

Sex Difference in Acute Renal Dysfunction Induced by Methylmercury in Mice

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ABSTRACT

To investigate the sex-related difference of susceptibility of renal function to methylmercury (MeHg) toxicity, various doses of MeHg chloride (MMC, 20–200 $\mu\text{mol/kg}$) were orally administered to C57BL/6N mice of both sexes. On days 1, 3, 5, and 7 after MMC administration, the extent of damage to renal function and the renal Hg levels were examined. After dosing, female mice survived much longer than males. With the increase in the dose level to 200 $\mu\text{mol/kg}$, the changes of the renal Hg levels 24 h after administration showed biphasic features with a plateau of around 85 $\mu\text{g/g}$. The renal Hg in male mice increased more rapidly to the plateau than in females. The doses by which the renal Hg level reached the plateau were 80 and 120 $\mu\text{mol/kg}$ for males and females, respectively. The time-dependent decrease of the renal Hg became much slower with dose levels exceeding 80 and 160 $\mu\text{mol/kg}$ for males and females, respectively. Inhibition of phenolsulfonphthalein excretion and increase of plasma creatinine after the MMC administration were more marked in males than in females. Inorganic Hg levels in the kidney of MeHg-intoxicated mice were much lower than that of HgCl_2 -intoxicated mice, indicating that the involvement of inorganic Hg, a product of biotransformation of MeHg, in the renal failure caused by MMC treatment would be negligible. Although pathological changes in the renal proximal tubules of HgCl_2 -intoxicated mice were marked, those of the MeHg-intoxicated group were slight. The results obtained here suggest that the kidney is one of the primary target organs in MeHg acute toxicity, and that the kidney of male mice has a higher susceptibility to MeHg toxicity than that of females.

INTRODUCTION

A series of experiments on sex-related differences in the response to methylmercury (MeHg) in mice yielded the following results. (a) After administration of MeHg chloride (MMC) at a dose level of negligible toxicity (20 $\mu\text{mol/kg}$), male mice showed markedly higher renal uptake and urinary excretion rates of MeHg than females in various mouse strains, which might account in part for lower Hg levels in various tissues except for the kidney in males (1). (b) The sex-related difference in the renal handling of MeHg was closely related to the difference of glutathione turnover rate in this tissue, and these features were suggested to be under hormonal control (2). (c) In the case of successive administration, rapid renal uptake of MeHg in male mice sometimes caused an adverse effect; the kidney was quickly saturated with MeHg, leading to inhibition of urinary Hg excretion at an earlier time than in females (3). Accordingly, the kidney might be one of the critical tissues by which susceptibility to MeHg toxicity was determined. In this case, however, the involvement of inorganic mercury in renal dysfunction could not be neglected, because it took more than 10 days to manifest the inhibition of Hg excretion after the first MMC administration (3).

In the present study, to obtain further information about the susceptibility of the kidney to toxicity of MeHg itself, renal Hg levels, biotransformation, and extent of renal failure were examined after a single administration of various doses of MMC in C57BL/6N mice.

MATERIALS AND METHODS

Chemicals. MMC and HgCl_2 were purchased from Tokyo Chemical Co. (Tokyo) and Wako Chemical Co. (Osaka), respectively. Phenolsulfonphthalein (PSP) was obtained from Difco Laboratories (Detroit, Michigan).

Animals. Male and female C57BL/6N mice (aged 7 weeks) were obtained from CLEA Japan Co. (Tokyo) and housed in polycarbonated cages for a week before use, at $23 \pm 1^\circ\text{C}$ with a 12/12-h light cycle, and were allowed to free access to water and laboratory chow (rodent diet CE-2, CLEA Japan Co.). MMC was dissolved in distilled water at concentrations of 1, 2, 4, 6, 8, and 10 mM, and orally administered to mice at a volume of 20 mL/kg body weight (20, 40, 80, 120, 160, and 200 $\mu\text{mol MMC/kg}$ of doses, respectively) on day 0; then the mice were housed in metabolism cages (1 mouse/cage).

