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National Institute for Minamata Disease

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(as of March, 2002)

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Research Activities

1. Clinical study on Minamata disease

1. Pathological and biochemical study for all (30) autopsy cases of Niigata Minamata disease (methylmercury poisoning)

Komyo Eto (Clinical Medicine)
Akira Yasutake (Basic Medical Sciences)
Atsuhiro Nakano (Basic Medical Sciences)

An additional 3 autopsy cases of Niigata Minamata disease patients were sent to NIMD. The clinical and pathological evidence was reported precisely. The pathological changes of the spinal sensory nerves were prominent all 3 cases. One case showed severe pathological changes of spinal sensory nerves due to the complicated disease of diabetes mellitus. Additionally, another 12 autopsy cases of Niigata Minamata disease will be examined from the next year.

2. The study on clinical features of Minamata disease patients

Junji Wakamiya (Clinical Medicine)

The multivariate analysis of neurological findings between the residents in an organic mercury unpolluted district and the applicants in Kumamoto, Kagoshima, and Niigata until 1975 was conducted. The degree was light in order of Kumamoto, Kagoshima, and Niigata, and there were many complications in Kumamoto. Two diagnostic models were calculated using significant differences of neurological findings as indices and using the difference between the typical Minamata disease patients and normal residents. Both showed excellent discrimination and the discrimination was the same. Those who recognized as Minamata disease patients showed high predicting index score, and this result was corresponding to the result by these multivariate analyses well. It was thought that the differential diagnosis among organic mercury poisoning and other neurological diseases was important in applicants who didn't recognize as Minamata disease. Moreover, we make the model using only sensory disturbance, but this model showed low discrimination.

3. Study on the occurrence mechanism of nerve cell injury through the NMDA receptor of methylmercury

Junji Wakamiya (Clinical Medicine)
Ken-ichiro Miyamoto (Clinical Medicine)
Tatsumi Adachi (Basic Medical Sciences)

Methylmercury 5 mg/kg/day was administered orally for 12 consecutive days to a mature rat, AIDA which is a metabotropic type inhibitor and kynurenic acid which is glycine site inhibitor of the NMDA receptor were administered simultaneously, and nerve cell death was examined. As a result, the apoptosis of cerebellar granule cell and cerebral cortex was suppressed by kynurenic acid, AIDA administration. It was considered that the NMDA receptor agrees with lesion place by the methylmercury.

4. A study on effects of the immune system of methylmercury

Ken-ichiro Miyamoto (Clinical Medicine)
Junji Wakamiya (Clinical Medicine)
Koji Murao (Clinical Medicine)
Tatsumi Adachi (Basic Medical Sciences)

Rats plasma cytokine IL－2, IL－4, IL－12, IF－γ with MeHg treated were measured. IL－2, IL－4, IL－12 were not changed, but IF－γ was significantly decreased It was shown that IF－γ was related to protects to early bacteria infection. From above these data, it was clear that humoral and cyto-immunity ability are depress to bacteria infection at early stage of MeHg exposure.

5. A study on objective quantity of sensory disturbance in Minamata Disease patient with neurometer

Ken-ichiro Miyamoto (Clinical Medicine)
Junji Wakamiya (Clinical Medicine)
Koji Murao (Clinical Medicine)

For healthy subjects 12 persons, the CPT in 9 a.m., 11:30, 16:30 of the identical person was measured, and the circadian variation was examined. That result, the CPT in morning, daytime, point of time in the evening 2000 Hz and 250 Hz and 5 Hz at each frequency, CV was within 10% on the dispersion in normal-range. There was abnormal sensation after third finger and lateral malleolus of 17 inhabitants on the measurement result of the CPT of the lower for the 3 inhabitants Amazon river fishing village mercury pollution area. As a method for evaluating Minamata disease sensory disturbance for the human in view of the reproducibility in view of the evaluation method, it was clarified that the excellent possibility was high.

6. Study on the effect of the unsaturated fatty acid on methylmercury poisoning

Ken-ichiro Miyamoto (Clinical Medicine)
Junji Wakamiya (Clinical Medicine)
Koji Murao (Clinical Medicine)
Atsuhiro Nakano (Basic Medical Sciences)

By the experimental system equal to last year in n-3 system unsaturated fatty acid and the group which added DHA and not adding group, following experiments were carried out: 8 direction radiation maze task, water maze task, positive choice reaction time measurement. As the result in the group which was made to be n-3 system unsaturated fatty acid and DHA lacking spatial learning failure of 8 direction radiation maze task and water maze task it has have. With it reversely, in the addition group, spatial learning failure by the methylmercury was significantly suppressed.

