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Effects of Methylmercuric Chloride of Low Concentration on the Rat Nervous System

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Methylmercury chloride (MeHg) poisoning, well known as Minamata disease, is characterized by symptoms and signs of neuropathy, such as cerebellar ataxia and sight and hearing loss (Takeuchi 1961). Experimentally, the crossing of the hind legs on being held by the tail (Suzuki and Miyama 1971) or a rotating movement of the tail (Ohi et al. 1978) have been considered to be typical signs of MeHg poisoning in the rodents. In addition, Berlin et al. (1975) have demonstrated the MeHg-induced visual impairment is an early sign of preclinical changes. Mattson et al. (1981) reported that a subtle distortion of Visual Evoked Response (VER) occurred in dogs fed MeHg over 2 months, in spite of a relatively low concentration of MeHg in the brain tissue.

In an earlier study we reported the effects of 20 μg/g of MeHg on the rat (Yamamura et al. 1986). The results were as follows. After 2-week exposure to 20 μg/g MeHg, effects on behavior, pathological changes of brain and prolongation of EEP (early potential of evoked potential) latency were observed. So, in this experiment, we planned to expose rats to lower concentrations of MeHg. We therefore investigated the effects of MeHg exposure at a low concentration on behavioral indices, neurological signs, the circadian rhythm of behaviors, EEP, and pathology of the visual cortex and the sciatic nerve in rats.

MATERIALS and METHODS

Fifty-nine wistar adult male rats were fed water and diet ad libitum. The rats were housed in a room having an ambient temperature of 21-25°C.

The commercial powder chow was purchased from Nihon Clea Co. (CE-2). A methylmercury chloride (MeHg) diluted by a small amount of ethyl alcochol (1-2 ml)

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was mixed in the above chow (250 g) with water of 250 ml so as to obtain a content of 10 μg Hg/g. Small dumplings were made from the above diet and soon after they were deposited in a deep freezer (Revco. Co., at -80°C) in order to prevent vaporization of the MeHg. Animals were fed a diet containing MeHg (10 μg/g) from 10 weeks of age when the body weight of the rats exceeded 250 g. The experimental schedule of MeHg administration was as follows.

Exp. 1 was a schedule of 4 week exposure to MeHg.
Exp. 2 was that of 6 week exposure to MeHg.
Exp. 3 was that of 8 week exposure to MeHg.

Eleven rats were exposed to MeHg in Exp. 1, 11 in Exp. 2 and 10 in Exp. 3. Seven to 10 control rats were observed for each experiment.

The rats were observed every five days for neurological signs, namely crossing of the hind legs on being held by the tail and the rotating movement holding the tail as an axis. Spontaneous movements of MeHg exposed rat and control rats were continuously measured by an Automex II. The counts of spontaneous activity were summarized every ten minutes and, using a minicomputer (HP-1000). Power spectral analysis was performed after auto-correlation analysis of time series data of spontaneous activities of ever 10 minutes.

The EEP of the animals was measured electrophysiologically. The rat was fixed to a stereotaxic apparatus (SR-6, Narishige Co.) after narcosis with nembutal (0.5 cc/kg: 50 mg/ml). Enamel coated tungusten wire (diameter: 100 μ) as a unitary electrode in which the cross section was uncoated, was placed on the rat cortex through a small hole. The small hole in the rat skull was made by a dental burr (Volber-5, NSK). The hole was located on the interaural line and 3 mm lateral to the midline in the adult rat skull. A reference electrode was placed on the rats' ear.

From the outside of a shield box, brief (20 μ sec) light flashes (0.64 J) were produced by a photostimulator (NEC-SANEI, 1A12) placed 25-30 cm in front of the rat's head. Ten to 20 minutes before above examination, one or two drops of midriatic (mydrin P) was applied to rat's eyeball. The stimulating frequency was 3 Hz. To evaluate EEP, two hundred evoked potentials were amplified (Nihonkoden, Model Vc-10); each one was summed by computer (signal processor 7T07, NEC-SANEI Co.) and the results of these average summed data were plotted on the memory scope of the above computer. Two or three such summations were observed for each rat.