Survival Rates. For 7 days following MMC administration, changes of body weight and survival rates of the mice (6 for each dosing group) were examined.

PSP Excretion Test. On days 1, 3, 5, and 7 after MMC administration, distilled water (22.5 mL/kg) was given to mice (4 for each experimental group) 30 min prior to PSP injection. Aqueous solution of PSP sodium salt was intravenously injected to mice (4 $\mu\text{mol/mouse}$) under pentobarbital anesthesia. Urine excreted for the following 30 min was collected, and the amount of PSP in the urine thus collected was determined by the absorption at 560 nm after alkalification.

Hg Analysis. After PSP excretion test, blood was collected from the central caval vein. Then the mice were perfused with saline and the kidneys were excised. One of each pair of kidneys was homogenized in water for Hg analysis and the other was used in histological experiment. An aliquot of blood was centrifuged at $5000 \times g$ for 5 min to separate plasma. Hg content in each sample was determined by the oxygen combustion-gold amalgamation method (4). Selective determinations of inorganic Hg levels in the kidney were carried out according to the method described before (5).

Plasma Creatinine. An aliquot of plasma deproteinized with the aid of acetonitrile was chromatographed using a Waters SCX cation exchange column with 25 mM sodium phosphate buffer (pH 3.65, 2.5% acetonitrile) as an eluent at a flow rate of 2 mL/min, and absorption at 215 nm was determined.

Effects of Sex Hormones. Male and female mice (5 for each experimental group) were castrated and treated by estradiol benzoate or testosterone propionate as described previously (2), then orally administered MMC (120 $\mu\text{mol/kg}$). Twenty-four hours after MMC administration, PSP excretion rate, plasma creatinine, and renal Hg were examined.

Induction of Nephrotoxicity by HgCl_2 . Four groups of mice of both sexes (5 in each group) were intravenously injected by HgCl_2 (2, 4, 10, or 20 $\mu\text{mol/kg}$). Twenty-four hours after the administration, mice were subjected to PSP excretion test; then plasma and kidney were excised for Hg analysis as described above.

Effects of Selenite on Nephrotoxicity. Five individual male mice in each of 5 groups were administered the following: MMC (120 $\mu\text{mol/kg}$, p.o.); MMC (120 $\mu\text{mol/kg}$, p.o.), + Na_2SeO_3 (20 $\mu\text{mol/kg}$, i.v.); HgCl_2 (20 $\mu\text{mol/kg}$, i.v.); HgCl_2 (20 $\mu\text{mol/kg}$, i.v.), + Na_2SeO_3 (20 $\mu\text{mol/kg}$, i.v.) or Na_2SeO_3 (20 $\mu\text{mol/kg}$, i.v.). Twenty-four hours after the administration, PSP excretion rates were examined and Hg levels in plasma and kidney were determined.

Histological Examination. The kidneys were fixed in 10% neutral formalin, embedded in paraffin, and sectioned at 8 μm thickness. Then the sections were stained by hematoxylin and eosine for a microscopic examination.

Statistical Analysis. Statistical analysis of the data obtained was performed according to Student's *t* test.

RESULTS

In the previous results, male C57BL mice survived much longer than females after successive administration of 20 μmol MMC/kg/day (3). Following single administration of a higher dose, however, females showed markedly higher survival rates than males, as shown in Table 1. All the females survived for more than 7 days after MMC administration at dose levels up to 160 μmol MMC/kg body weight, and only 1/3 of the mice receiving the highest dose (200 μmol /kg) died on day 7. On the other hand, 1/3 of males died on day 6 at a dose level as low as 80 μmol /kg, and no mouse survived more than 4 days after a dosage of 200 μmol /kg.

With the increase in the dose level to 200 μmol /kg, the changes of the renal Hg levels 24 h after the administration showed biphasic features in both sexes of mice (Fig. 1). Although the Hg concentration linearly increased in the first phase up to the level of around 85 $\mu\text{g/g}$ with the increasing dose, further increase of the dose resulted in

much less increase in the Hg levels. The doses by which the renal Hg level reached the plateau were 80 and 120 μmol /kg for males and females, respectively.