7. Peripheral nerve findings of Minamata disease (methylmercury poisoning): An immunohistochemical study of autopsy cases

Komyo Eto (Clinical Medicine)
Akira Yasutake (Basic Medical Sciences)

To evaluate peripheral nerve involvement in Minamata disease patients (methylmercury poisoning), we examined the peripheral nerves of human autopsy and control cases. The first group of 14 cases was autopsied around 1974 and the second group of 10 cases was autopsied around 1985. For histochemical examination, we used antibodies to PG-M1 (CD68, macrophage), KP-1 (CD68), protein product 9.5(PGP-9.5), neurofilament 210 (NF-210) and S-100. In the spinal posterior ganglions of the first group of Minamata disease patients, the infiltration of macrophages were prominent surrounding the ganglion cells, and mild infiltration of macrophages were found in the second group of them. The infiltration of macrophages in the spinal posterior root was stronger than the anterior root of the first group in the Minamata disease patients. We conclude that a pathogenesis of sensory nerve lesions was one of the causes from the pathological changes of the spinal posterior ganglions. Usually the spinal posterior ganglions were not shown severe changes. For the study of the complete or incomplete regeneration of the peripheral nerves, it is important to use an electron microscope.

2. Research concerning the mechanisms on methylmercury toxicity and the development of new treatments

1. Methylmercury cytotoxicity and distribution in a cell

Ryoji Aramaki (Basic Medical Sciences)

Reduced amino acid incorporation is thought about as a cause of the MeHg resistance of plateau phase cell. We studied the C-14 amino acid incorporation. Plateau phase cells low level incorporation compared that of log phase cells.

2. Cell line dependency of methylmercury cytotoxicity

Ryoji Aramaki(Basic Medical Sciences)
Akira Yasutake (Basic Medical Sciences)

The death mechanism of MeHg induced V79 cells were examined by use of Annexin V FITC fluorescence method. The result, apoptosis was not thought about in a cause of the death.

3. Study on Function of brain metallothionein

Akira Yasutake (Basic Medical Sciences)

Previously we found that exposure to mercury vapor effectively induced brain metallothionein (MT) in rats. Here, we examined time-dependent alterations following 3 weeks of exposure in the MT isomers, MT-I/II and MT-III, using FPLC-gel chromatography. Rats were exposed to mercury vapor of 8.3 mg/m^3 for 15 hr in total during 5 consecutive days. Total MT levels in rat cerebrum and cerebellum increased by 65% and 155%, respectively, 24 hr after the final exposure. The increased levels remained unchanged for at least 2 weeks after termination of exposure in both tissues. Interestingly, most MT in control rat cerebrum and cerebellum was accounted for by MT-III with MT-I/II being less than 10%. Through mercury vapor exposure, MT-I/II was quickly induced to a significant extent in both tissues, reaching a level comparable to MT-III. The induction rate of MT-I/II in the cerebellum was somewhat higher than in the cerebrum. Chromatograms showed that the MT-I/II thus induced began to decline at an early stage in both tissues. In the cerebrum, the amount of MT-I/II on day 22 was about 30% of the maximum level on day 1. On the other hand, the induction of MT-III was not that dramatic, but it did become evident, at least in the latter stage, when MT-I/II had begun to decrease. Thus, though the induction rate of MT-III was not as high as MT-I/II, it was sustained throughout the experimental period.

4. Study on injury mechanism of the methylmercury poisoning brain neuronal death

Ken-ichiro Miyamoto (Clinical Medicine)
Junji Wakamiya (Clinical Medicine)
Koji Murao (Clinical Medicine)

The band of 150 kDa which is a degradation product of the fodrin in methyl mercury administration rat brain in postnatal 16^{th} was detected, it proves the involvement of the NMDA receptor and there was a chromatic figure in which the antibody for the fodrin is strong for Occipital cortex to Parietal cortex of the rat cerebrum even in the immunostaining. From this result, decomposition of the fodrin by the methyl mercury is NMDA receptor stimulation, Ca influx, by a series of passing route of inflow, calpain activation, decomposition of the fodrin, the

promotion was considered.

5. Study on the effect of the sex hormone on methyl mercury poisoning

Ken-ichiro Miyamoto (Clinical Medicine)
Junji Wakamiya (Clinical Medicine)
Koji Murao (Clinical Medicine)

The effect was equal to that of the model rat last year. It was administered with the methyl mercury, and as it was administered the estradiol was electrophysiologically examined. In the week of final administration (post-seventh), current perception threshold (CPT) was measured in order to evaluate the sensory disturbance by Neurometer. The effect for the methylmercury toxicity was strongly observed in the group which administered ovariectomy and estradiol by returning prediction in the measurement result of the CPT. As a result, the consideration is being carried out at present.

