

Differences in the Fate of Methylmercury between Mice with and without Hair

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We investigated time-dependent changes in the fate of methylmercury (MeHg) in both sexes of hairy and hairless mice during 6 days after its single administration to clarify whether the presence of hair could affect tissue distribution and excretion of MeHg. Despite the excretion of mercury (Hg) into hair of hairy mice, Hg concentrations in several but not all tissues were higher in hairy than in hairless mice, especially in the brain, liver, kidney and testes in males and in the brain, liver and blood in females. The cumulative amounts of Hg excreted in feces in males and in both urine and feces in females were lower in hairy than in hairless mice, whereas there was no significant difference in the urinary level in males. However, significant differences in the tissue Hg levels were observed earlier than those in the excretory Hg levels. Accordingly, the higher Hg levels in various tissues of hairy mice as compared to those in hairless mice would not have resulted from the lower Hg excretion levels. These results suggest that the presence or absence of hair markedly affects the fate of MeHg, but the differences in its fate between hairy and hairless mice would not be directly due to the fact that hair provides an excretion route.

Key words — methylmercury, hair, excretion, distribution

INTRODUCTION

Mercury (Hg) compounds including methylmercury (MeHg) are major hazardous environmental pollutants, and human intake is often from fish. MeHg is easily absorbed from the intestine, and distributed to many tissues including brain.¹⁾ Many reports using experimental animals have revealed that MeHg is transported into the brain through the neutral amino acid transport system,^{2–4)} and induces neuronal cell damage.^{1,5–7)} Since MeHg can also easily pass through the blood-placenta barrier,^{1,8)} and the developing brain is more vulnerable to it than the adult brain,^{1,6)} its effects on human health, especially on maternal and infant health, should be given close attention.¹⁾

Hg compounds, especially MeHg, distribute in hair as well as in liver, kidney and brain, and the Hg content in human hair is considered to be a good indicator of MeHg exposure from foods.¹⁾ Although there are many reports on the fate and toxicity of MeHg including these modifying factors, studies are generally performed using hairy experimental animals. Therefore, the influence of the presence and the amount of hair remains unclear, although hair that provides an excretion route might play important roles in the fate and toxicity of MeHg.

In the present study, we examined time-dependent changes in the fate of MeHg in hairy and hairless mice after a single administration of MeHg to clarify whether the presence of hair could affect tissue distribution and excretion of MeHg.

MATERIALS AND METHODS

Animals — Hairless male mice (Hos : HR-1) and C57BL/6N female mice were obtained from Hoshino Laboratory Animals Co. (Saitama, Japan) and CLEA Japan Co. (Osaka, Japan), respectively. The animals were maintained at $24 \pm 1^\circ\text{C}$ and 50–60% relative humidity, and on standard laboratory chow (CE-2, CLEA Japan Co.) and tap water *ad libitum*. They were crossbred at 10–12 weeks of age. The second generation of mice had black, gray or white hair, and some of them lost all their hair within a few days at approximately 3 weeks of age. Only the mice with black hair were weaned and divided into males and females at 4 weeks, and hairy males and hairless females, or hairless males and hairy females, were crossbred at 10–12 weeks. After similar crossbreeding several times, all mice obtained

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had black hair within a few weeks after birth even in hairless mice as described above. Both sexes of hairy and hairless mice were used for the experiments at 8 weeks of age as described below. All experimental procedures were approved by the Ethics Committee on Animal Experiments of the National Institute for Minamata Disease (NIMD).

MeHg Administration and Hg Determination

MeHg administration and Hg determination were performed as previously described⁹⁾ with minor modifications. Methylmercuric chloride (Tokyo Chemical Industry Co., Tokyo, Japan) was dissolved in distilled water and administered orally to mice at a dose of 20 $\mu\text{mol}/\text{kg}$ on day 0. The animals were housed in metabolism cages (1 mouse/cage), and urine and feces were collected on day 1, 3, 5, and 6. On day 1, 3 and 6, each mouse was anesthetized using pentobarbital, and hair was collected. Blood was then collected from the inferior caval vein in a heparinized syringe, and plasma was obtained from the aliquot after centrifugation at 3000 rpm for 3 min. After perfusion with ice-cold saline *via* the heart, kidney, liver, gonads and brain were excised. The Hg content of each sample was determined by the oxygen combustion-gold amalgamation method¹⁰⁾ using a Rigaku Mercury Analyzer MA-2 (Nippon Instruments Co., Tokyo, Japan) and expressed as total Hg (organic Hg + inorganic Hg).

