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REDUCED DEGRADATION OF METHYL MERCURY CHLORIDE IN INTESTINAL CONTENT OF GERMFREE MICE

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ABSTRACT

Degradation and fecal excretion of methyl mercury chloride in germfree mice was compared to that of conventional mice after intraperitoneal administration of methyl mercury chloride. The relative amount of inorganic mercury in liver, kidney and small intestinal content of germfree mice was similar to that of conventional mice. However, in large intestinal contents and feces it was lower in germfree mice than in conventional mice. The amount of inorganic mercury excreted in feces during seven days was much lower in germfree mice (2.5% of total mercury administered) than in conventional mice (16.8%), while the amount of organic mercury excreted was about the same.

These results suggest a reduced degradation of methyl mercury in the intestinal contents of germfree mice and the importance of intestinal flora to the amount of inorganic mercury in the feces of mice.

It has been reported that methyl mercury compounds administered to animals are cleaved and inorganic mercury is released within the animal body[3,5,8,9]. Since inorganic mercury is preferentially excreted in feces, it is considered that the release of inorganic mercury from methyl mercury in the body may play an important role in the excretion of methyl mercury compounds[10]. It has also been reported that methyl mercury compounds become degraded in human feces[1] and by microorganisms isolated from environmental samples[2,11,12,13].

In our previous study[4], when methyl mercury chloride (MMC) was singly administered orally, the amount of mercury excreted with the feces of germfree mice during 10 days was about half that of the control conventional mice. This fact suggested the difference of the release of inorganic mercury from MMC in the animal body (tissues and intestine) between germfree and conventional mice.

In this paper, the amounts of organic and inorganic mercury in feces, kidney, liver, intestine and intestinal content of germfree mice to which MMC was administered are compared with those of conventional mice.

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MATERIALS AND METHODS

Germfree ICR-JCL female mice (from CLEA, Tokyo) approximately 10 weeks of age and 27–31 g in weight were used. Conventional ICR-JCL female mice, whose ages and body weights were similar to the germfree mice, were used as control group. Both the germfree and the control mice were allowed to feed freely on a sterilized pellet diet and sterilized water. The germfree mice were fed and treated in a vinyl isolator disinfected by hyperacetic acid spraying. The absence of germs was confirmed by incubating feces at 20 °C and 30 °C for one week in thioglycolate medium, heart infusion broth and potato dextrose broth. No microorganisms were detected.

Each mouse was put in a metabolic cage for 2 or 3 days so that it could become accustomed to its surroundings prior to the intraperitoneal administration of 2 mg of mercury/kg body weight as MMC. Feces and urine were collected every 24 hours. The mice were sacrificed by taking blood samples from the hearts seven days after the mercury administration. The contents of the intestines, intestinal tracts (small intestine, caecum and large intestine), liver and kidney were removed. The small intestine was divided into three equal lengths. The intestinal tracts were cut open and washed with 10 ml of one percent NaCl solution. The washings were taken for the content of the intestinal canal. Samples were maintained at −20 °C prior to mercury determination.

The feces, crushed in a mortar, were kneaded homogeneously with distilled water. About one gram of this fecal paste was mixed with 2 ml of one percent cysteine, 2 ml of 20 percent sodium chloride, 2 ml of 45 percent sodium hydroxide and distilled water added to 10 ml. After over-night refrigeration, total and inorganic mercury were determined separately by atomic absorption as described by Magos[3].

The intestinal content (10 ml) was mixed with 2 ml of one percent cysteine, 2 ml of 20 percent sodium chloride and 2 ml of 45 percent sodium hydroxide. The mixture was homogenized in a teflon homogenizer and distilled water was added to 20 ml. After over-night refrigeration, total and inorganic mercury were determined separately.

Each part of the intestinal tract was weighed and homogenized with five-volumes (v/w) each of one percent cysteine, 20 percent sodium chloride and 45 percent sodium hydroxide. The final volume was supplemented with distilled water to yield a 2% (w/v) solution. The mixture was homogenized and refrigerated over night. Total and inorganic mercury were determined separately.

For liver and kidney, 100–200 mg of tissue was homogenized in one percent sodium chloride to give a 100-fold suspension. Total and inorganic mercury were determined separately.

