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An Attempt to Assess the Inheritable Effect of Methylmercury Toxicity Subsequent to Prenatal Exposure of Mice

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A human epidemic of methylmercury poisoning occurred in certain areas around Minamata Bay, Japan in 1953 - 1960, through the consumption of fish and shellfish contaminated by an effluent from an acetaldehyde plant. Such poisoning has since been known as "Minamata Disease" (Tsubaki and Irukayama 1977). To date, more than 2,000 persons have been officially declared to have Minamata Disease, and 630 of these have already died (Nomura et al. 1986). During the outbreak dozens of babies who were affected by methylmercury in utero were born in these areas following maternal ingestion of contaminated foods. The clinical features of this "Congenital Minamata Disease" were mental retardation and motor disorders (Harada Y 1977; Harada M 1978). Besides the actual damage to the fetus when mothers ate methylmercury-contaminated foods, the specter was also raised that irreversible genetic damage could produce malformations or more subtle deviations in subsequent generations. Consequently, some parents refused to let their sons and daughters marry persons from Minamata (D'Itri and D'Itri 1977).

In the previous experiment (Inouye et al. 1985), we treated pregnant mice with 20 mg/kg methylmercuric chloride and observed the growth of their offspring. After the termination of the experiment, three male offspring were mated with ten untreated females. Four fetuses with neural tube defects (exencephaly and brain hernia) and two with minor malformations were found among 73 offspring when examined at term. Although it was hoped that this high percentage of malformations might be just a chance occurrence, we decided to examine the effect of maternal exposure to methylmercury on the reproduction of the second filial generation (F2). Since many offspring died during the perinatal period in the previous experiment when pregnant mice were treated with 20 mg/kg methylmercuric chloride, dams were given 15 mg/kg of the chemical to obtain a viable first filial generation (F1) in the present experiment. The F1 males were used to determine the potential of methylmercury to produce male-mediated fetal effects, and female littermates were examined for mercury retention.

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MATERIALS AND METHODS

Animals employed were inbred C3H/HeN mice commercially supplied by Kyudo Co., Ltd., Kumamoto. They were kept in a room at 22 ± 2° C and 50 - 60 % relative humidity. A solid diet of CA-1 (total mercury less than 0.01 ppm and selenium less than 0.5 ppm), produced by CLEA Japan, Tokyo, and tap water, were made available ad libitum.

Nulliparous females of more than 12 weeks were caged with males in pairs overnight, and the next morning females with vaginal plugs were taken to be in day 0 of pregnancy. Thirty-five pregnant females were divided into three groups. The females of two groups were orally administered methylmercuric chloride, dissolved in distilled water, at a dose of 15 mg/kg (12 mgHg/kg) on day 14 or 17 of pregnancy. The females of the third group were not treated during pregnancy. Twenty pregnant females were also set aside for foster mothers.

All pregnant females were allowed to give birth. The newborn offspring (F1) were foster-mothered by untreated dams (6 pups for each dam) to avoid intake of mercury via breast milk. Twenty-five female offspring were taken from each group for mercury analysis on 1, 7, 14, 21 and 28 days after birth. Total mercury levels in the blood, brain, liver and kidney were determined by the oxygen combustion-gold amalgamation method (Jacobs et al. 1960). More than half of the males were killed for neurochemical analysis of the brain at 9 weeks of age (data to be reported separately).

F1 males of 12 weeks were caged singly and mated with females of the same strain commercially supplied. The females were examined for vaginal plugs every morning. In addition, 11 commercially supplied males of the same strain were also paired with females.

Pregnant females were put to death by cervical dislocation on day 18 of pregnancy. The uterus was opened and examined for resorption sites. The fetuses (F2) were counted, removed, examined for external malformations under a dissecting microscope, and weighed. Abnormally small fetuses were defined as those which weighed less than 0.80 g (mean − 3SD of controls). They were fixed in 95 % ethanol, eviscerated, cleared with KOH and stained with alizarin red S for demonstration of the skeleton. These specimens were examined for skeletal abnormalities and for the number of ossified vertebrae under a dissecting microscope.