6. Development of laboratory animals for metabolic analysis of methylmercury

Takashi Kuwana (Basic Medical Sciences)
Tatsumi Adachi (Basic Medical Sciences)
Atsuhiro Nakano (Basic Medical Sciences)

Hairless mouse line was compared with normal hair mouse as a model of human. Hairless mice had not a tendency of different metabolism on methylmercury though they could not waste mercury with their hair.
The functions of plasmids, which had been inserted *merA* and *merB* genes, were analyzed whether or not these inserted genes are functional in mammalian cells. Unfortunately, these genes did not act functionally, so we had to decide that this project may require rearrangement.

3. Experimental study on the toxic effects of methylmercury upon offspring

1. Methylmercury poisoning in common marmosets

Komyo Eto (Clinical Medicine)
Akira Yasutake (Basic Medical Sciences)

Common marmosets were used as model animals for methylmercury (MeHg) poisoning. Six marmosets were given MeHg of 5 ppm Hg in drinking water. The animals were divided into 3 groups of 2 each. The first group was examined for acute symptomatic MeHg poisoning. They were given MeHg for 70 and 90 days, respectively, to manifest severe symptoms. The second group was sacrificed after 38 days of MeHg exposure,

when they had acute-subclinical MeHg poisoning. The third group of animals was exposed for 21 days, and then observed for 2.5 years without MeHg exposure. One of them showed typical symptoms of MeHg poisoning after MeHg exposure had ended, but the other showed only slight symptoms without ataxia. This experiment demonstrated that MeHg causes pathological changes in neural tissues including the peripheral nerves in common marmosets. Furthermore, common marmosets were found to show MeHg-induced pathological changes similar to those in humans in the cerebrum and cerebellum.

2. Experimental study on the effect of methylmercury for offspring reproduction

Takashi Kuwana (Basic Medical Sciences)
Hideaki Tsuchiya (Basic Medical Sciences)
Keiko Matsuda (Basic Medical Sciences)
Dongfeng Zhao (Basic Medical Sciences)

Mouse PGCs marked with a transfected GFP gene had been injected into mouse embryos and the fate of the injected PGCs were traced using histological procedure. After 2 days, few of them were recognized at the root of dorsal mesentery in which there are many host PGCs closed to gonadal anlage suggesting the possibility that foreign PGCs would be able to introduce into host gonads with our techniques and that our method would be a good for the direct analysis of methylmercuric toxicity to the germline.

3. Development study on the new assessment methods of toxic effects regarding differentiation and development of neural cells

Tatsumi Adachi (Basic Medical Sciences)

(1) To determine whether the timing of the differentiation of oligodendrocytes can be controlled through PDGF produced by astrocytes, the influence of astrocyte-inducing factors, leukemia inhibitory factor (LIF) and bone morphogenetic protein (BMP)-2, on the differentiation and development of glial cells were investigated. The GFAP expression level markedly increased by the addition of either LIF or BMP-2, and was further enhanced by the addition of both proteins. The number of oligodendrocytes increased by the addition of LIF, whereas decreased by the addition of BMP-2. The number did not change by the addition of both proteins. These results suggest that the timing of oligodendrocyte differentiation cannot be completely controlled, even when astrocyte development is accelerated by the addition of LIF and BMP-2.

(2) When astrocytes were maintained in serum-free medium containing dibutyryl cAMP (dbcAMP), the morphology of astrocytes showed satellite shape for relative long time.

4. Epidemiological study for Minamata disease

1. Epidemiological study on mercury effect fetus in Minamata City

Xiao jie Liu (Epidemiology)
Mineshi Sakamoto (Epidemiology)
Miyuki Matsumoto (Clinical Medicine)
Kiyoka Miyamoto (Clinical Medicine)
Hirokatsu Akagi (International Affairs and Environmental Sciences)
Ryou Nomura (Director General)

Recently in some Fetal Minamata Disease patients, functional impairment is reported to rapidly progress. We conducted symptom surveillance, especially activities of daily living (ADL), in 23 Fetal Minamata Disease patients for the purpose of studying the cause of disease progression and for establishing possible medical and health assistance. Results show that the ability for social interaction is preserved in about three fourths of the patients. However, their motor function in performing ADL is impaired. More than one third of patients need health assistance due to impairment of ADL, especially one half of them need help in bathing themselves. In conclusion, we need to determine the cause of the relatively rapid progression of symptoms and to establish the appropriate treatment in some of these patients.