Statistical Analysis — Data on Hg levels, except for hair, were analyzed by two-way analysis of variance (ANOVA). Significant differences between hairy and hairless mice at the specified times and in the specified sex were determined by Student's *t*-test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Both in males and females, there was no significant difference in body weight between hairy and hairless mice at 8 weeks of age, although the weight was higher in hairy than in hairless mice at 4 weeks (Table 1).

Time-dependent changes in tissue and excretory Hg were investigated in both sexes of hairy and hairless mice orally administered MeHg at a dose of 20 $\mu\text{mol}/\text{kg}$. In both hairy and hairless mice regardless of sex, the Hg concentration in the brain was highest on day 3 (Fig. 1A and 1B), whereas the concentrations in other tissues continued to decrease during the 1st to the 6th day (Fig. 1C–1L). In contrast,

Table 1. Body Weights in Both Sexes of Hairy and Hairless Mice

	Body weight (g)	
	Hairy mice	Hairless mice
Aged 4 weeks		
Male	20.30 \pm 2.62	16.41 \pm 3.19*
Female	16.80 \pm 2.13	13.33 \pm 2.44*
Aged 8 weeks		
Male	28.37 \pm 2.28	27.28 \pm 1.57
Female	21.72 \pm 1.56	21.11 \pm 1.26

Values represent the mean \pm S.D. obtained from 12 mice. Significant differences from hairy mice at * $p < 0.01$.

the hair Hg concentration continued to increase in hairy mice (Table 2). Hg concentrations in the brain and liver tended to be higher in hairy than in hairless mice regardless of sex, although significant differences were observed only on day 3 in males and on day 6 in females (Fig. 1A–1D). The Hg concentration in the kidney in males was also higher in hairy than in hairless mice except on day 6 (Fig. 1E), whereas that in females was identical between hairy and hairless mice (Fig. 1F). The Hg concentration in the testes was significantly higher in hairy than in hairless mice only on day 3 (Fig. 1G), although the level in the ovary was similar during 6 days (Fig. 1H). In both sexes, there were no significant differences in Hg concentrations in blood and plasma between hairy and hairless mice except for the blood in females on day 1 and 6 (Fig. 1I–1L). The cumulative amount of Hg excreted in feces tended to be lower in hairy than in hairless mice both in males and females (Fig. 1O and 1P), although that in urine did so only in females (Fig. 1M and 1N). Significant differences were observed from the 5th day in fecal excretion in males (Fig. 1O), and from the 3rd day in urinary and fecal excretion in females (Fig. 1N and 1P).

In the present study, Hg levels in several but not all tissues were higher in hairy than in hairless mice (Fig. 1A–1L), in spite of the excretion of Hg into the hair of hairy mice (Table 2). In addition, although hairy mice showed lower Hg excretion in feces in males and in both urine and feces in females than hairless mice, significant differences in tissue Hg concentrations were observed earlier than those in excretory Hg levels (Fig. 1A–1P). Accordingly, the higher Hg levels in various tissues in hairy rather than in hairless mice would not have resulted from the lower Hg excretion levels. Although the reasons for the differences in the fate of MeHg remain un-