Control C57BL mice excreted about 30% of injected PSP in urine for 30 min after injection in both sexes. PSP excretion at 24 h after MMC administration was markedly inhibited (96%) in males at a dose level of 80 μmol /kg, at which the renal Hg reached the plateau level, and was inhibited completely by higher doses (Fig. 1). In females, however, no such inhibition was observed even when the kidney was saturated with Hg after treatment with 120 μmol MMC/kg. The plasma creatinine values drastically increased concomitantly with the inhibition of urinary PSP excretion in male mice, whereas its increase was rather small in females even when PSP excretion was inhibited after dosing of 160 μmol /kg or more (Fig. 1).

Biological half-lives of the renal Hg (3.2–3.5 d for males and 6.5–6.9 d for females) were unchanged with dose levels up to 40 and 120 μmol /kg for males and females, respectively. However, the decreases became much slower with higher doses (Fig. 2), at which significant inhibition of PSP excretion was observed. Accordingly, the doses less than 40 and 120 μmol /kg might be considered to be nontoxic at least for the kidney.

Effects of sex hormones following castration on the renal response to MeHg toxicity are summarized in Table 2. Hg levels and susceptibility of the kidney were drastically modified by hormonal treatment. Since these

Table 1
Survival Rates (%) of MMC-Treated Mice for 7 Days after Administration

Dose ($\mu\text{mol}/\text{kg}$)	Sex	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
20	M	100						100
	F	100						100
40	M	100						100
	F	100						100
80	M	100			100	67	33	33
	F	100						100
120	M	100			100	33	33	33
	F	100						100
160	M	100		100	67	67	33	33
	F	100						100
200	M	100	100	17	0			
	F	100					100	67

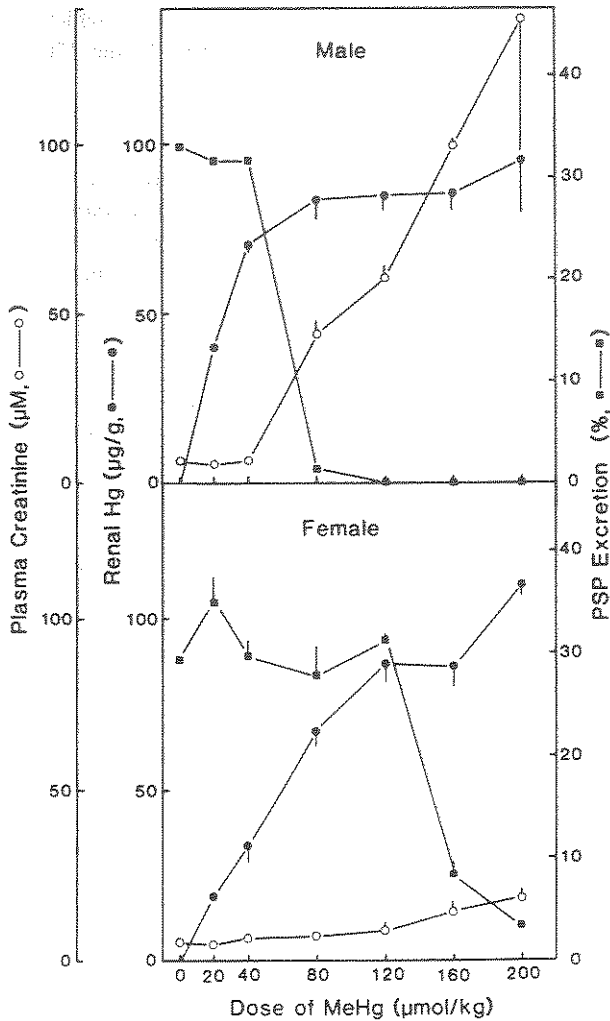


Figure 1. Dose-dependent changes of renal Hg, PSP excretion, and plasma creatinine levels in MeHg-treated mice. Twenty-four hours after MMC administration, mice were subjected to PSP excretion test; then plasma and kidney were excised. The values represent mean \pm SD obtained from 4 mice.

indexes of males approached that of females after castration only, whereas that of castrated females remained unchanged, renal susceptibility to MeHg toxicity might be under androgen control to a great extent.