The data for litter size, fetal mortality, and incidence of malformations and abnormally small fetuses were analyzed using the chi square test with Yates' correction for comparisons between control and experimental values. The data on the body weight and the number of ossified vertebrae of fetuses were analyzed using Student's t-test (two-tailed) or Welch's test. P values less than 0.01 were considered to be significant.

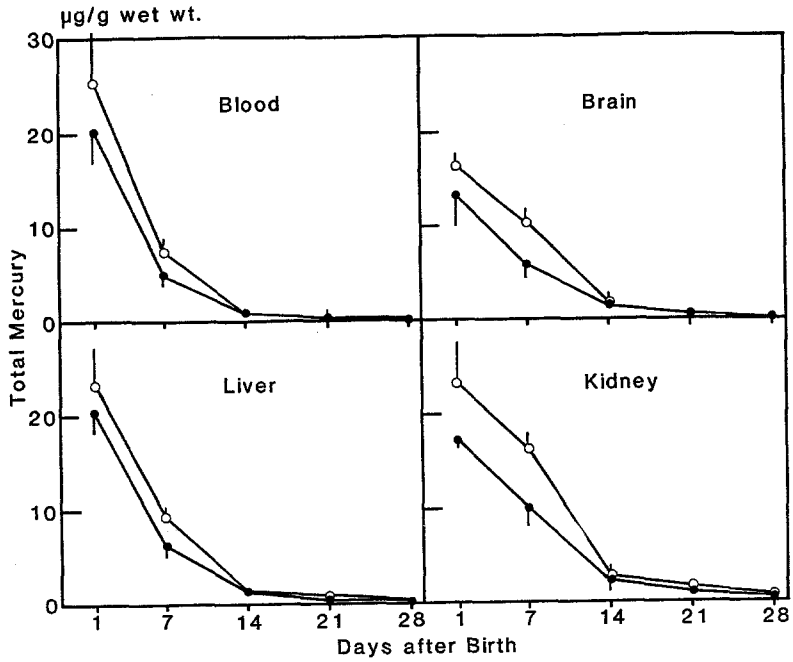

Figure 1. Total mercury contents in the blood and organs of F1 female offspring of which dams were treated with 15 mg/kg (12 μgHg/g) methylmercuric chloride on day 14 (●—●) or 17 (○—○) of pregnancy. Each point indicates an average ± SD of five samples.

Table 1. Production of F1 generation by maternal treatment with 15 mg/kg methylmercuric chloride

Pregnant day treated(No. dams)	Live birth (F1) Male	Female	Growth of F1 males Died	Weaned	F1 mated
Day 14 (12 dams)	28	31	4	24	10(group A)
Day 17 (12 dams)	41	27	4	37	16(group B)
Untreated(11 dams)	32	39	0	32	8(group C)

RESULTS AND DISCUSSION

As shown in Figure 1, mercury was retained in the body of F1 females for weeks after birth following maternal exposure to methylmercury during pregnancy, suggesting the retention of mercury in the male littermates. However, more than 85 % of male offspring survived to adulthood (Table 1), and all males examined were fertile.

The pregnancy status of female mice mated with F1 males was presented in Table 2. Fetuses with exencephaly were detected among

live fetuses of group A. No surviving fetuses were involved in the malformation in control group C, but there were two dead cases. No statistically significant difference was recognized in the incidence of exencephaly, when dead cases were included, between the two groups. Abnormally small fetuses were found in groups A, B and C, but the frequencies were not significantly different among groups. Umbilical hernia was a common malformation occurring both in the treated and the untreated groups. Micophthalmia and polydactyly of the hindlimb appeared as single cases in group B. Frequencies of abnormal fetuses, including both those involved in any kind of malformations and having abnormally low weights, were not significantly different among groups A, B and C. There were a small number of fetuses with slight skeletal variation (e.g., 14th rib) in all groups.