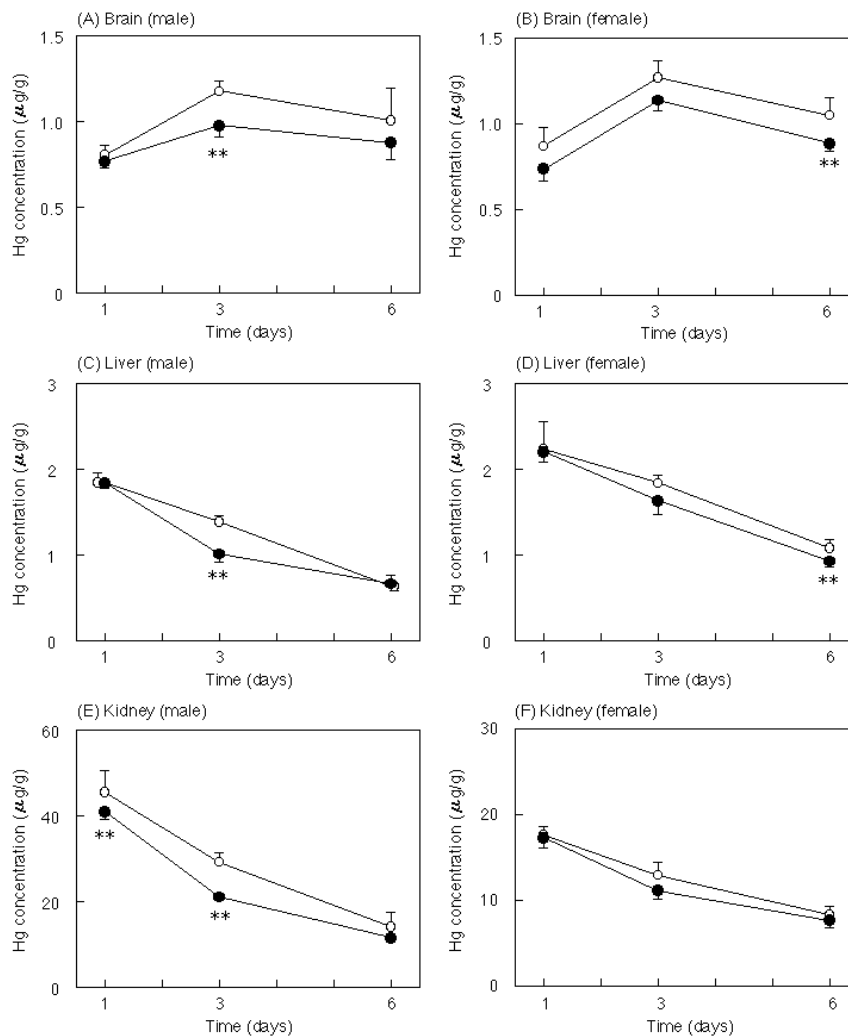


Fig. 1. Time-Dependent Changes in Tissue and Excreted Hg in Both Sexes of Hairy (○) and Hairless Mice (●).

Mice were orally administered MeHg at a dose of 20 $\mu\text{mol/kg}$ on day 0. The values represent the mean \pm S.D. obtained from 3 to 4 mice. Two-way ANOVA: groups of mice (hairy and hairless mice); days [1, 3, (5) and 6]. Male. Brain: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, not significant (NS). Liver: groups, $p < 0.05$; days, $p < 0.01$; group and day interactions, $p < 0.01$. Kidney: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. Testes: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. Blood: groups, NS; days, $p < 0.01$; group and day interactions, $p < 0.01$. Plasma: groups, NS; days, $p < 0.01$; group and day interactions, $p < 0.01$. Urine: groups, NS; days, $p < 0.01$; group and day interactions, NS. Feeces: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. Female. Brain: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. Liver: groups, $p < 0.05$; days, $p < 0.01$; group and day interactions, NS. Kidney: groups, NS; days, $p < 0.01$; group and day interactions, NS. Blood: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, $p < 0.05$. Plasma: groups, NS; days, $p < 0.01$; group and day interactions, NS. Urine: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. Feeces: groups, $p < 0.01$; days, $p < 0.01$; group and day interactions, NS. (*) and (**) indicate significant differences from hairy mice at the specified times at $p < 0.05$ and $p < 0.01$, respectively.

clear, they must be caused by the presence or absence of hair, probably through some alteration of the metabolism that is important for not only tissue accumulation of MeHg but also its excretion.

It should be noted that tissue distribution and excretion of MeHg sex-dependently differ between mice with and without hair, especially in the kidney, blood, gonads and urine (Fig. 1A–1P). We recently revealed that, regardless of the marked sex difference in the fate of MeHg in intact C57BL mice,^{11–13} its fate was similarly affected by a dietary modifica-

tion (dietary protein deficiency) regardless of sex, except for the gonads.¹⁴ We, therefore, suggested that some factors that affect the fate of MeHg might be similarly influenced by this dietary modification both in males and females.¹⁴ The candidates would be the metabolism of low molecular weight thiol compounds and/or transport activity of the neutral amino acids, which are markedly affected by a dietary protein deficiency.^{9,15–19} In contrast, in the hairy and hairless mice in this study, there might be sex-dependent differences in the alterations in those fac-