The latencies (ms) of EEP during 10 ms from the onset

of the light flash were measured from the above memory scope using Polaroid Instantfilm. The latencies of EEP were measured as follows. The time from flash stimulus to initial upward deflection of potentials was measured. Above potential of short latency is called "EEP" by authors.

Estimation of mercury in the blood and the brain: Cerebral (excluding the cerebellum) and blood specimens were collected immediately after decapitation. Each total mercury level was measured according to the method of Taguchi (1971) using a Beckman-Toshiba model MV-253 mercury vapormeter. The values were expressed as micrograms per gram wet sample. The mercury concentration in the brain as a whole (excluding the cerebellum) was estimated. One hemisphere of collected brain was used to obtain total mercury and the sciatic nerves was used for pathological examination.

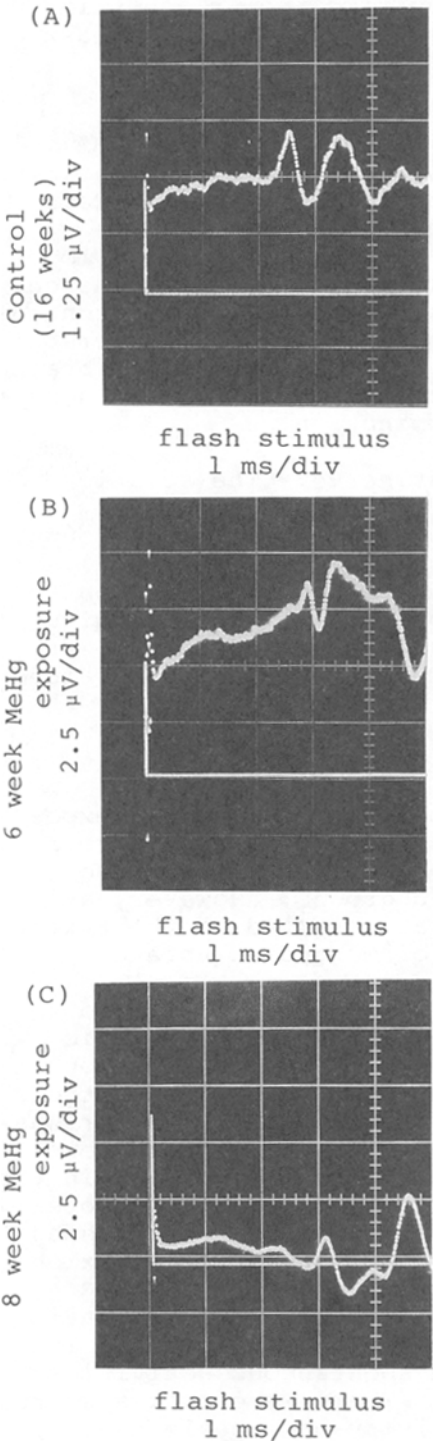
Pathological study of MeHg rat nerve: The sciatic nerves of 35 rats were fixed in 10 % formalin for histophathological examination (4 from control groups, 11 from Exp. 1, 10 from Exp. 2 and 10 from Exp. 3). The sciatic nerves were dehydrated and embedded in paraffin as usual by method of Ambo (1973). Longitudinal and transverse 8 μm sections of the nerves were prepared and stained with H.E. The myelin was stained with luxol fast blue and the axon with Bodian.

RESULTS and DISCUSSION

The body weight of rats in both the 4 week MeHg exposure and the control groups increased. There were no significant differences in body weight between the control groups and the exposed groups. However, a decrease of rat body weight was observed in the 8 week exposure group after 7 weeks of MeHg diet uptake.

