Reduced Mercury Excretion with Feces in Germfree Mice After Oral Administration of Methyl Mercury Chloride

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Methyl mercury salts are degraded to inorganic mercury in vivo (NORSETH et al 1970a, b) and also by isolated microorganisms in vitro (SPANGLER et al 1973a, b, TAIRA 1975, TONOMURA & KANZAKI 1969). This degradation probably plays an important role in mercury excretion of animals exposed to methyl mercury salts. Preferential inorganic mercury excretion in feces was observed in rats after intravenous administration of methyl mercury salts (NORSETH et al 1971). Methyl mercury chloride was also anaerobically degraded in a human fecal specimen (EDWARD et al 1975). Consequently, intestinal microorganisms are thought to be concerned with the degradation of methyl mercury in an animal body. NORSETH (1971) studied the release in feces of inorganic mercury from methyl mercury, subcutaneously given to germfree and control rats.

In this paper, fecal and urinal mercury excretion and mercury retention in several tissues of germfree mice, orally given methyl mercury chloride, is compared with that of control mice.

MATERIALS AND METHODS

Germfree ICR-JCL female mice (from CLEA, Tokyo) approximately six weeks of age and 20 grams in weight were used. Each mouse was fed under standard germfree conditions in a metabolic cage. Two or three metabolic cages were set up in one vinyl isolator disinfected by hyperacetic acid spraying. Conventional ICR-JCL female mice, whose ages and body weights were similar to the germfree mice, were used as a control group. The control mice were fed under the same conditions as the germfree mice in another isolator. Absence of germs was confirmed by incubation of the feces at 37°C for one week in thioglycolate medium.

The germfree and the control mice were allowed to take freely 1.0 ml of drinking water containing 20 μg mercury as methyl mercury chloride on the first day. Feces and urine in every 24 hours were collected excluding the feces in the first 4 hours which were collected separately. The mice were killed by blood sampling from the heart on the 10th day after the mercury administration. Measurement of total mercury in feces, urine and several tisses was carried out by atomic absorption analysis using combustion method in an electric furnace supplied with oxy-

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gen gas flow on the Hitachi Zeeman Effect Mercury Analyzer 501 (detection limit - 0.5 ng/ 50 mg or 50 ul of tissues or urine).

RESULTS AND DISCUSSIONS

Mercury absorption:

Mercury absorption in five germfree mice as well as in four control mice was more than 99 percent of the administered methyl mercury chloride, assuming the amount of mercury absorption to be the difference between the amount of mercury administered, and the amount of mercury excreted within four hours, which was supposed to be unadsorbed to the intestinal wall (TABLE I).

TABLE I

Absorption of methyl mercury chloride administered orally in germfree and control mice

	Amount of Hg absorbed (μg)*	Rate of Hg absorbed**
Germfree	19.97	0.998
Control	19.95	0.997

* Amount of Hg absorbed = Amount of administration (20μg) — amount of Hg excreted within 4 hours with feces.

** Rate of Hg absorbed = amount of Hg absorbed / amount of administration.

TABLE II

Mercury excretion in 10 days after administration

	Germfree	Control	Germfree/control
Amount of mercury excretion in	(μg)		
feces*	4.805	9.144	0.525
urine	4.529	3.543	1.280
sum	9.334	12.687	0.736
Rate of mercury excretion in			
feces	0.240	0.457	
urine	0.226	0.177	
sum	0.466	0.634	

* Amount of fecal mercury excreted within 10 days of administration except the first 4 hours.

Our results on methyl mercury absorption calculated by this procedure support the fact that gastrointestinal absorption of methyl mercury chloride administered in food is almost complete (NORDBERG et al, 1972).

Mercury excretion:

Germfree mice excreted 24 percent of the administered mercury with feces within 10 days of administration, while the control mice excreted 46 percent (TABLE II). Mercury excretion with feces in germfree mice was about half that of the the control mice. The amounts of fecal mercury excretion per day decreased linearly in the semilogarithmic scale (Figure 1). Figure 2 shows the comparison of the daily fecal mercury excretion with the total mercury residue in the body. It was approximately 7.5 percent in the control mice, while in germfree mice only 2 percent was excreted daily. The amount of the total urinal mercury excretion of germfree mice was similar to that of the control mice (TABLE I, Figure 1).

Our results show that germfree conditions affect mercury excretion. NORSETH (1971), however, described that no difference in the release of inorganic mercury in the gastrointestinal tract