In our previous study[4], total mercury was directly vaporized by burning samples in an oxygen flow. In the present study, total and inorganic mercury were vaporized by reducting samples with tin (II) as described by Magos[3]. A comparison was made of the values obtained by the previous and the present method. The total mercury concentration of fecal paste samples from the

germfree and the conventional mice to which MMC had been administered was determined both by the previous method and by that used by Magos. No differences were observed (Fig. 1).

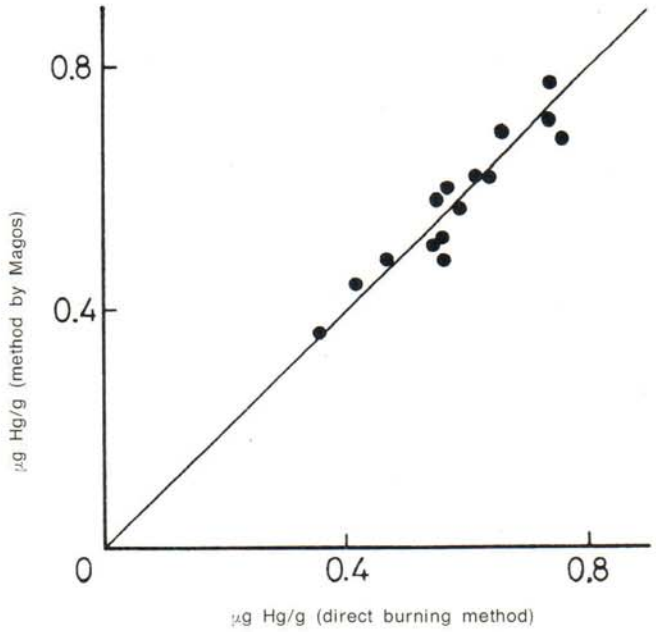

FIG. 1 — Comparison of two methods of mercury determination. Total mercury concentrations in 15 samples of fecal paste determined by both the direct burning method (horizontal scale) and the method described by Magos[3] with a slight modification (vertical scale). The straight line in the figure represents "y = x".

RESULTS

Mercury excretion

The amounts of total and inorganic mercury in feces per day is shown in Figure 2. During the experimental period, the germfree mice excreted less than half the amount of mercury excreted by the conventional mice. For seven days the germfree mice excreted fecally about 10 percent of the mercury administered, while conventional mice excreted about 27 percent. The fecal mercury excretion in germfree mice was evidently smaller than that of the conventional mice. The amount of fecally excreted inorganic mercury in germfree mice for seven days was smaller than that of the conventional mice (germfree: 2.5 percent of the administered mercury, conventional: 16.8 percent). The amount of fecally excreted organic mercury in the germfree mice was similar to that of conventional mice (germfree: 7.3 percent of the administered mercury, conventional: 10.1 percent).