2. MeHg transportation from mothers to offspring throughout gestation and lactation periods (Exposure assessment of offspring throughout gestation and lactation periods)

Mineshi Sakamoto (Epidemiology)
Hirokatsu Akagi (International Affairs and Environmental Sciences)
Atsuhiro Nakano (Basic Medical Sciences)

MeHg is one of the most risky substances which comes through fish consumption. Docosahexaenoic acid (DHA, C22:6-n3), which is one of the most important fatty acids for normal brain development and function, is also derived from fish consumption. Our objective in this study was to examine the relationships between maternal Hg concentrations in red blood cells (RBCs-Hg) and fetal plasma fatty acid composition. Venous blood samples were collected from forty-eight pairs of mothers and fetuses (umbilical cord blood). The differences in RBCs-Hg concentrations between paired samples were determined by paired *t*-test. The associations between RBCs-Hg and plasma fatty acid concentrations were studied by correlation analysis. In all 48 cases fetal RBCs-Hg were higher than maternal RBCs-Hg. The geometric mean of fetal RBCs-Hg was 13.8 ng/g, which was significantly ($p<0.01$) higher than that of maternal RBCs-Hg (8.61 ng/g). While the average fetal/maternal RBCs-Hg rate was 1.6, the individual rate varied 1.08 - 2.32, suggesting considerable individual differences in MeHg transfer from mothers to fetuses through the placenta. A significant correlation was observed between maternal and fetal DHA concentrations ($p<0.05$, $r = 0.39$). Further, a significant correlation was observed between maternal RBCs-Hg fetal plasma DHA ($p<0.05$, $r = 0.34$). These results confirm that MeHg and DHA which

originated from fish consumption simultaneously transferred from maternal to fetal circulation. Fish consumption poses both risks and benefits for people from the standpoint of MeHg and n-3 PUFA. Pregnant women in particular would do well to at least consume smaller fish, thereby enjoying the benefits while reducing the possible risk.

3. Experimental study for risk assessment of the brains of fetus and neonates

Mineshi Sakamoto (Epidemiology)
Hirokatsu Akagi (International Affairs and Environmental Sciences)
Atsuhiro Nakano (Basic Medical Sciences)

Methylmercury (MeHg) can be transferred to newborn offspring through milk, in addition to passage through the placenta during their intrauterine life. Higher MeHg accumulation and susceptibility to toxicity in the fetus than in the mother during the gestation period is well known. However, the MeHg accumulation in the brain during the late pregnant period when the human brain is most vulnerable is not clear. Also, changes in methylmercury accumulation in the developing rat tissues with consecutive exposure throughout gestation and lactation periods were not well studied. The purposes of this study were to evaluate the changes in MeHg accumulation in the brain and other tissues of offspring, based on consecutive doses of MeHg to mothers throughout gestation and lactation. Adult female rats were given a diet containing 5 ppm Hg (as MeHg) for 8 weeks. Then they were mated and subsequently given the same diet throughout gestation and lactation periods. The newborn offspring were placed with the mothers until postnatal day 20; the offspring were exposed to MeHg throughout their intrauterine life through the placenta, and during the postnatal developing phase via contaminated breast-milk. On the days of embryonic days 18, 20, 22 and at parturition, the concentrations of Hg in the brains of infants was approximately 1.5-2.0 times higher than those in the mothers throughout the period of late gestation. On the other hand, during the suckling period the concentration in the brain of the offspring rapidly declined to about 1/10 of that during late pregnant periods. Changes in MeHg accumulation in blood and liver after parturition were similar to those in the brain. These results will be explained by limited MeHg transport by milk and rapid increase in the volumes of the brain and body. However, changes in MeHg accumulation in kidney were quite different from other tissues, and maintained the low levels throughout the periods. Thus, although mothers are subjected to constant and prolonged MeHg throughout both the gestation and lactating periods, the risk to offspring may be especially high throughout late gestation period but rapidly decrease during suckling period.

4. Study on role of selenium and iodine in development of methylmercury toxicity

Atsuhiro Nakano (Basic Medical Sciences)
Mineshi Sakamoto (Epidemiology)
Ken-ichiro Miyamoto (Clinical Medicine)

The effect of selenium compound for the toxicity of the methylmercury was examined in rats. Male rats of 10

weeks-old were used as experimental animals. Methyl mercury was administered to male rats at proportion of 5mgHg/B.W.-kg/day for 12 days. These rats died all within 2 weeks after the end of the administration. The selenomethionine was administered with methylmercury to the rats of the other group at the proportion of 1mgSe/Kg or 5mgSe/Kg. The rats that both of methyl mercury and selenomethionine were administered were also weak on the lowering of the body weight, and survived over 30th all. In addition, only the methylmercury is continuously administered to the rats of next group at proportion of 5mgHg/B.W.-Kg/day for 12 days, and selenomethionine was administered at proportion of 1mgSe/B.W.-Kg/day from the 13th. Intake of bait and water gently rose, when the selenomethionine was administered to these rats, and the body weight was also recovered, and then it began to increase. The rat over 80% survived over 30th. As above, it was clarified that the selenomethionine suppressed very strongly methylmercury toxicity for rats.