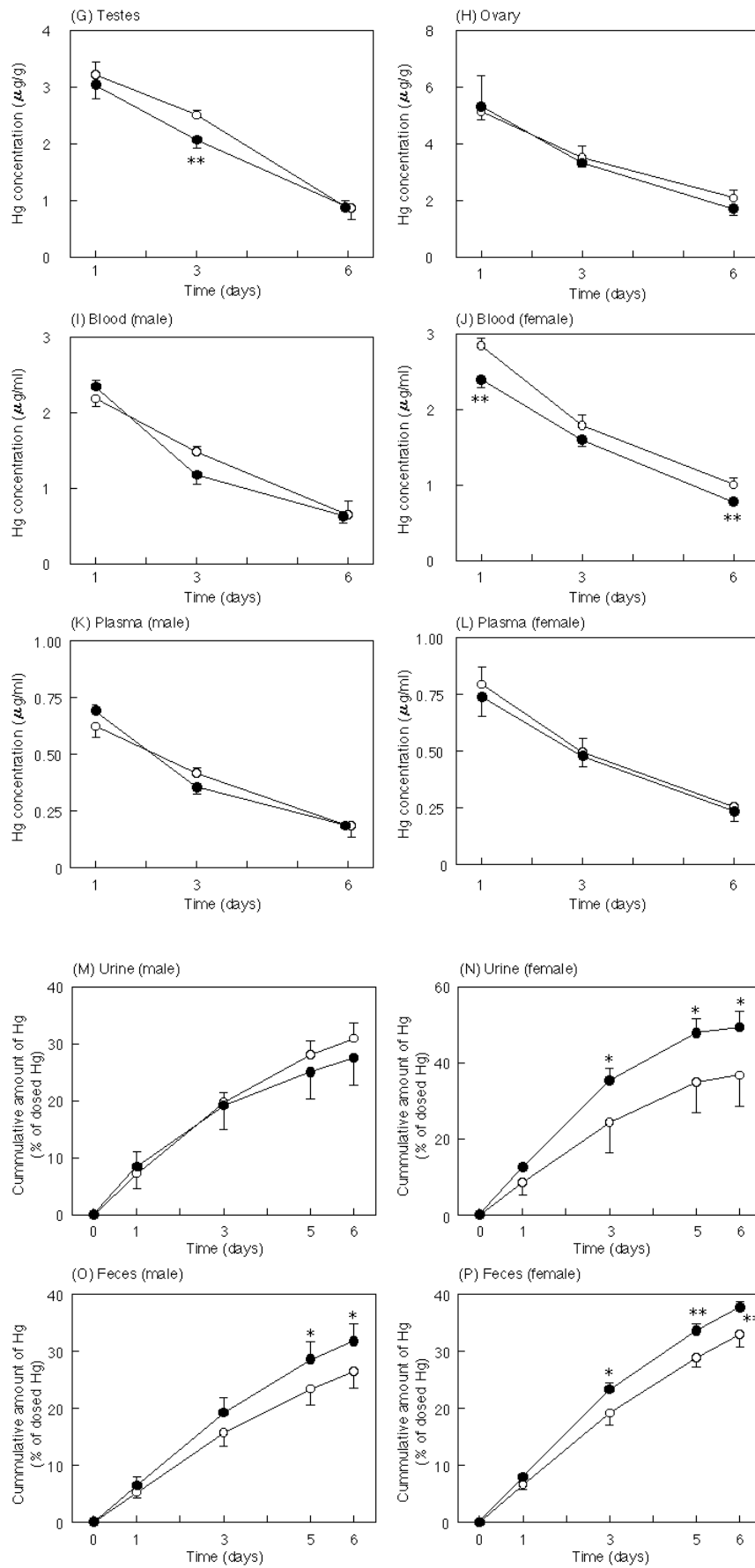


Fig. 1. Continued

Table 2. Changes in Hair Hg Concentration in Both Sexes of Hairy Mice

Days after MeHg Administration	Hg concentration (mg/g)	
	Male	Female
1	0.286 ± 0.077	0.295 ± 0.159
3	0.539 ± 0.099	0.484 ± 0.156
6	0.700 ± 0.313	0.883 ± 0.335

Values represent the mean ± S.D. obtained from 4 mice.

tors caused by losing hair.

In conclusion, the fate of MeHg markedly differs between mice with or without hair, probably due to important differences in metabolism and not to the presence or absence of hair as an excretion route.

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