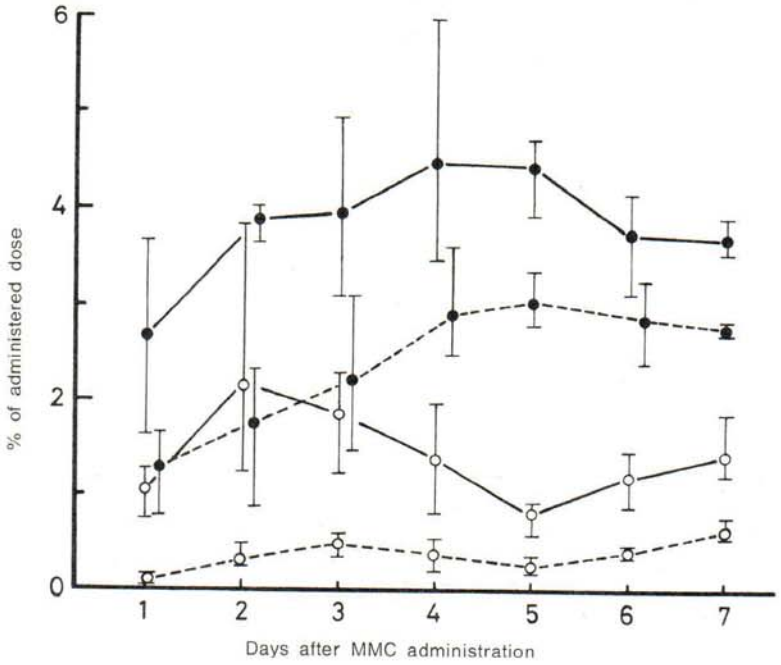

FIG. 2 – Daily mercury excretion with the feces in germfree and conventional mice after a single intraperitoneal administration of MMC (2 mg Hg/kg body weight). Amounts of mercury excreted are expressed as percentages of mercury administered. Open circles: excretion by germfree mice; solid circles: excretion by conventional mice; solid line: total mercury excretion; dotted line: inorganic mercury excretion. Each point represents a mean of single determinations in four germfree or in three conventional mice and range of values is indicated with a bar.

The amount of inorganic mercury relative to total fecal mercury amount per day is shown in Figure 3. In germfree mice, the relative amount of inorganic mercury was about 8 percent one day after mercury administration, and about 43 percent seven days after administration. In conventional mice, the relative amount was about 47 percent one day after administration and 75 percent seven days later. The relative amount of inorganic mercury increased daily both in germfree and conventional mice, while the relative amount in germfree mice was always smaller than that in the conventional mice.

Mercury amount and relative amount of inorganic mercury in intestinal content

The amounts of total mercury in the contents of various parts of the intestine were determined on the 7th day after mercury administration (Fig. 4). As for the small intestine, the total mercury amount in the intestinal contents in germfree mice was larger than that in conventional mice. In germfree mice, the lower the location in the intestine, the lower the amount of the total mercury. In

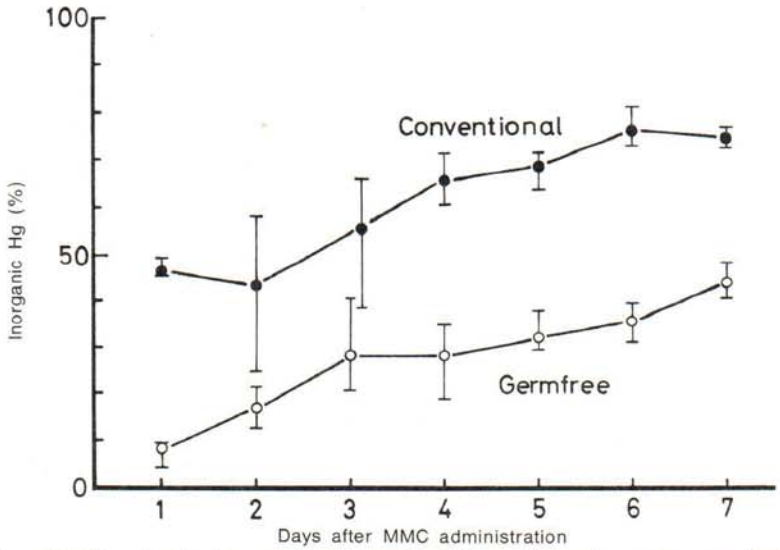

FIG. 3 – Relative amount of inorganic mercury in the feces of germfree and conventional mice after a single intraperitoneal administration of MMC (2 mg Hg/kg body weight). Values are expressed as percentages of total mercury in the samples. Each point represents a mean of single determinations in four germfree or in three conventional mice, and range of values is indicated with a bar.