5. Study on monitoring procedure of exposure of heavy metals including mercury in period of fetus to infant

Atsuhiro Nakano (Basic Medical Sciences)
Megumi Yamamoto (Basic Medical Sciences)
Mineshi Sakamoto (Epidemiology)
Ken-ichiro Miyamoto (Clinical medicine)

The extraction method of methylmercury from the sample for analyzing methyl mercury by the gas chromatography method was examined. The iodide was the highest for extraction efficiency of methylmercury halide to organic solvent (toluene), and the order of bromide and chloride was left. It was proven that methylmercury iodide could be extracted into toluene even in neutral region. The hydrolyzed solution of the sample was neutralized by hydrochloric acid. The hydrolyzed solution of the sample was neutralized by hydrochloric acid. The hydrolyzed solution of sample was neutralized by hydrochloric acid, and methylmercury iodide was formed by adding monoiodoacetate. The interference of fat component would be able to be considerably reduced, since the methylmercury would be able to be extracted as an iodide in neutral region.

6. Hair mercury concentration of Japanese people

Masako Yamaguchi (Epidemiology)
Noriyuki Hachiya (International Affairs and Environmental Sciences)
Miyuki Matsumoto (Clinical Medicine)
Akira Yasutake (Basic Medical Sciences)

Additional 2100 samples were collected in Ishinomaki and Fukuoka.

In the laboratory experiment, we found that the treatment of hair samples with a lotion for artificial waving caused a 30%-reduction in the mercury content. Furthermore, longitudinal hair analysis showed a marked

difference in the concentration between the hair root and the tip of the hair taken from artificially waved females; higher values were observed at the hair root. These results suggested that artificial waving significantly removes hair mercury and that hair analysis at the hair root should be necessary to estimate and accurate methylmercury exposure for waved persons.

7. Time course distribution of Minamata disease patients around Yatsushiro Sea and the severity of lesions in the brain —analysis using the data from autopsy —

Mineshi Sakamoto(Epidemiology)
Kiyoka Miyamoto (Clinical Medicine)
Miyuki Matsumoto (Clinical Medicine)
Komyo Eto (Clinical Medicine)

Time course distribution of Minamata disease patients around Yatsushiro Sea and the severity of lesions in the brain were studied using the data from 450 cases of autopsy. The data are valuable to estimate the time course distribution and the degree of the disease. However, the data contains a big bias since most of patients were recognized as Minamata disease patients after the pathological examination in Kumamoto University. On the assumption that this data contain big bias, this study was carried out. Incidence of the disease was considered by the appearance of the sensory disorder. The severities of the lesions were assessed from light to severe. In Fukuro area, Minamata City, the number of the incidence was highest at 1956 and decreased to 1959. On the other hand, the incidence increased after 1959 in Tunagi and Ashikita Towns. Patients with the severe lesions were found in Fukuro area. On the other hand, most of the cases in Tunagi and Ashikita showed light lesions. These data of autopsy cases were consistence with the geographical distribution and severity of the pollution. Historically, Chisso Company discharged methylmercury to Minamata Bay at first and caused sever pollution during 1955 to 1959. The company changed the outlet to Minamata River in 1958, then the pollution spread to northern area, such as Tunagai and Ashikita Town.

5. Environmental sciences on mercury

1. Behavior of mercury in Minamata Bay after sludge reclaims completion

Yoshiaki Yasuda (International Affairs and Environmental Sciences)
Masako Yamaguchi (Epidemiology)

A boulder shore index was figured out using data of monitoring investigation of benthos community structure done every other year from 1997 in four stations of Minamata Bay, eleven control stations around western Japan. That is, rate of number of a specific species to the total number of each station was calculated and compared with

each other. For example, the value of this index is high in the species dominant in boulder shore such as *Gaetice depressus, Pagurus filholi, Ischnochiton comptus* etc. On the contrary, value is low in the species dominant in the rocky shore such as mussels, or barnacles. Under the analysis of similarity of species composition in each station (total 11 points), for example, C_λ which is drown from the probability of a randomly extracted species to be same species, tau-index which is an comparing method using ranking correlation of dominance, some unexpected tendency was found in some stations. That is, in a two stations far from each other, such as boulder shores of Noto Island and Minamata Bay, the species composition showed larger similarity than those near Minamata Bay. In rocky shore similarity decreased along distance from Minamata Bay. Such a mosaic structure in similarity in the boulder shore could be caused by some environmental factors such as a particle size distribution of sediment other than distance or latitude. Then sampling of sediment and pore water at each station was started in the end of this fiscal year.

2. Mercury accumulation by freshwater and sea water fishes

Masako Yamaguchi (Epidemiology)
Yoshiaki Yasuda (International Affairs and Environmental Sciences)

To estimate participation by osmoregulatory system in mercury accumulation by fish, opercular epithelia and anterior and posterior intestine from FW- or SW-adapted eel were isolated and MeHg uptake was tested with or without antagonist for ion transport. Mercury uptake was higher in organs of SW eels than FW one. Mercury uptake was decreased by ouabine in gill and intestine in SW environment but increased in gill tissue in FW. DIDS drastically accelerated mercury uptake in gill and intestine in both FW and SW.

