Intestinal Absorption of Mercury *in Vitro* from Intestinal Contents of Methylmercury Administered Mice

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Intestinal flora plays an important role in the decomposition and fecal excretion of methylmercury. The assumed mechanism is that decomposition of organic mercury (o-Hg) to inorganic mercury (i-Hg) by intestinal flora in cecum decreases the reabsorption of mercury in large intestines. To confirm this hypothesis, we examined the large intestinal mercury absorption *in vitro* from intestinal contents of methylmercury administered mice. Methylmercury (2 mg Hg/kg) was administered intraperitoneally to adult female mice, and the contents of small intestine (ileum) and cecum were taken out 24 hr after the administration. Ratios of organic mercury to total mercury in small intestinal content and cecal content were 86% and 49%, respectively. Contents fo both samall intestine and cecum were packed into cecum and colon of normal mice, respectively, and were incubated in a medium (Tyrode's solution) at 37°C for 2 hr. 73% of total mercury (t-Hg) was absorbed from small intestinal content into the cecum and medium after the incubation. On the other hand, only 34% of t-Hg was absorbed from cecal content; most i-Hg remained unabsorbed. However, the absorption rate of t-Hg increased to 57% when the cecal content from antibiotics treated mice was used, because of the high percentage (90%) of o-Hg contained. These results suggest that o-Hg in small intestinal and cecal contents can be reabsorbed by cecum and colon *in vivo*, and that the decomposition of o-Hg to i-Hg by intestinal flora decreases the intestinal reabsorption rate of t-Hg in methylmercury exposed mice.

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INTRODUCTION

Decomposition of organomercurials to inorganic mercury (i-Hg) is important for mercury excretion by animals,[1] because i-Hg is excreted faster than organomercurials.[2,3] Intestinal flora plays important roles not only in decomposition of organic mercury (o-Hg) but also in fecal excretion of total mercury (t-Hg) in animals administered methylmercury as reviewed by Rowland[4] and Tanaka-Kagawa,[5] though animal tissues can decompose methylmercury *in vitro*[6–8] and *in vivo*.[9–11] In the case of phenylmercury, the mercury was decomposed much faster than methylmercury, and intestinal flora did not participate in the decomposition nor in the fecal excretion of mercury.[12]

Total amount and percentage of i-Hg to t-Hg in feces were less in germfree mice,[11,13] antibiotic-treated animals,[14,15] and cecum-resected mice[16] than in control animals after methylmercury administration. The percentage of i-Hg was also less in large intestinal contents of these mice than in those of control mice,[11,15,16] but not in small intestinal contents. These reports suggest that o-Hg, possibly methylmercury, in large intestinal contents will be reabsorbed if the mercury is not decomposed to i-Hg by intestinal flora. However, it may be possible that the reabsorption of o-Hg is inhibited by the incorporation of o-Hg within bacterial cells. To confirm the role of bacterial decomposition of o-Hg in large intestine in the fecal excretion of methylmercury, it is important to demonstrate that the o-Hg in large intestinal content can be absorbed by the intestines. Thus, in this paper we preliminarily studied the absorption of mercury *in vitro* from intestinal contents of methylmercury administered mice. The intestinal contents of antibiotic-treated mice were also used to examine the effect of reduction in intestinal flora on mercury absorption.

MATERIALS AND METHODS

Methylmercuric chloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) dissolved in saline was administered intraperitoneally (2 mg Hg/kg) to adult female ICR mice weighing about 25 g, and the

mice were killed under ether anesthesia 24 hr after the mercury administration. Small intestine (ileum) and cecum were resected, and were cut open to collect contents without touching the inner wall of the tracts. Some mice were given neomycin sulfate (1 mg/ml) and chloramphenicol (1 mg/ml) (both obtained from Sigma Chemical Co., St. Louis. Mo., U.S.A.) through drinking water from two days before the mercury administration until content sampling.