The dosage parameters of MeHg exposure used did not result in overt signs of toxicity such as paralytic gait. Slight flexion of the hind legs and rotation on holding the tail as an axis were not observed in the animals treated with MeHg for 4 weeks (Exp. 1) nor was crossing of hind legs observed. However, there were 3 cases with flexion and crossing of the hind legs in the animals treated with MeHg for 6 week (Exp. 2). Moreover, the rotation movement on holding the tail as an axis was observed frequently. Six cases with flexion and crossing of the hind legs were observed in the 8 week MeHg exposed group (Exp. 3: n=10). The rotation movement was not present in any of the controls. In rats of every experiment, the spontaneous activities were dominant in dark period. There were peak spectral values of the spontaneous activity on 1 cycle/day.

Figure 1
The time pattern of EEP



The EEP of adult rats more than 10 weeks old was obtained by stimulation with a strobe flash of 200 times. In Figure 1, the figure (A); an example of EEP of a control rat, (B); an example of the EEP of a 6 week MeHg exposed rat and (C); an example of the EEP of a 8 week MeHg exposed rat. The EEP data for the control cases were obtained from controls for both the 4 week exposure group and 6 week exposure group. In this figure, the abcissa is latency of EEP (1 ms/div). The above datam of EEP obtained during 10 ms after the light flash stimulus was summed and averaged 200 times, and the primary response induced by light flash was observed. The data for latency of the primary peak waves in these experiments are expressed in Table 1. The latency of primary response of EEP was observed to lengthen in the order of control, 6 week MeHg exposed rats and 8 week MeHg exposed rats.

There was a wide difference between the total cerebral mercury content in the control rats and in those exposed to MeHg ($P < 0.05$). The studies on blood mercury levels showed similar results. In the rats exposed to MeHg for 8 weeks (Exp. 3), the total average of cerebral mercury content (0.49 μg Hg/g, n=10) was not significantly higher than that in rats exposed for 6 weeks (0.47 μg Hg/g, n= 11). However, the total

Table 1 Latency (msec) of the rat's EEP in MeHg exposure

t=8.21 ($P < 0.01$)

t=3.70 ($P < 0.01$) t=2.19 ($P < 0.05$)

Control (M±S.D.)	4 week exposure (M±S.D.)	6 week exposure (M±S.D.)	8 week exposure (M±S.D.)
2.60±0.26 (n=27)	2.75±0.44 (n=18)	3.08±0.62 (n=30)	3.38±0.43 (n=30)

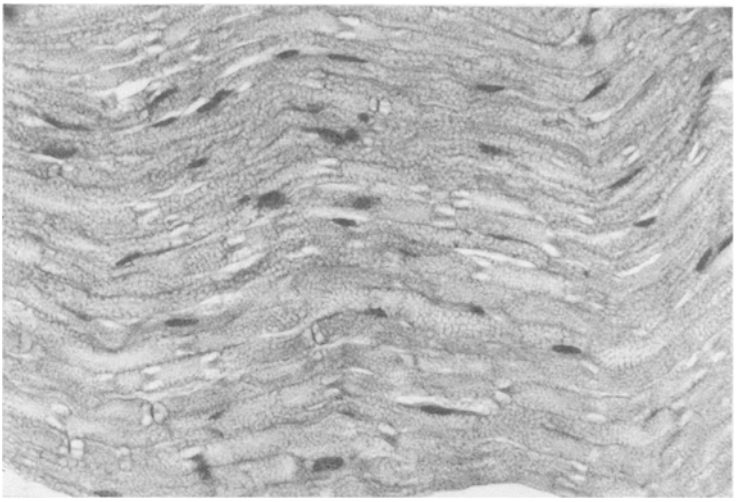
n=number of samples (2~3 samples/1 rat)

average cerebral mercury contents in Exp. 2 and Exp. 3 were higher than that of Exp. 1 (0.18 μg Hg/g, n=11, $p < 0.01$). But, there were not significant difference of the blood concentration of MeHg among those of Exp. 1, Exp. 2 and Exp. 3 (ca 3 μg Hg/g). The total mercury content in the diet decreased by an average of 3.0 percent at ambient temperature and humidity after 2 days.