3. Molecular ecological study on the mercury-volatilizing bacteria from Minamata Bay

Kunihiko Nakamura (Basic Medical Sciences)

The regions encoding organomercurial resistance of *Pseudomonas haloplanktis* M-1, isolated from Minamata Bay, was identified. *Mer B* gene encoding organomercurials lyase was not located near the *mer* determinant previously reported. The 3.9-kb sequence includes four open reading frames (OFRs). These four OFRs were identified as m*erB*, *merT2*, *merP2*, and mer*T3*, respectively. These genes constitute a second operon following a *tnpA* gene. The complete genetic structure of the mercury resistance module of M-1 is organized as *merR-merT1-merP1-merC-merA-merD-merT2-merP2-merT3-merB*.

4. Improved analytical methods for determining total mercury and methyl mercury in biological and environmental samples

Hirokatsu Akagi (International Affairs and Environmental Sciences)
Akito Matsuyama (International Affairs and Environmental Sciences)

Highly sensitive and systematic methods for determining total mercury and methylmercury in various biological and environmental materials have been established to study and evaluate the environmental factors influencing the dynamics of mercury in aquatic system. For the analysis of total mercury, a biological or sediment sample is digested in a 50ml thick walled digestion flask using HNO_3–$HClO_4$–H_2SO_4 (1:1:5) mixture by heating at 200±5℃ on a hotplate for 30 minutes. In the analysis of water, the sample is treated first with $KMnO_4$ and H_2SO_4 and then extracted with dithizone in toluene. The extract is evaporated to dryness and digested in the same manner as described above. After cooling, the digested sample is filled up to the 50 ml mark with mercury-free water. The analysis of mercury in the sample solution is done by cold-vapor atomic absorption spectrometry using a semi-automated system recently developed in our laboratory. With this system, sensitivity and accuracy are substantially improved and the determination of the sample is completed within one minute. The detection limit for this method is 0.5 ng Hg in the sample solution.

Analytical procedure for methylmercury in biological samples consists of : (1) alkaline digestion with 1N KOH in ethanol; (2) washing out fatty materials with hexane after slightly acidified with 1N HCl; (3) extraction with dithizone in toluene ; (4) clean-up with Na_2S; (5) re-extraction with dithizone in toluene; and (6) measurement of methylmercury by ECD-gas chromatography. For methylmercury in sediment or water samples, the sediment is treated with 1N KOH in ethanol, whereas the water sample is treated with $KMnO_4$ and H_2SO_4. After these pretreatments, methylmercury is extracted with dithizone in toluene and then followed by clean-up with Na_2S, re-extraction with dithizone in toluene and measurement of methylmercury in the same way as described above. The detection limit of these procedures is around 0.1ng/g in a 0.5 g sample on a wet weight basis.

5. The developments of remediation technology for mercury polluted soil/ sediment using low temperature thermal treatment and efficient recovery technology of vaporized mercury by heating treatment

Hirokatsu Akagi (International Affairs and Environmental Sciencs)
Akito Matsuyama (International Affairs and Environmental Sciences)

We are using RA –915(RA-915 made by Lumex Co. Ltd.) as a semi automatic mercury analyzer. This analyzer has special mode for mercury analysis. This special mode is called the thermo-scanning mode. It is able to continuously analyze mercury content in soil sample at variable heat temperature. We analyzed mercury content in mercury-polluted sediment (Minamata bay sediment) with thermo scanning mode. As a result, we observed four peaks of mercury release from mercury polluted soil by heating 300 ℃ to 700 ℃. In general, decomposition point of β－mercury sulfide by heating is 446℃. These facts mean that mercury species in sediment is not only β－mercury sulfide. On the other hand, we developed new additive as a recycle system of waste of fluorescent lamps waste production. This recycle system becomes more efficient as a treatment system

using this new additive.

6. Development of mercury removal method by mercury-volatilizing bacteria

Kunihiko Nakamura (Basic Medical Sciences)

The bacterial volatilizations of mercury compounds and the removal of methylmercury from a fish broth were examined in detail by using 20 methylmercury-volatilizing *Alteromonas* strains from the seawater of Minamata Bay. The optimal mercury concentrations for the bacterial volatilization of mercuric chloride ($HgCl_2$) and methylmercuric chloride (CH_3HgCl) were observed in these strains. The volatilization rates for CH_3HgCl by these strains were extremely higher than those for $HgCl_2$. When the concentration of organic compounds in the fish broth was low, the volatilization rates of CH_3HgCl increased. The removal of methylmercury from the fish broth was examined using a laboratory column packed by immobilized methylmercury-volatilizing bacteria in *k*-carrageenan beads. Over 75% of mercury was removed from the fish broth containing 1 μg /ml of methylmercury in 19 hrs.