Since mercuric mercury, such as HgCl_2 , is well known to cause severe damage to the renal tubules (6-8), the possibility of participation of this mercurial species, which was produced by biotransformation of MeHg (9, 10), in the renal failure observed here was examined. The ratios

of inorganic Hg (Hg_i) in the kidney of MMC-treated mice were 2% to 4% of total Hg (MeHg plus Hg_i) at 24 h after the administration, and were independent on sex and dose except for the groups of males given 120 and 160 $\mu\text{mol/kg}$, which showed a 2 to 3 times higher ratio of Hg_i than the others (data not shown). The renal Hg_i levels and PSP excretion rates of MMC- and HgCl_2 -treated mice are compared in Table 3. After HgCl_2 administration, in contrast to MeHg treatment, females showed somewhat higher susceptibility to this mercurial species than males. Male and female mice treated with MMC showed renal failure (indicated by reduced PSP excretion) with Hg_i levels as low as 2.45 and 2.74 $\mu\text{g/g}$, respectively, whereas accumulated Hg_i after HgCl_2 administration did not disturb the renal functions even with levels as high as 12.2 and 6.9 $\mu\text{g/g}$, respectively. This indicates that the renal Hg_i levels of MMC-treated mice are much lower than the toxic levels even when the doses were increased to damage the renal function. Accordingly, the renal dysfunction caused after MMC administration would not be due to Hg_i which was transformed from MeHg. Furthermore, although coadministration of Na_2SeO_3 , which was reported to decrease the acute nephrotoxicity of Hg_i (11, 12), resulted in increased plasma Hg, decreased renal Hg, and recovered PSP

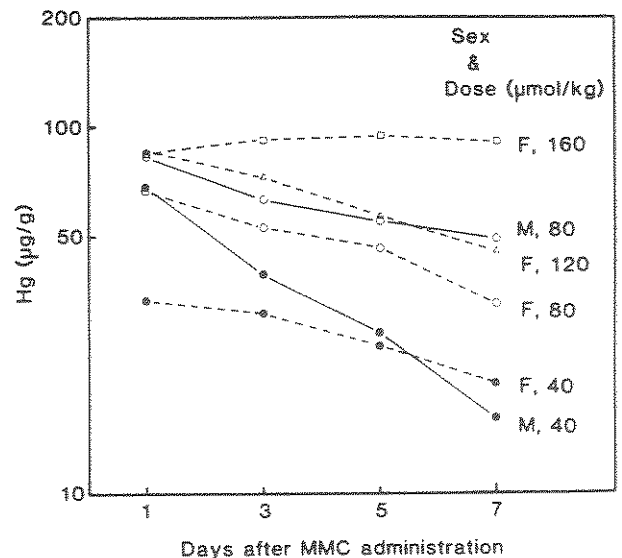


Figure 2. Time-dependent changes of renal Hg after oral administration of MMC. On days 1, 3, 5, and 7 after MMC administration, mice were sacrificed and the kidneys were excised for Hg analysis. Values represent mean \pm SD obtained from 4 mice. Data from the representative dose groups are shown to avoid complexity.

Table 2
Effects of Castration and Sex Hormones on Renal Hg Levels, PSP Excretion Rates,
and Plasma Creatinine Levels in MMC-Treated Mice^a