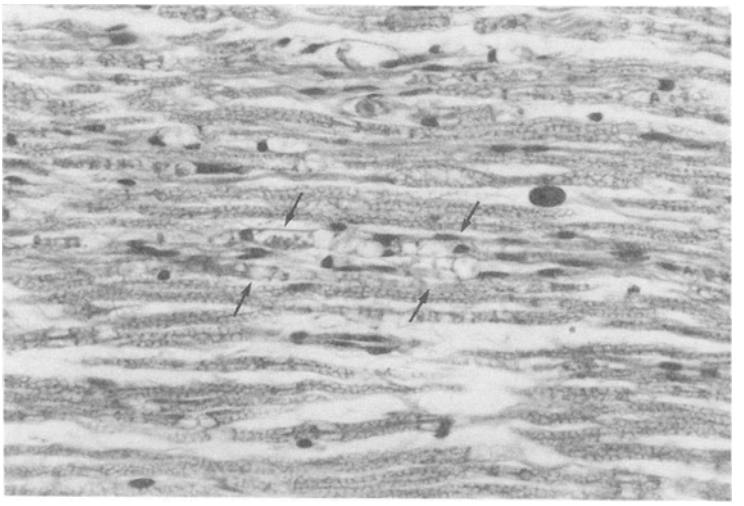
No histological abnormalities were recognized in the sciatic nerves of the 4 week exposure group (11 rats; Exp. 1). Endoneurial edema was not observed in the 6 week exposure group (10 rats; Exp. 2) but sporadic destruction of the myelin sheath (demyelination) and degeneration of axons were observed in all rats of this group. The changes, however, were less severe than those in 8 week exposure group. Sporadic destruction of myelin sheath and degeneration of axons were observed in all 10 rats of the 8 week exposure group (Exp. 3). Mild endorneurial edema was also observed in all of this group (Miyakawa 1970; Cavanagh 1971). An example of the sciatic nerve of Exp. 3 is shown in Figure 2.

A decrease in body weight was observed in the 8-week MeHg-exposed rats. Flexion and/or crossing of the hind legs were observed in the 6 week exposure group. Moreover, crossing of the hind legs and rotation of a tail were observed in the rats of Exp. 3. The above changes of behaviour of rats in Exp. 2 and Exp. 3 were considered to be MeHg poisoning.

Suzuki and Miyama (1971) reported that in an experiment on single or repeated oral administration of MeHg to mice, death followed the occurrence of neurological



longitudinal section of a sciatic nerve of control rat (H.E., ×330)



sciatic nerve of 8 week exposed rat showing endoneurial edema and myelin sheath-breakdown with degenerated axon (H.E., ×330)

Figure 2 Sciatic nerve of control and 8 week exposed rat

symptoms, such as an ataxic gait, when amount of mercury was more than 30 μg Hg/g of diet, while the lowest daily dose group (10 μg Hg/g of diet) did not cause loss of body weight or neurological symptoms during the experimental period up to the 41st day of MeHg feeding.