7. X-ray induced degradation of methylmercury in aqueous solution

Ryoji Aramaki （Basic Medical Sciences）
Akira Yasutake (Basic Medical Sciences)
Masako Yamaguchi (Epidemiology)

Relationships were found in an X-ray dose of X-irradiated MeHg in various aqueous solutions and the appearance of inorganic mercury. But, this decomposition was not found in the case of pH4 buffer. This buffer includes organic acid. Then methylation is thought about as a cause of existence of organic acid in X-irradiated aqueous solution. But, methylation of X-irradiated inorganic mercury in pH4 buffer was not observed.

6. Social scientific study on Minamata disease

1. Study on social impacts of a disease name “Minamata disease” as a specific case of methylmercury poisoning and related problems

Noriyuki Hachiya (International Affairs and Environmental Sciences)
Koumyo Eto (Clinical Medicine)
Kiyoka Miyamoto (Clinical Medicine)
Miyuki Matsumoto (Clinical Medicine)
Hirokatsu Akagi (International Affairs and Environmental Sciences)

Ryou Nomura (Director General)

When first patient of Minamata disease was discovered, the causative agent of the disease was unknown. The term “Minamata disease” was used first in 1957, with notice saying “tentatively by the time when the toxic agent is confirmed”. In 1970, the research team of Ministry of Health and Welfare concluded that the term Minamata disease should be used in Cabinet orders, because of the fact that the name has already become established worldwide. Minamata disease was defined at that time as a disease of the nervous system resulting from the ordinal ingestion of organic mercury accumulating fishes and shellfishes and did not include the organic mercury poisoning induced by the direct exposure to the chemical, including by inhalation or by oral or percutaneous exposure. The disease name has been considered to include a disease caused through environmental pollution. However, The inclusion of “Minamata” in the disease mane has been a matter of controversy for long time, especially in the local districts of Minamata and neighboring area. Some citizens of the districts have been insisting that they have been forced to accept disadvantages such as discrimination and prejudice, and the disease name, a disgrace to the district, should be changed. On the other hand, others emphasize the advantage of the fact that the disease name contains “Minamata”. They say that Minamata became famous over the world owing to the disease name, and it is favorable to Minamata City, which is aiming at taking a leading contribution to solving environmental problems as an environmental model city.

Publications and Scientific meetings

1. Department of International Affairs and Environmental Sciences

(1) Publication

(1) Akagi H
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(2) Akagi H, Sakamoto M, Ikingura JR
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(5) Ikingura JR, Akagi H
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(2) Scientific meeting

(1) Akagi H
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A survey of environmental pollution by mercury derived from a chemical factory drain in Guizhou, China.
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(4) Ikingura JR, Akagi H
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International Workshop on Health and Environmental Effects of Mercury.
Nov 2002, Dar es Salaam, Tanzania.

2. Department of Clinical medicine

(1) Publication

(1) Eto K

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(2) Eto K, Yasutake A, Korogi Y, Akima M, Shimozeki T, Kuwana T, Kaneko Y

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(3) Zhang J, Miyamoto K, Hashioka S, Hao HP, Murao K, SaidoTC, Nakanishi H
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Dev Brain Res **142 (1)**, 105-110, 2003.

(2) Scientific meeting

(1) Eto K
Pathogenesis of methylmercury poisoning in the primates.
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3. Department of Basic Medical Sciences

(1) Publication

(1) Adachi T, Takanaga H, Sakurai Y, Ishido M, Kunimoto M, Asou H
Influence of cell density and thyroid hormone on glial cell development in primary cultures from embryonic rat cerebral hemisphere.

J Neurosci Res **69**, 61-71, 2002.

(2) Adachi T, Yasutake A, Hirayama K
Influence of dietary levels of protein and sulfur amino acids on metabolism of glutathione and related amino acids in mice.
J Health Sci **48**, 446-450, 2002.

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J Health Sci **49**, 160-165, 2003.

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Effect of methylmercury on histamine release from rat mast cells.
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Nov 2002, Orlando, USA.

(2) Arizono K, Ishibashi H, Shinkura R, Yamamoto M
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e-hormone 2002.
Oct 2002, New Orleans, USA.

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4. Department of Epidemiology

(1) Publication

(1) Sakamoto M, Kakita A, Wakabayashi K, Takahashi H, Nakano A, Akagi H
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Brain Res **949**, 51-59, 2002.

(2) Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H
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(3) Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H
Exposure assessment during gestation lactation: declining risk in human infants during lactation.
In: Proceedings of the International Workshop on Health and Environmental Effects of Mercury.

