# Decomposition and Fecal Excretion of Phenylmercury in Mice Treated with Antibiotics: A Study on the Roles of Intestinal Flora

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**Intestinal flora plays important roles in the decomposition and excretion of organomercurial methylmercury, and these roles in decomposition and excretion of organomercurial phenylmercury were examined in mice given antibiotics orally. Female ICR mice were given antibiotic neomycin and chloramphenicol in drinking water for 2 d before phenylmercury administration in order to decrease intestinal flora, and the antibiotics were given throughout the experiment. Phenylmercuric acetate (2 mg Hg/kg body weight) was administered to mice intraperitoneally, and feces and urine were collected daily for 4 d. Mice were sacrificed 5 d after phenylmercury administration. There were no differences between the control and the antibiotic-treated mice in the percentage of inorganic mercury to total mercury in feces, urine, liver or kidney. Total mercury excreted in feces and urine did not differ between the antibiotic-treated mice and the control mice. These results suggest that intestinal flora does not play a role in the decomposition and excretion of phenylmercury in mice.**

**Key words** —— phenylmercury, decomposition, intestinal flora, excretion, mice, antibiotics

## INTRODUCTION

Biotransformation, especially decomposition to inorganic form, is important for excretion of organomercurial methylmercury in animals,[1] because inorganic mercury is excreted faster than organomercurials.[2,3] Although animal tissues can decompose organomercurials *in vitro*[4-6] and *in vivo*,[7-9] intestinal flora plays important roles not only in decomposition of methylmercury but also in fecal excretion of mercury in animals administered methylmercury as reviewed by Rowland[10] and Tanaka-Kagawa[11]; after methylmercury administration the amount and percentage of inorganic mercury to total mercury in feces were less in germfree mice,[9] antibiotic-treated animals,[12,13] and caecum-resected mice[14] than in respective control animals; and total amount of mercury excreted in feces was less in these animals with reduced intestinal flora than in control animals. Thus, the retention of mercury in the body is greater in animals with reduced intestinal flora than in conventional animals,[9,12-15] resulting in increased toxicity of methylmercury in animals with reduced intestinal flora.[12]

Organomercurial phenylmercury was used as pesticides and fungicides. This compound is decomposed and excreted much faster than methylmercury.[3,7,16] Although phenylmercury is decomposed by tissue homogenate or tissue slices *in vitro*,[5,17] it is also decomposed by microorganisms *in vitro*.[18,19] Thus it is possible that intestinal flora take part in decomposition and excretion of phenylmercury in animals; however, the roles of intestinal flora in these actions have not yet been elucidated. In the present pilot investigation, we examined the effect of antibiotic treatment on the decomposition and excretion of phenylmercury in mice to estimate the role of intestinal flora.

## MATERIALS AND METHODS

**Animal Experiment** —— ICR female mice, weighing 26—31 g and 10 weeks of age, were housed individually in metabolic cages, and were given neomycin sulfate and chloramphenicol (both obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.) through drinking water which was freely available to them. Each antibiotic was dissolved in distilled water at a concentration of 1 mg/ml. Distilled water was given to control mice. After 2 d of antibiotic administration phenylmercuric acetate (Wako Pure Chemical Industries, Ltd., Osaka) (2 mg Hg/kg body weight) was

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administered intraperitoneally, and feces and urine were collected every 24 h for 4 d. Mice were sacrificed by heart puncture under ether anesthesia 5 d after the mercury administration. Samples were stored at −20℃ until mercury determination.

**Mercury Determination** —— Inorganic and organic mercury in feces, urine, and organs were determined as described in our report[13] using the selective atomic absorption method by Magos.[20] Reliability of our method for mercury determination was checked by comparing the total mercury concentrations obtained by our method with those obtained by a direct burning method using a Hitachi Zeeman effect mercury analyzer, and the measured values by these two methods agreed well.

## RESULTS

The amount of total mercury and inorganic mercury excreted in feces and urine did not differ between the antibiotic-treated and the control mice (Fig. 1 A and B). About 70% of total mercury in feces excreted the first 24 h was inorganic mercury in both control and antibiotic-treated mice (Fig. 2 A). It increased to about 100% the following days. The percentage of inorganic mercury in urine of both mouse groups was about 60% the first day, and increased to more than 90% on days 3 and 4.

The concentrations of total mercury in the liver and kidney of antibiotic-treated mice were similar to those of the control mice (Table 1). More than 90% of the mercury in these organs was in inorganic form in both the antibiotic-treated mice and the control mice on day 5, and no difference was observed between the two groups.