Treatment	Sex	Renal Hg (dose %)	PSP excretion (%)	Plasma creatinine (μ M)
Intact + SO	M	4.45 \pm 0.26	0.3 \pm 0.2	80.0 \pm 3.1
	F	4.51 \pm 0.10	21.8 \pm 7.2	7.13 \pm 3.81
Intact + EB	M	5.17 \pm 0.44	13.5 \pm 2.8*	15.4 \pm 2.8*
	F	4.12 \pm 0.38	15.5 \pm 0.9	7.43 \pm 1.57
Intact + TP	M	5.00 \pm 0.12	0.1	107.0 \pm 7.5*
	F	5.10 \pm 0.52	0.3 \pm 0.1*	68.2 \pm 16.2*
Castration + SO	M	4.20 \pm 0.28	19.5 \pm 8.3	8.6 \pm 0.95*
	F	3.34 \pm 0.41*	26.1 \pm 0.7	6.13 \pm 1.33
Castration + EB	M	3.75 \pm 0.24	17.8 \pm 3.1*	10.3 \pm 3.5*
	F	3.23 \pm 0.19*	16.3 \pm 2.6	8.58 \pm 1.27
Castration + TP	M	4.58 \pm 0.52	0.1	84.0 \pm 10.1
	F	5.66 \pm 0.81	0.3 \pm 0.1*	65.4 \pm 2.8*

^aMice were subcutaneously injected with estradiol benzoate (EB, 1 mg/kg/day), testosterone propionate (TP, 50 mg/kg/day) or sesame oil (SO, 5 mL/kg/day) for 7 days. Effects of hormonal manipulation were examined 24 h after MMC (120 μ mol/kg) oral administration. Values represent mean \pm SD obtained from 5 mice.

*Significantly different ($p < 0.01$) from control (Intact + SO) mice.

excretion in HgCl₂-treated mice; these indexes in the MMC-treated group were not affected by Na₂SeO₃ at all (Table 4).

The pathological changes in the renal cortex were evident in the proximal tubules of HgCl₂ (20 μ mol/kg)-treated mice; the epithelial cells were swollen and exfoliated into the tubular lumen (Fig. 3). This observation was compatible with the findings reported before (7, 8). On the other hand, the changes were slight in MMC (120 μ mol/kg)-treated animals (Fig. 3), though the renal function in males was suppressed similarly to HgCl₂-treated group (Table 3).

DISCUSSION

The present study demonstrated that a single toxic dose of MeHg caused severe renal dysfunction, indicated by the reduced PSP excretion and increased plasma creatinine level, within 24 h in C57BL mice. Although the renal function of male mice was readily inhibited after MMC dosing by which the kidney was saturated with Hg, that of females was not inhibited when the renal Hg reached

saturation. Previously, we found that the efflux rate of renal glutathione, which has a high affinity for MeHg, was 2 times higher in male C57BL mice than in females (2), and that most of the renal MeHg would be secreted in the luminal space as its glutathione conjugate (2, 13). Therefore, the renal tubules of male mice would be damaged more easily by a higher concentration of MeHg metabolite(s) in the lumen because of the higher efflux of glutathione-MeHg conjugate from the renal cells, even if renal MeHg levels were comparable to that of females. Since turnover rate of renal glutathione was closely controlled by sex hormones (2), the fact that castration and hormonal treatment drastically modified Hg accumulation and susceptibility of the kidney was consistent with the notion described above.

Although neurotoxic action of MeHg is well documented in various animal species including man (14-17), its nephrotoxic action is still not well defined. A few investigators reported renal injury by MeHg in rats 8 days after successive administration of MeHg for 7 days (18) or after feeding an MeHg-containing diet for a long period (19-21). Since the ratio of Hg_i in the kidney increased to more than 40% of the total Hg as early as 10 days after

Table 3

Inorganic Hg Levels in the Kidney and PSP Excretion Rates of MMC- or HgCl₂-Treated Mice^a

Treatment	Sex	Inorganic Hg ($\mu\text{g/g}$)	PSP excretion (%)
Control (saline)	M	—	33.3 \pm 1.5
	F	—	29.4 \pm 1.3
MMC ($\mu\text{mol/kg}$)			
80	M	2.45 \pm 0.82	1.3 \pm 1.0*
	F	1.90 \pm 0.57	27.8 \pm 9.2
120	M	9.09 \pm 1.11	0.1*
	F	3.02 \pm 0.13	31.2 \pm 2.4
160	M	7.36 \pm 2.55	0*
	F	2.74 \pm 0.89	8.3 \pm 3.7*
HgCl ₂ ($\mu\text{mol/kg}$)			
2	M	8.22 \pm 0.45	42.7 \pm 4.2*
	F	3.97 \pm 0.37	35.7 \pm 3.2
4	M	12.22 \pm 0.27	42.2 \pm 1.6*
	F	6.89 \pm 1.35	37.5 \pm 5.1
10	M	19.35 \pm 1.55	26.3 \pm 18.0
	F	18.11 \pm 1.50	1.9 \pm 0.6*
20	M	28.56 \pm 0.87	0.3 \pm 0.1*
	F	32.22 \pm 4.37	0.4 \pm 0.2*