The intestinal contents of 10 mice were mixed and packed into a 1 ml plastic syringe. Intestinal tracts, cecum and colon, were prepared from normal mice after gentle washing in Tyrode's solution to remove intestinal content. About 200 mg of the small intestinal content and the cecal content were injected into the cecum and the colon, respectively; The tracts were closed by ligation with string, and were incubated in 2 ml medium (Tyrode's solution) at 37°C for 2 hr. After incubation the tracts were cut open, and were washed in saline to separate intestinal content and tract. i-Hg and o-Hg in the samples were determined as described before[15] by the selective atomic absorption method by Magos.[17] Absorption rates of mercury were calculated by dividing the amount of mercury detected in both intestinal tract and medium by the amount in all fractions (content, tract, and medium).

RESULTS AND DISCUSSION

Mercury Absorption from Small Intestinal Content by Cecum

About 90% of mercury was in organic form, possibly methylmercury, in small intestinal contents irrespective of whether or not antibiotics were administered to content donors. After the incubation about 70% of t-Hg was detected in the cecum and medium in experiments using the contents from both antibiotic-treated mice and non-treated mice (Fig. 1-A), suggesting that mercury in the contents can be absorbed by cecum. Absorption rate of o-Hg in the content was about 70% (Table 1), if mercury in the tract and medium is regarded as absorbed mercury. The absorption rate of i-Hg was calculated as about 60%. This seems too high , as the intestinal absorption rate of i-Hg is usually several percent or less *in vivo*. During the determination of i-Hg by the method used in the present investigation, several percent of o-Hg in a sample is decomposed, and is detected as i-Hg. For this reason, the method is not suitable for the a precise determination of small amount of i-Hg in the presence of relatively a large amount of o-Hg. Thus, the calculated high absorption rates of i-Hg might have been caused by error in mercury determinations, because amounts of o-Hg relative to i-Hg in samples were high.

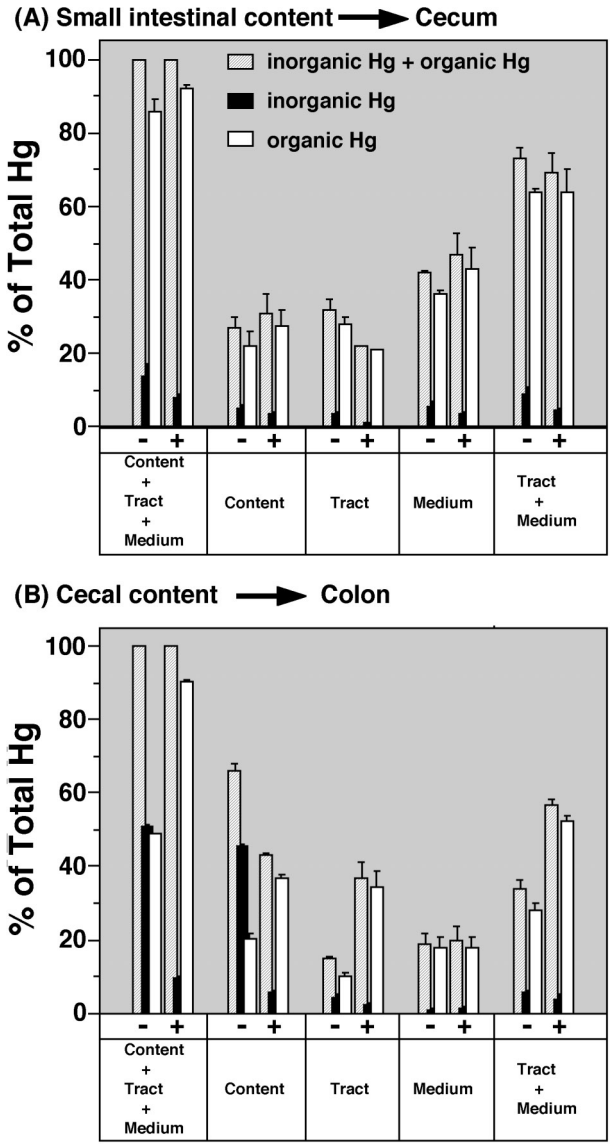

Fig. 1. Relative Amount of Mercury Detected in Each Fraction after the Incubation of Intestinal Contents Packed in Cecum and Colon *in Vitro*