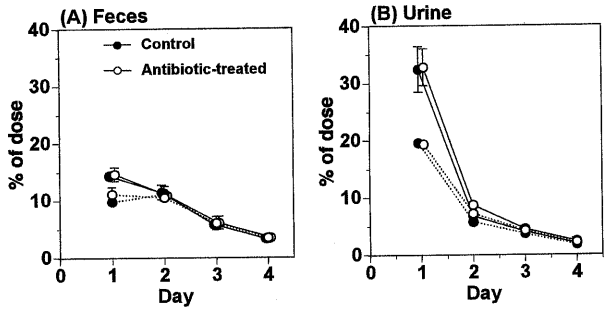


**Fig. 1.** Total (———) and Inorganic (·····) Mercury Excreted in Feces (A) and Urine (B) of Antibiotic-treated Mice Administered Phenylmercury (2 mg Hg/kg body weight)

Each point represents a mean of data from two mice. The range of two data is indicated by vertical line.

## DISCUSSION

Decreased excretions of total and inorganic mercury in feces was observed in germ free mice administered methylmercury, and the role of intestinal flora in the intestinal decomposition of this compound has been demonstrated.[9] The reduction of intestinal flora by the treatment with antibiotic neomycin and chloramphenicol also decreased both the amount and the percentage of inorganic mercury in feces in methylmercury administered mice.[13] In the present investigation, however, the same treatment with the antibiotics did not affect either the total amount or the percentage of inorganic mercury in the feces of phenylmercury administered mice (Figs. 1, 2). These results suggest that intestinal flora thus does not take part in the phenylmercury decomposition and fecal excretion in these ani-

**Table 1.** Total Mercury Concentration and Percentage of Inorganic Mercury to Total Mercury in the Liver and Kidney of Mice 5 d after Phenylmercury Administration

| Antibiotics | Liver | | Kidney | |
|---|---|---|---|---|
| | Total Hg ($\mu$/g)[a] | Inorganic Hg (%) | Total Hg ($\mu$g/g)[a] | Inorganic Hg (%) |
| + | 1.2 | 98 | 6.4 | 97 |
| | 1.1 | 93 | 5.9 | 95 |
| − | 1.1 | 99 | 7.6 | 94 |
| | 1.1 | 100 | 6.2 | 97 |

Each value is data from one mouse. *a*) Concentration is based on wet weight.

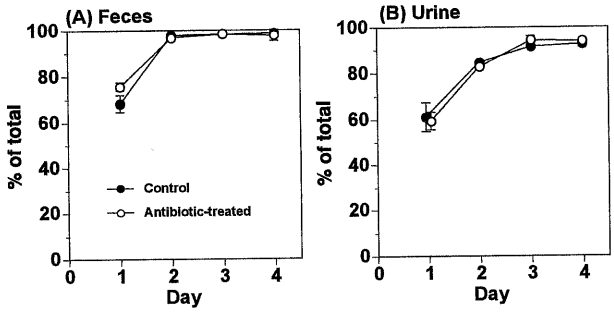


**Fig. 2.** Percentage of Inorganic Mercury to Total Mercury Excreted in Feces and Urine of Antibiotic-treated Mice Administered Phenylmercury (2 mg Hg/kg body weight)

Each point represents a mean of data from two mice. The range of the data is indicated by vertical line.

mals.

The antibiotics treatment we used reduced intestinal flora to about 10% that of untreated mice as tested by number of colonies formed on an agar plate, and the treatment did not completely eliminate intestinal flora. Therefore, we cannot eliminate the possibility that some residual microorganisms decomposed organic mercury derived from phenylmercury in the intestinal content. However, the percentages of inorganic mercury to total mercury in the liver and the kidney were more than 90% 5 d after phenylmercury administration. This is in contrast to the case of methylmercury, in which less than 10% of the mercury was in inorganic form in the liver and kidney of mice sacrificed 4 d after the administration.[13] Gage[7] reported that phenylmercury decomposed much faster than methylmercury in rats. Furthermore, whereas methylmercury is decomposed only slightly by tissue homogenate *in vitro*, a substantial amount of phenylmercury can be decomposed.[5]

Amount of inorganic mercury excreted in urine (about 20% of dose) was more than that in feces (about 10%) in both antibiotic-treated mice and control mice on the first day after phenylmercury administration (Fig. 1). This again contrasts with methylmercury, where urinary excretion of inorganic mercury is much less than fecal excretion in mice (unpublished data). Since the rate of intestinal absorption of inorganic mercury is lower than organomercurials,[2,3] the high amount of inorganic mercury in the urine of phenylmercury administered mice cannot be accounted for by the amount of inorganic mercury in feces. Therefore, most of the inorganic mercury in the urine is assumed to be generated within organs by the decomposition of phenylmercury, and it is possible that most inorganic mercury in feces is also derived from tissues.

In conclusion, it is suggested that most of the inorganic mercury detected in feces, urine and organs of mice administered organomercurial phenylmercury is not derived from intestinal flora but from tissues, and that intestinal flora has no function in the decomposition and excretion of phenylmercury in these animals.

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