In the study of Arito et al. (1982), MeHg-dosed rats

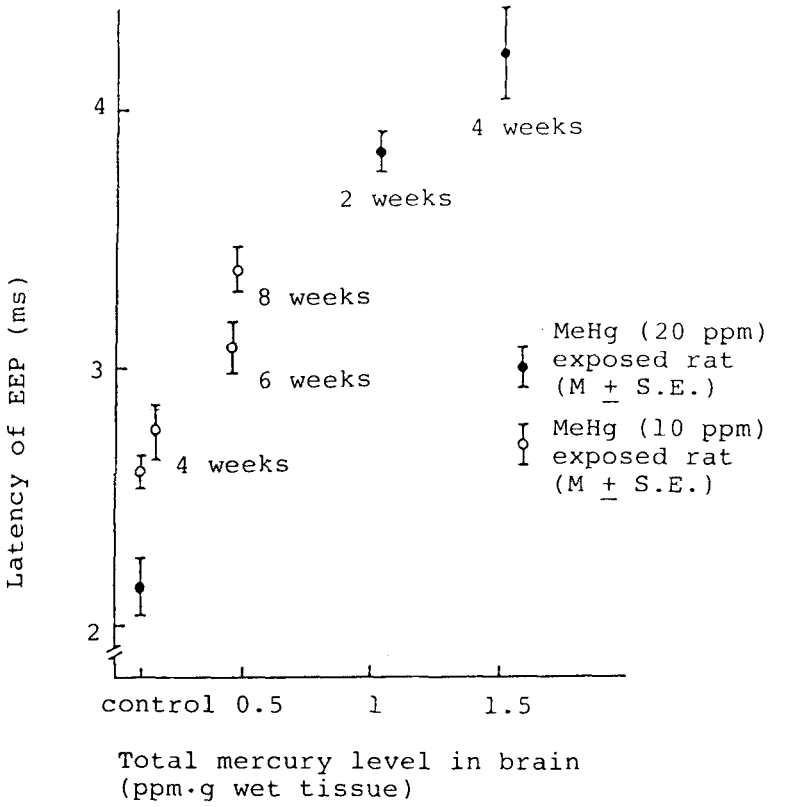


Figure 3 Latency of EEP

exhibited an increase in both dark-phase slow wave sleep and paradoxical sleep but a decrease in light-phase paradoxical sleep, suggesting that the activity of rats in the dark phase decreases while in the light phase it increases. However, in this study, the peak of spontaneous activity was observed in the dark phase. In this experiment, a change of the latency of the EEP induced by MeHg poisoning was observed. However, data on latency and potential in EEP of man showed variation among investigators. In our experiment, a fine enamel-coated tungsten electrode was passed through a small hole in the rat skull and the EEP to a strong flash stimulus as an indicator of evoked potential was obtained. The response of EEP was considered to be a small potential (within 1-2 μV), so processing was performed 200 times. Takahashi (1980) has reported that the latency of EEP obtained from the rat visual cortex was 2-3 ms.

Yonemura et al. (1967) reported that the latency of primary response of VEP (PPP; positive primary potential) obtained from an electrode on the rabbit cortex

was 13 ms and in particular that the latency of EVEP was only 6.5-7 ms. From the latency of potential, the EVEP of short latency contained the SCP (potential of superior colliculus) as a component. Above mentioned reason, the EEP, namely, small potential of short latency was also considered to represent a peripheral function of visual system of the rat.

In Figure 3, we expressed the relationship between total mercury level in the brain and the latency of EEP (ms) using data of a previous experiment (Yamamura 1986) and that of this experiment. A dose-response relationship may thus exist between total mercury level and latency of EEP.

There is a considerable risk of accumulation of methylmercury in the tissues of mammals by prolonged exposure to methylmercury compounds. This risk is mainly due to its very slow elimination and a biological half-life which varies between twenty and seventy days in different species.

In our experiment, mercury concentration in the brain was lower than is reported in the report of Ohi et al. They have reported that the onset of tail rotation is an early mainfestation of neurological symptoms induced by MeHg feeding. Mattson et al. (1981) reported that dogs fed MeHg for 2 months showed a subtle distortion of VER and that the brain mercury content was 1.28 μg Hg/g in the occipital cortex. Our data of MeHg were obtained from hemisphere of brain excluding cerebellum and the concentration of MeHg was 0.5 μg Hg/g.

Total mercury concentrations in the blood were about 3 ppm in our experiments. It was observed in other experiments (Nordberg and Skerfving 1974, Chang 1977) that the mercury concentration in the blood of rats fed MeHg increased more than tenfold over mercury concentration in the brain. The rat, markedly different from other species, has a low content of mercury in the brain under MeHg poisoning compared to other organs. The result of our experiment showed approximately the same trend.

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