels of exhaled metalic mercury over time among normal, hypocatalasemic and acatalasemic mice injected with 10 μCi of mercury (II) chloride.

Subject	Time (h)	Mean value[a] (%)			ANOVA F value
		A[b]	H[b]	N[b]	
Hourly	1	0.01974	0.00795	0.00385	11.37*
	2	0.02550	0.01503	0.00906	5.15*
	3	0.03806	0.01618	0.00773	12.30*
	4	0.03285	0.01974	0.00604	14.72*
	5	0.04690	0.01676	0.00646	9.25*
	6	0.04072	0.01714	0.00936	15.97*
	7	0.05884	0.01752	0.00932	7.71*
	8	0.04056	0.01798	0.00954	14.72*
Cumulat	1	0.01974	0.00795	0.00385	11.37*
	2	0.04524	0.02293	0.01291	10.42*
	3	0.08330	0.03915	0.02064	12.85*
	4	0.11615	0.05888	0.02668	14.53*
	5	0.16305	0.07564	0.03314	14.40*
	6	0.18263	0.09278	0.04250	12.96*
	7	0.23939	0.11031	0.05182	11.42*
	8	0.28104	0.12827	0.06136	13.19*

Subject: Hourly; the amount of hourly exhaled metallic mercury over time, Cumulat; cumulative amount of exhaled metalic mercury over time. ANOVA: Analysis of variance. *: $p < 0.05$. A: Acatalasemic mice, H: Hypocatalasemic mice, N: Normal mice.

[a]: (μCi of mercury in exhaled air/μCi of mercury injected) $\times$ 100. [b]: Significant differencs were shown in all the comparisons (N *vs.* H, H *vs.* A, and N *vs.* A) at any time ($p < 0.05$).

exhaled air. The total amounts of metallic mercury in the exhaled air of normal, hypocatalasemic and acatalasemic mice are shown in Fig. 1 and Table 1. A cumulative amount of the exhaled metallic mercury was observed in the descending order from acatalasemic, hypocatalasemic and normal mice.

The results of one-way ANOVA of cumulative exhaled metallic mercury levels among the normal, hypocatalasemic and acatalasemic mice injected with $^{203}HgCl_2$ are shown in Table 1. Significant differences were seen over the entire time course ($p < 0.05$). Differences between mean values for the cumulative amounts of exhaled metallic mercury for each group were analysed by using Tukey's method for multiple comparison (14). Data gathered indicated that the cumulative mercury levels were significantly different ($p < 0.05$) throughout the total time course after injection of $^{203}HgCl_2$ between acatalasemic and hypocatalasemic, and between acatalasemic and normal, and between hypocatalasemic and normal.

Relation between catalase activity in blood and lungs, and cumulative amounts of exhaled mercury. A negative correlation was recognized between the logarithm of catalase activity in the blood or lungs and the logarithm of cumulative levels of the exhaled mercury among the three groups of mice (Fig. 2). The regression equation between the blood catalase activity or pulmonary catalase activity and the cumulative levels of exhaled mercury were determined as follows:

$$\text{Blood}: Y = 23.23 - 0.022X \quad (r = -0.83)$$
$$\text{Lungs}: Y = 20.70 - 0.018X \quad (r = -0.74),$$

where Y represents logarithm of cumulative level of exhaled metallic mercury (nCiHg), and X represents logarithm of catalase activity of blood (PV/gHb) or of lungs (PU/g).

in acatalasemic, hypocatalasemic and normal mice.

The data indicate that the catalase activity in the blood and lungs was influenced by the exhaled levels of metallic mercury among the three groups

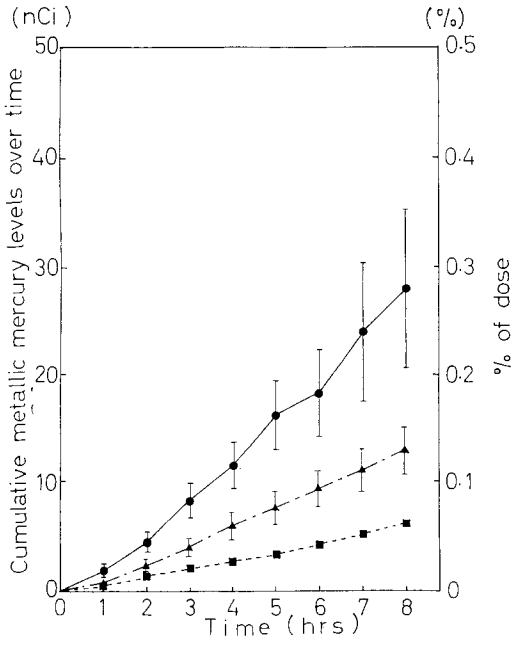

Fig. 1 Cumulative amount of metallic mercury exhaled from acatalasemic, ●—●: hypocatalasemic ▲—▲: and normal mice ■—■: after intraperitoneal injection of $^{203}HgCl_2$ (10 μCi) (m ± SEM). Amounts of metallic mercury are expressed as percent of injected amounts of mercury (II) chloride.