Both body weight and the number of ossified vertebrae were significantly reduced in group B when compared with the control group C, and they were also significantly different between group C and the breeder's group (Table 2).

In the present experiment, F1 male mice of which dams were exposed to methylmercury during pregnancy were mated with untreated females, to assess the inheritable effect of the toxicity. This method has been applied ever since Nomura (1975) showed the possibility that the teratogenic effect following maternal exposure to chemicals would be transmitted to F2 generation, and also cases were reported in which paternal exposure to X-rays and chemicals produced malformations in the offspring (Nomura 1982; Kirk and Lyon 1984; Nagao 1987). In the testes of male mouse type A cells, the ancestral stem cells of the spermatogonia, first appear at 3 days after birth, and meiotic divisions begin at about 8 days after birth (Rugh 1962). During this period F1 mice retained a high concentration of mercury in the body (Figure 1), and hence the ancestral stem cells of the spermatogonia could be affected by mercury.

Some malformations were detected in the F2 offspring (Table 2). The incidence of exencephaly was not significantly different among offspring of treated and control F1 males. Exencephaly, as well as the microphthalmia, open eyelid and polydactyly seen in the present experiment, is known to be a spontaneously occurring malformation in mice (Nagao 1987; Morita et al. 1987). The umbilical hernia manifested both in the treated and the untreated groups, seemed to be spontaneous defect in this strain of mice.

Reduction both in the body weight and the number of ossified vertebrae of fetuses in group B suggested some intra-uterine growth retardation. The mechanism of this effect is obscure. But both the indices were significantly different between the two control groups (Table 2), so this reduction might be just a chance occurrence. When one reflects that some hazardous effects induced by maternal exposure to chemicals are transmitted to F2 generation (Nomura 1975), and also that paternal treatment with chemicals is able to affect the subsequent generation (Nomura 1982; Kirk and

Table 2. Pregnancy status of females mated with F1 or breeder's males

Group Males	Females pregnant	Corpora lutea	Implants	Fetuses (F2) Dead	Fetuses (F2) Live	Anomalies	Body weight mean ± SD g	Ossified vertebrae
A 10	32	297	280 (94.3 %)	22 (7.8 %)	258	5: Exencephaly, 2 Umbilical hernia, 2 Small, 3	1.05 ± 0.092	35.5 ± 1.02
B 16	41	387	368 (95.1 %)	34 (9.2 %)	334	14: Microphthalmia, 1 Polydactyly, 1 Umbilical hernia, 4 Small, 9	1.02 ± 0.109*	35.3 ± 1.11*
C 8	14	125	114 (91.2 %)	18 (15.8 %)	96	1: Small, 1 (Dead exencephaly, 2)	1.06 ± 0.085	35.7 ± 0.96
Breeder's 11	28	259	243 (93.8 %)	32 (13.2 %)	211	1: Umbilical hernia, 1	1.15 ± 0.123*	36.8 ± 1.39*

Percentages: Ratios of implants to corpora lutea and dead fetuses to implants.
*Significantly different from group C (control).

Lyon 1984; Nomura 1987), the possibility that methylmercury affected spermatogonial stem cells of F1 males during the perinatal period and thus subsequently induced growth retardation of F2 offspring, cannot be altogether dismissed. However, reduction in the body weight of F2 was less than 4 %, even when F1 males were suggested to have retained a high concentration of mercury from the data of female littermates (Figure 1). The highest methylmercury level in the blood of the umbilical cord was 4.65 μg/g dry weight in patients of congenital Minamata disease, and more than 1 μg/g in many cases (Harada 1975, 1978). They were less than 1 μg/g and 0.1 - 0.2 μg/g wet weight, respectively, since values calculated in dry weight are usually equivalent to 5-10 times those in wet tissue. These values are considerably lower than those in the present experiment. Therefore, even if the slight growth retardation noted in F2 fetuses of the present experiment could be caused by methylmercury, male-mediated fetal effects subsequent to the intra-uterine exposure to the chemical may be practically nil in humans.

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