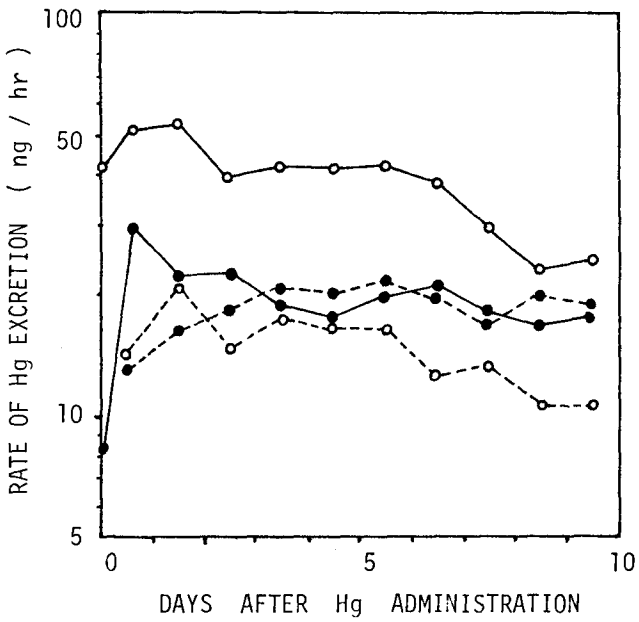

Figure 1.
Rate of total mercury excretion per hour in feces and urine of germfree and control mice after administration of methyl mercury chloride (20μg). —●— : feces of germfree mice, —○— : feces of control mice, --●-- : urine of germfree mice, --○-- : urine of control mice.

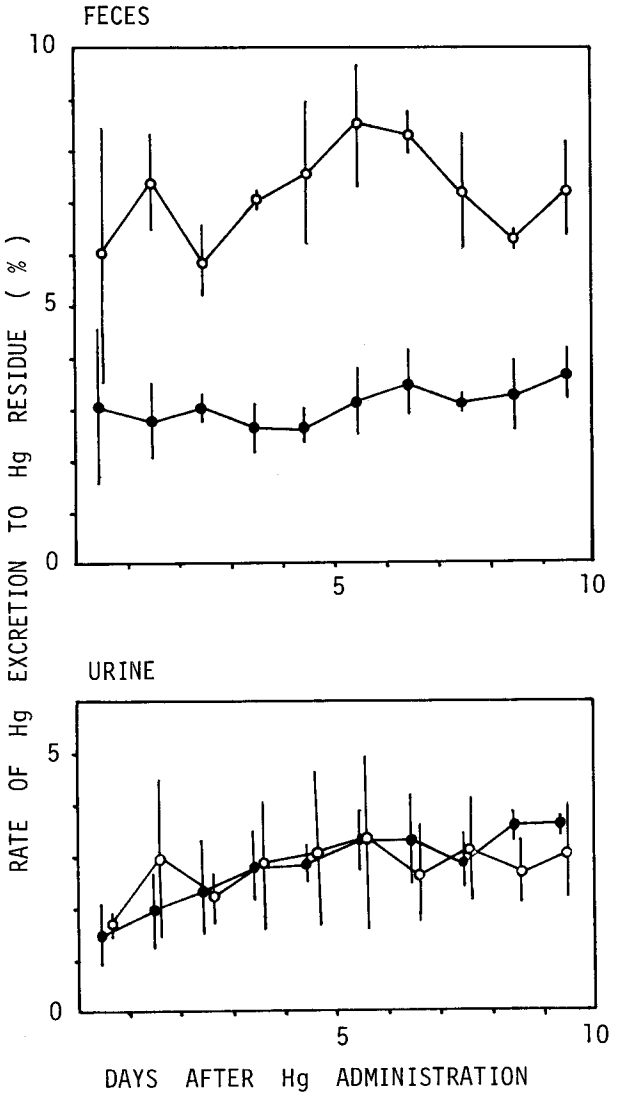

Figure 2.
Percentage of total mercury excretion to mercury residue in germfree (—●—) and control (—○—) mice after oral administration of methyl mercury chloride (20 μg). Each point represents the mean and the range for two experiments.

could be detected in germfree rats. In his experiments, mercury was given to rats subcutaneously and must have permeated directly into the blood stream without passing through the intestinal wall. Therefore, his results indicated that there was no detectable difference in reabsorption of mercury excreted with bile between germfree and conventional rats when mercury was given parenterally. However, mercury compounds given orally could be modified differently by the presence or absence of intestinal microorganisms, when initially introduced into the gastrointestinal tract. Thus modified mercury compounds may be differently metabolized, reabsorbed or excreted in germfree mice.

Mercury concentration in tissues:

Mercury concentration in the brain, kidney, liver and spleen of the germfree mice were about 1.5-1.8 fold of the control mice (TABLE III). Though these organs of the germfree mice were slightly smaller than those of the control mice, the total amount of mercury in each organ was still about 1.3 fold of the control mice (TABLE IV). Higher mercury retention in tissues of the germfree mice probably resulted from the lower mercury excretion in feces.

TABLE III

Total mercury concentration in several organs (ppm).

	Germfree	Control	Germfree/control
Brain	0.301	0.207	1.45
Spleen	0.423	0.244	1.73
Liver	1.349	0.843	1.60
Kidney	3.632	1.957	1.86

TABLE IV

Total mercury amount of several organs (ng).

	Germfree	Control	Germfree/control
Brain	118	91	1.30
Spleen	30	21	1.43
Liver	1695	1198	1.41
Kidney	1025	606	1.69

SUMMARY

When methyl mercury chloride was administered orally the amount of mercury excretion with feces of germfree mice was noticeably lower than that of the control mice. Germfree mice excreted 24 percent of the administered mercury within 10 days of administration while the control mice excreted 46 percent. Mercury retention in the organs of germfree mice was slightly higher than in the control mice. These results suggest that the existence of microorganisms in animal intestines are concerned with mercury excretion in the animal body.

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