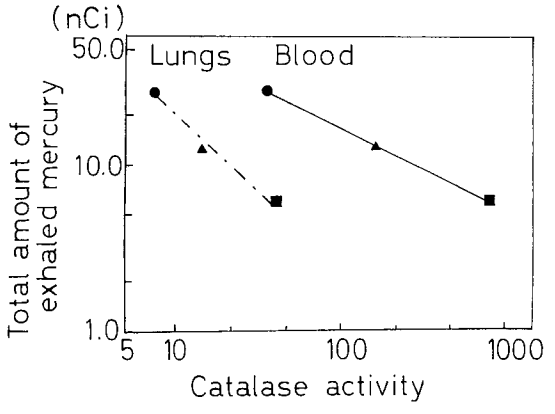

Fig. 2 Relationship between the catalase activity in the lungs (PU/g w. w) or the venous blood (PU/gHb) and the cumulative amounts of metallic mercury (10 μCi) (m ± SEM).
●: Acatalasemic, ▲: Hypocatalasemic and ■: Normal mice.

of mice injected with mercuric chloride.

Discussion

Our previous report regarding exhalatin study confirmed that the reduction of mercuric ion to metallic mercury occurs in the tissues by superoxide anion and NADPH or NADP (15), and postulated that the resulting metallic mercury is reoxidized by catalase as follows:

$$\text{mercuric ion} \xrightarrow[\text{superoxide anion}]{\text{reduction}} \text{metallic mercury}$$

$$\text{metallic mercury} \xrightarrow[\text{catalase and peroxidase}]{\text{oxidation}} \text{mercuric ion}$$

Dunn *et al.* (7) showed that the rate of exhalation of metallic mercury in mice injected with mercuric chloride is increased by ethanol, thereby, suggesting that the oxidation of metallic mercury is metabolized by catalase and inhibited by ethanol. The total amount of mercury exhaled from acatalasemic mice was significantly higher than that of the normal mice, explainable by the assumption that the oxidation of metallic mercury by catalase in acatalasemic mice is less than that of the normal mice.

In the present study, the increased catalase activity in the lungs or in the blood of normal, hypocatalasemic and acatalasemic mice resulted in the decreased levels of metallic mercury exhaled. Further, in our previous study (16), the ratio of mercuric ion concentration in the lungs of acatalasemic mice to mercury (II) chloride injected was 3.86 % which was higher than 3.67 % in normal mice, while its ratio in the blood of acatalasemic mice was 6.06 % which was almost similar in normal mice, 6.00 %. The authors have also reported that the concentration of metallic mercury in the arterial blood (18) of acatalasemic mice was highr than that of normal mice. Therefore, data suggested that concentrations of metallic mercury were higher in both the lungs or blood of mice having less catalase activity compared to those with increasingly high catalase activity. Our results (17) levels agreeably with those reported by Dunn *et al.* (7), which described that ethanol treatment led to an eight-fold

increase of mercury accumulation on a filter over a four-hour period, compared with those placebo, water-treated mice. Thus, a negative linear relationship was obtained between the logarithm of catalase activity in the lungs or blood in the three kinds of mice and the logarithm of the amounts of exhaled metallic mercury.

As for the amount of exhaled metallic mercury, a discrepancy was shown between our data in this present study with those of the former data (15). Percent of injected dose of the hourly exhaled metallic mercury in this study showed higher amounts than the data of our former study (15) *i. e.,* a remarkable increase from within 2 to 4 h after injection. The amounts of metallic mercury exhaled by mice injected with mercury (II) chloride were 0.027 % for until 4 h, which was almost similar to the results of Dunn *et al.* (7). And also amounts of metallic mercury exhaled from mice were also higher than those in our previous report (15) primarily due to Hopcalite (I), which has a higher trapping activity of metallic mercury than that of $KMnO_4$. Until now, the motivating factor behind the amounts of mercury exhalation being decreased for up to about 3 h is not clear. It should be emphasized that the former experiment was carried out at 28 °C (15), while the present one was done at 20 °C. Further investigation must be done to clear this out.

As mentioned above, mercuric ion was reduced to the metallic mercury by superoxide anion and NADPH or NADH and the reduction of mercuric ion to metallic mercury occurred by recycling of mercury in the tissues and the metallic mercury was reoxidized to mercuric ion by catalese. The amount of metallic mercury oxidized by catalase in lung was less in acatalasemic mice than in normal mice. The metallic mercury escaping from reoxidization may be exhaled.

Furthermore, these results suggest that catalase plays an important role in the oxidation of metallic mercury yielded from the mercuric ion.

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