^aMMC and HgCl₂ were administered orally and intravenously, respectively. Values represent mean \pm SD obtained from 5 mice.

*Significantly different ($p < 0.01$) from control mice.

Table 4

Effect of Selenite Coadministration on Hg Levels in Plasma and Kidney and on Renal Function 24 h after Treatment in Male Mice^a

Treatment	Plasma Hg ($\mu\text{g/mL}$)	Renal Hg ($\mu\text{g/g}$)	PSP excretion (%)
MMC	5.76 \pm 1.25	93.8 \pm 9.0	0.6 \pm 0.3
MMC + Na ₂ SeO ₃	5.54 \pm 0.52	104.2 \pm 9.8	0.6 \pm 0.4
HgCl ₂	1.04 \pm 0.28*	28.7 \pm 3.0*	0.2 \pm 0.1*
HgCl ₂ \pm Na ₂ SeO ₃	5.40 \pm 0.54	17.7 \pm 1.4	20.1 \pm 3.9
Na ₂ SeO ₃	—	—	36.0 \pm 2.3

^aMMC (120 $\mu\text{mol/kg}$) was administered orally, and HgCl₂ and Na₂SeO₃ (20 $\mu\text{mol/kg}$ for each) were administered intravenously. Values represent mean \pm SD obtained from 5 mice.

*Significantly different ($p < 0.01$) from selenite coadministered group.