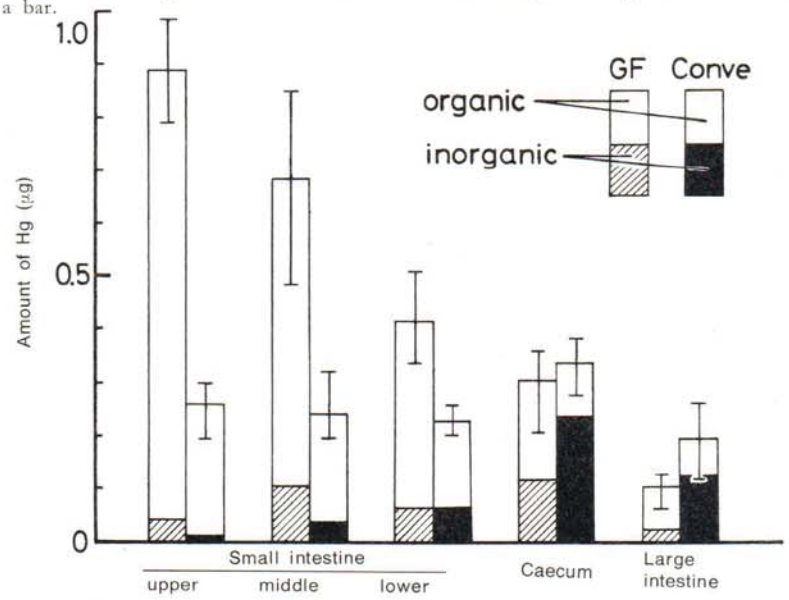

FIG. 4 – Amount of organic and inorganic mercury in intestinal contents of germfree and conventional mice seven days after a single intraperitoneal administrations of MMC (2 mg Hg/kg body weight). Each value is a mean of single determination in four germfree or in three conventional mice and range of total mercury amount is indicated with a bar.

conventional mice, the amount of total mercury of the intestinal contents was similar in all three parts of the small intestine. As for the contents of the large intestine, the total mercury amount in the germfree mice was lower than that in the conventional mice.

Their relative amount of inorganic mercury in the intestinal content is shown in Table 1. There was no difference in the relative amounts of inorganic mercury in the upper and the middle parts of the small intestine, in germfree or conventional mice. In both germfree and conventional mice, the lower the section of the small intestine, the higher the relative amount of inorganic mercury. The relative amounts of inorganic mercury in the contents of the caecum and large intestine of germfree mice were evidently lower than those of the conventional mice.

Similar results were also observed when the mice were sacrificed four days after mercury administration.

TABLE 1

Relative amount of inorganic mercury to total mercury in intestinal content of germfree and conventional mice seven days after a single intraperitoneal administration of MMC (2 mg/kg body weight).

Mouse	Small intestine			Caecum	Large intestine
	upper	middle	lower		
Germfree	4.5* (3.3–6.1)**	15.4 (8.8–19.2)	14.7 (11.8–20.5)	38.2 (35.2–40.4)	21.1 (17.3–23.6)
Conventional	3.5 (0.8–6.5)	16.4 (10.3–23.9)	27.4 (22.1–32.6)	71.1 (69.2–74.8)	62.4 (58.5–68.3)

Values are expressed as percentages to total mercury in the samples.
* Mean and ** range of single determinations in four germfree or in three conventional mice.

Total mercury concentration in the liver and kidney

The concentrations of total mercury and relative amounts of inorganic mercury in the liver and kidney seven days after mercury administration are shown in Table 2. The total mercury concentrations in the germfree mice were higher than those in the conventional mice in both the liver and the kidney. There were little differences in the relative amounts of inorganic mercury between the germfree and the conventional mice.

Total mercury concentrations and relative amounts of inorganic mercury in the intestinal tract

The total mercury concentrations and relative amounts of inorganic mercury in the intestinal tracts are shown in Table 3. The concentrations in the three parts of the small intestinal tract of the germfree mice were slightly higher than those found in conventional mice. However, the concentrations in the tracts of the caecum and the large intestine of the germfree mice were slightly lower

TABLE 2

Total mercury concentration and relative amount of inorganic mercury to total mercury in the liver, kidney and blood of germfree and conventional mice seven days after a single intraperitoneal administration of MMC (2 mg/kg body weight).

Mouse	Liver		Kidney		Blood
	μg Hg/g	% inorganic	μg Hg/g	% inorganic	μg Hg/g
Germfree	3.3* (2.9–4.0)**	26 (24–28)	11.7 (7.2–13.5)	18 (13–32)	1.7 (1.5–2.0)
Conventional	2.1 (1.9–2.2)	27 (25–29)	6.9 (2.4–10.1)	23 (19–27)	1.1 (1.0–1.2)

*Mean and **range of single determinations in four germfree or in three conventional mice.