Small intestinal content (A) and cecal content (B) were packed into cecum and colon, respectively, and were incubated in a medium (Tyrode's solution) at 37°C for 2 hr. After the incubation inorganic and organic mercury in content, tract and medium were determined. Total amount of mercury detected in all fractions was regarded as 100%. Mean from two samples and the range are indicated by columns and a vertical bar on the top of column, respectively. –: Intestinal content from mice treated with methylmercury alone was used. +: Intestinal content from mice treated with antibiotics and methylmercury was used.

Table 1. Absorption Rate of Mercury from Intestinal Contents of Methylmercury Administered Mice *in Vitro*

Content from	Packed in	Antibiotics	Absorption rate (%)		
			T-Hg[a)]	I-Hg[b)]	O-Hg[c)]
Small intestine	Cecum	–[d)]	73 (70–76)	–	75 (71–78)
		+[e)]	69 (63–74)	–	70 (64–75)
Cecum	Colon	–	34 (31–36)	11 (9–13)	58 (54–61)
		+	57 (56–57)	–	58 (57–60)

Intestinal contents from methylmercury administered mice were packed in cecum and colon, and were incubated in a medium at 37°C for 2 hr. After the incubation inorganic and organic mercury in content, tract, and medium were determined. *a*) (T-Hg in tract and medium) / (T-Hg in content, tract and medium) ×100. *b*) (I-Hg in tract and medium) / (I-Hg in content, tract, and medium) ×100. *c*) (O-Hg in tract and medium) / (O-Hg in content, tract and medium) ×100. *d*) Intestinal content from mice treated with methylmercury alone. *e*) Intestinal content from mice treated with antibiotics and methylmercury. Mean and range of two data readings are indicated.

Mercury Absorption from Cecal Content by Colon

About 50% of t-Hg was in organic form in cecal contents prepared from methylmercury administered conventional mice, where as that from antibiotic-treated mice was 90%, similar to that in small intestinal contents (Fig. 1-B). This difference suggests the bacterial decomposition of o-Hg in cecal content of the conventional mice. Absorption rate of t-Hg from cecal contents by colon was estimated at 34% when the content from methylmercury administered conventional mice was used (Table 1). This result also suggests that mercury in cecal content can be absorbed by colon, but the absorption rate is lower than that from small intestinal content (73%). This lower absorption rate is related to the high amount of inorganic mercury in the cecal content, because the absorption rate of i-Hg (11%) was lower than that of o-Hg (58%); in this case, the calculated absorption rate of i-Hg is considered to be reliable, because the relative amount of i-Hg in the content fraction was large enough for reliable determination. On the other hand, when cecal content from antibiotic-treated mice was used, the absorption rate of t-Hg was 57%; this value was higher than that from the cecal content of conventional mice (34%). The absorption rate of o-Hg from cecal contents obtained from antibiotic-treated mice did not differ from that from conventional mice, suggesting that the presence of intestinal flora does not affect the absorption rate of o-Hg.

In the present *in vitro* investigations it was suggested that o-Hg in the small intestinal and cecal contents of methylmercury administered mice could be absorbed by cecum and colon, respectively, at a rate of more than 50%. Moreover, the absorption rate of t-Hg from cecal content was less than that from small intestinal content, because of the large amount and low absorption rate of i-Hg in cecal content. In combination with the *in vivo* experiment in germfree mice,[11)] antibiotic-treated mice[15)] and cecum resected mice,[16)] the present results further confirm that the decomposition of o-Hg to i-Hg by intestinal flora in cecum decreases reabsorption of t-Hg in large intestine, resulting in the enhancement of mercury excretion into feces in mice administered methylmercury.

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