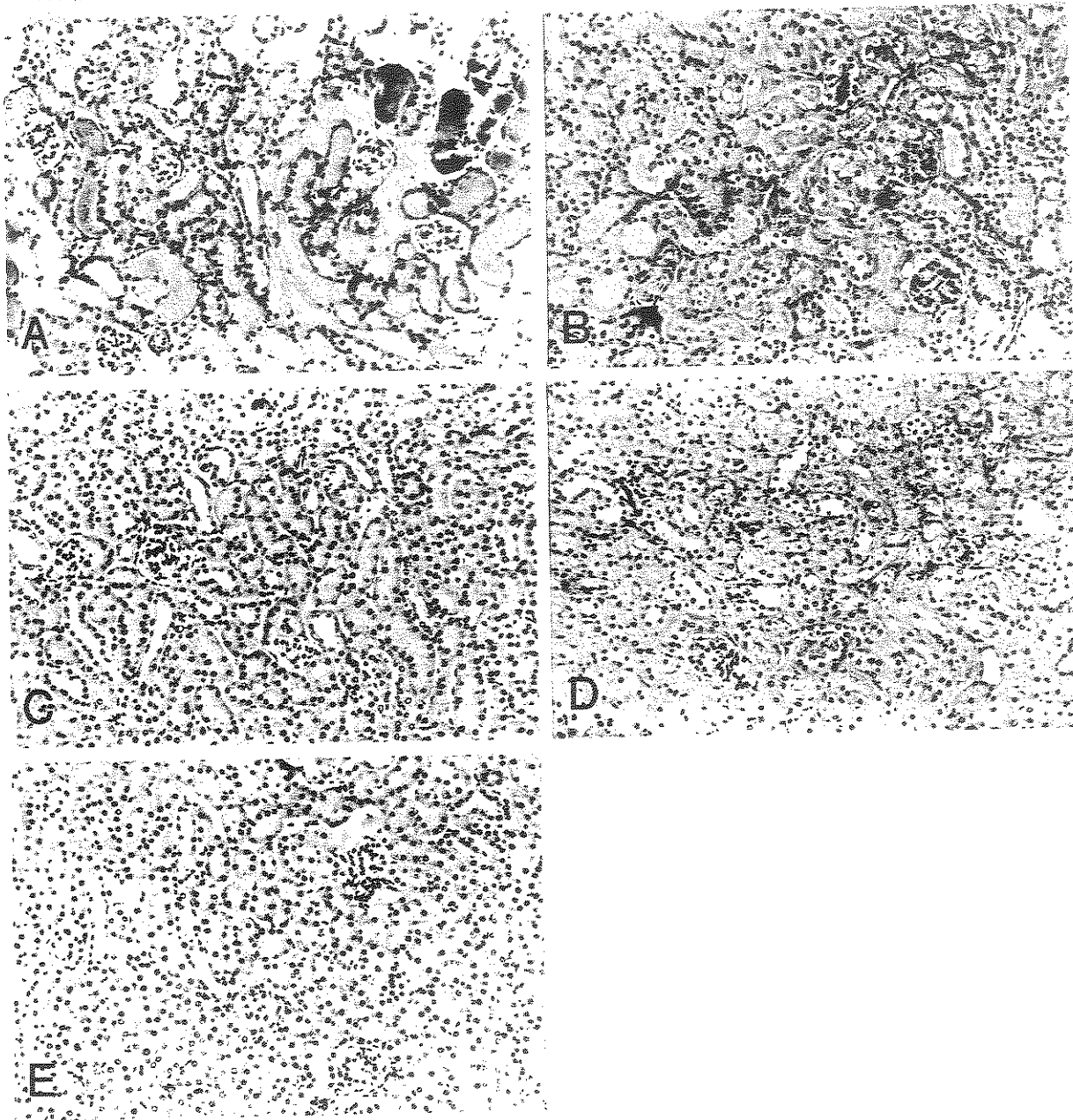


Figure 3. Photomicrographs of the renal cortex of C57BL/6N mice at 24 h after MMC (120 $\mu\text{mol/kg}$) or HgCl_2 (20 $\mu\text{mol/kg}$) treatment. The proximal tubular epithelium of HgCl_2 -treated female (A) and male (B) mice degenerated seriously, whereas changes in MMC-treated males (D) were less remarkable. The histological features of the kidney of MMC-treated females (C) were almost identical with those of control mice (E).

MeHg single administration in rats (9), and since Hg_i was a potent nephrotoxin (6–8), some contribution of Hg_i in the above-mentioned renal failure could well have been expected. Fowler (19) suggested the possibility of a toxic

effect of Hg_i on the proximal tubules in rats fed MeHg-containing diet.

In the present study, the renal function was already disturbed as early as 24 h after MMC treatment with

with dose levels of 80 and 160 $\mu\text{mol/kg}$ for males and females, respectively. Although a similar disturbance was observed after treatment with HgCl_2 with dose levels exceeding 10 $\mu\text{mol/kg}$ in both sexes, the renal Hg_i levels after toxic doses of MMC treatment were much lower than the levels after HgCl_2 administration of toxic doses (see Table 2). Thus, the involvement of Hg_i in the renal failure caused by MMC administration would be negligible at least at 24 h after the administration; MeHg itself possibly caused the renal disturbance observed here. There were a few differences between nephrotoxic actions of MeHg and Hg_i as follows: (a) the sex difference in the renal susceptibility was reversed between two mercurial species in C57BL mice; (b) although the nephrotoxic action of HgCl_2 was markedly depressed by the coadministration of selenite, that of MeHg did not change at all; and (c) the pathological changes in the renal cortex caused by MeHg were much slighter than those induced by Hg_i even though the renal function was damaged similarly. These observations indicate that the mechanism of nephrotoxic action of MeHg might be quite different from that of mercuric mercury.

The present results suggest that the kidney is one of the critical tissues by which susceptibility to MeHg acute toxicity is determined, and that the kidney of male mice has markedly higher susceptibility to MeHg toxicity than that of females, probably due to a higher glutathione turnover rate in the tissue.