TABLE 3

Total mercury concentration and relative amount of inorganic mercury to total mercury in intestine of germfree and conventional mice seven days after a single intraperitoneal administration of MMC (2 mg/kg body weight).

Mouse	Small intestine			Caecum	Large intestine
	upper	middle	lower		
Total Hg (μg/g)					
Germfree	2.4* (2.1–2.6)**	2.2 (2.0–2.3)	1.7 (1.5–1.9)	1.1 (1.0–1.2)	1.0 (0.9–1.2)
Conventional	1.7 (1.4–2.0)	1.4 (1.2–1.7)	1.1 (0.9–1.3)	1.4 (1.3–1.5)	1.2 (1.0–1.2)
Inorganic Hg (%)					
Germfree	2.3 (2.1–2.7)	2.1 (1.6–2.4)	1.6 (1.3–2.1)	3.2 (2.4–4.2)	3.2 (2.1–4.0)
Conventional	3.2 (3.1–3.3)	4.0 (2.7–5.1)	2.7 (1.9–3.5)	46.8 (36.8–52.0)	40.2 (33.3–47.9)

*Mean and **range of single determinations in four germfree or in three conventional mice.

than in conventional mice. The relative amount of inorganic mercury in the germfree mice was less than 5 percent in all parts of the intestinal tract. As for the conventional mice, the relative amount in the small intestinal tract was less than 6 percent, whereas that in the tracts of the caecum and the large intestine was 47 percent and 40 percent, respectively.

DISCUSSION

The results obtained in the present study in which MMC was administered to mice intraperitoneally are similar to those obtained in a previous study in which MMC was administered orally – namely, the fecal excretion of the total mercury in germfree mice was smaller than that in conventional mice and the total

mercury concentration in the kidney and liver seven days after mercury administration in the germfree mice was higher than that in the conventional mice.

There are several possibilities how these differences in mercury excretion between germfree and conventional mice may derive. The importance of the biotransformation of methyl mercury in tissues for mercury excretion has been reported[10]. However, no difference has been observed in the relative amounts of inorganic mercury in the liver and the kidney between germfree and conventional mice. The amount of mercury secreted in bile has been reported to be closely related to the amount of mercury excreted in the feces[7,10]. However, in view of the present results, it is difficult to understand why the amount of mercury secreted in the bile of germfree mice was smaller than that of conventional mice, because the total mercury amount in the small intestinal contents of germfree mice was larger than that of conventional mice, while the total mercury amount in the large intestinal contents and in the feces excreted seven days after MMC administration was smaller in germfree than in conventional mice.

Biotransformation in the intestinal content may also be important to mercury excretion. As for the relative amounts of inorganic mercury in the intestinal contents, no differences were observed between germfree and conventional mice in the upper and the middle parts of the small intestine. Their relative amounts of inorganic mercury were higher in the lower parts of the intestine both of germfree and of conventional mice. However, in conventional mice, the relative amount was much higher. Consequently, in the intestinal contents of the lower part of the intestine, both the amount and relative amount of inorganic mercury were lower in germfree mice than in conventional mice. Also in the feces, the relative amounts of inorganic mercury in germfree mice were evidently smaller than those in conventional mice. These results imply that a part of the fecal inorganic mercury must be transformed from methyl mercury in the intestinal canal, probably by the intestinal flora. Furthermore, methyl mercury has been reported to be transformed into inorganic mercury in human feces[1] and by microorganisms[11,12,13,14].

There were no great differences in the amounts of organic mercury excreted in the feces between germfree (7.3 percent of the administered) and conventional mice (10.1 percent). The amount of inorganic mercury excreted in the feces of germfree mice (2.5 percent of the administered) was apparently smaller than that in the feces of conventional mice (16.8 percent). The smaller amount of inorganic mercury in the feces may explain the smaller excretion of total mercury in the feces.

Norseth[6] has reported that the relative amounts of inorganic mercury in the intestinal content of the caecum and the feces of germfree rats given MMC did not differ from those in conventional rats. The discrepancy of his report from our results may reflect a basic difference between rats and mice in the enterohepatic circulation of mercury (in discussion of Ref. 6) or in intestinal flora.

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