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REFERENCES

- Hirayama K, Yasutake A: Sex and age differences in mercury distribution and excretion in methylmercury-administered mice. *J Toxicol Environ Health* 18:49-60, 1986.
- Hirayama K, Yasutake A, Inoue M: Effect of sex hormones on the fate of methylmercury and on glutathione metabolism in mice. *Biochem Pharmacol* 36:1919-1924, 1987.
- Yasutake A, Hirayama K: Sex and strain differences of susceptibility to methylmercury toxicity in mice. *Toxicology* 51:47-55, 1988.
- Jacobs MB, Yamaguchi S, Goldwater LJ, Gilbert H: Determination of mercury in blood. *Am Ind Hyg Assoc J* 21:475-480, 1960.
- Yasutake A, Hirayama K: Selective quantification of inorganic mercury in tissue of methylmercury-treated rats. *Bull Environ Contam Toxicol* 45:662-666, 1990.
- Farah A, Kruse R: The relation of mercurial diuresis to cellular protein-bound sulfhydryl changes in renal cells. *J Pharmacol Exp Ther* 130:13-19, 1960.
- Ganote CE, Reimer KA, Jennings RB: Acute mercuric chloride nephrotoxicity. An electron microscopic and metabolic study. *Lab Invest* 31:633-647, 1974.
- McDowell EM, Nagle RB, Zalme RC, McNeil JS, Flamembaum W, Trump BF: Studies on the pathophysiology of acute renal failure. I. Correlation of ultrastructure and function in the proximal tubule of the rat following administration of mercuric chloride. *Virchows Arch B Cell Pathol* 22:173-196, 1976.
- Norseth T, Clarkson TW: Studies on the biotransformation of ^{203}Hg -labeled methyl mercury chloride in rats. *Arch Environ Health* 21:717-727, 1970.
- Mehra M, Choi BH: Distribution and biotransformation of methyl mercuric chloride in different tissues of mice. *Acta Pharmacol Toxicol* 49:28-37, 1981.
- Chmielnicka J, Komsta-Szumaska E, Jedrychowski R: Organ and subcellular distribution of mercury in rats as dependent on the time of exposure to sodium selenite. *Environ Res* 20:80-86, 1979.
- Naganuma A, Ishii Y, Imura N: Effect of administration sequence of mercuric chloride and sodium selenite on their fates and toxicities in mice. *Ecotoxicol Environ Safety* 8:572-580, 1984.
- Yasutake A, Hirayama K, Inoue M: Mechanism of urinary excretion of methylmercury in mice. *Arch Toxicol* 63:479-483, 1989.
- Tsubaki T: Organic mercury intoxication in the Agano River area studied by Niigata University Research Group. *Clin Neurol* 8:511-520, 1968.
- Iverson F, Downie RH, Paul C, Trenholm HL: Methylmercury. Acute toxicity tissue distribution and decay profiles in the guinea pig. *Toxicol Appl Pharmacol* 24:545-554, 1973.
- Takeuchi T, Eto K: Pathogenesis of chronic Minamata Disease (chronic methylmercury poisoning). *Adv Neurol Sci* 18:845-860, 1974.
- Tagashira E, Urano T, Yanaura S: Methylmercury toxicosis. I. Relationships between the onset of motor incoordination and mercury contents in the brain. *Folia Pharmacol Japon* 76:169-177, 1980.
- Klein R, Herman SP, Brubaker PE, Lucier GW: A model of acute methyl mercury intoxication in rats. *Arch Pathol* 93:408-418, 1972.
- Fowler BA: Ultrastructural evidence for neuropathy induced by long-term exposure to small amount of methyl mercury. *Science* 175:780-781, 1972.
- Mitsumori K, Takahashi K, Matano O, Goto S, Shirasu Y: Chronic toxicity of methylmercury chloride in rats: Clinical study and chemical analysis. *Jpn J Vet Sci* 45:747-757, 1983.
- Mitsumori K, Maita K, Shirasu Y: Chronic toxicity of methylmercury chloride in rats: Pathological study. *Jpn J Vet Sci* 46:549-557, 1984.