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(2) Scientific meeting

(1) Sakamoto M, Kubota M, Matsumoto S, Nakano A, Akagi H
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Political studies

1. Rehabilitation and Health Consultation for Minamata Disease Patients

Kiyoka Miyamoto (Clinical Medicine)
Miyuki Matsumoto (Clinical Medicine)
Fusako Usuki (Clinical Medicine)

Once nerve cells in brain or spinal cord are damaged, their regeneration is difficult even today. The brain, however, can compensate for damage in one region by using other regions. Rehabilitation promotes such brain plasticity. The main treatment for Minamata disease in chronic stage, where the symptoms become stable, is to control symptoms with drugs or rehabilitation. Our institute is opened to Minamata disease patients for daycare and rehabilitation (occupational therapy and physical therapy). Some patients used the rehabilitation room. In order to maintain and improve the power of skeletal muscle and to prevent the contracture of the joints, training by a therapist or training using various equipments is performed. Medication is needed to improve high muscle tonus. Standing and walking trainings are important in order to improve balance and prevent contracture of the Achilles tendon. In addition, low-frequency electrical stimulation therapy and transcutaneous electrical stimulation therapy are effective for relief from pain. Fetal type patients have difficulties in fine movements of hands and fingers caused by disturbed brain function. In such cases, occupational therapy including making handicrafts is performed for the training of finger movements. Some people visited to consult about the relation between their symptoms and Minamata disease. Some consultations concerning mercury and health were performed.

2. Making Databases for Sending Research Information in National Institute for Minamata disease

Masao Watanabe (Epidemiology)
Junji Wakamiya (Clinical Medicine)

Sending information on Minamata Disease is one of the major work for the NIMD. Many publications and documents of mercury are accumulated in research, and the NIMD operates to prepare and provide information as a nucleus. We think this is very useful for researchers of organic mercury. Currently the data of books are minimum data just for the inside researchers' needs, but we will change and organize the database of this books and documents to be used for the outside researchers and the general public. We made the input card and modify the date from the old data this year. The numbers of modification are 2,300 books in a Japanese language and 700 books in a foreign language. Inputting the same title and the same publisher was not always unified, therefore we looked the database again and change to the unified system.

3. Organizing the Date Related to Minamata Disease and Developing the System for Sending Information

Hirokatsu Akagi (International Affairs and Environmental Sciences)
Hideaki Teduka (International Affairs and Environmental Sciences)
Katsuhiro Nagai (International Affairs and Environmental Sciences)
Manabu Yamasaki (International Affairs and Environmental Sciences)
Noriyuki Hachiya (International Affairs and Environmental Sciences)
Yoshiaki Yasuda (International Affairs and Environmental Sciences)

It is one of the important duties that the National Institute for Minamata disease corrects and keeps a mass of information includes many different fields related to Minamata disease, and sending the information and lessons. However, that includes piece of information that is in danger of being scattered and lost or information that is not well-preserved because of where it is kept in a varied places such as a university, corporation, individual, administrative organ, etc. It is also necessary to correct and record the frank views like patients' experiences immediately because of those patients' aging. Based on such situations, the National Institute of Minamata Disease opened the Minamata Disease Archives in June 2001 and started the positive measure to organize and provide the information related to Minamata disease. In the year of 2002, NIMD had conferences with researchers, administration and groups of patients to make progress for unifying "information of information sources". NIMD also held the "Discussion for Organizing Information Related to the Minamata Disease" to examine the preparation for the Overall Research Project related to Minamata Disease which is planned to start in 2003. NIMD has tacked positively providing information and data related to Minamata disease by utilizing auditorium and exhibition hall. While advancing examination of the measure about the Minamata disease related data synthesis investigation enterprise, NIMD held the "U.N. child environmental meeting", "Report Meeting of Johannesburg Summit" and the open seminar as an independence enterprise are also held in an auditorium. NIMD also put the "Database of News Paper Article related to Minamata Disease" on display in an exhibition hall, and performed the donation and purchases of books related to Minamata disease. Moreover NIMD started the process of examining and works towards the further offer of the information by using the internet.

(Reference)

About the outline of gathering and providing information of the Minamata Disease Archives in a year of 2002

1. Total Visitors

   There were 30,776 people visited the Minamata Disease Archives in 2002.

2. The use of the auditorium

   The auditorium was used for lectures given by oral historians of Minamata Municipal Minamata Disease Museum, and as a place such as environmental study given by Kumamoto Environmental Education and Intelligence Center. One hundred and one events were hold in the auditorium.

3. Situation of data collection in a data room

Database-ization of data was advanced, while having recommended to purchase books about Minamata disease, and also performing the appeal of the data donation from researcher of Minamata disease. The Minamata Disease Archives keeps the data of 1,506 volumes now at the end of March 2003.

4. Training for Visitors from Developing Countries

Hideaki Teduka (International Affairs and Environmental Sciences)
Katsuhiro Nagai (International Affairs and Environmental Sciences)
Hirokatsu Akagi (International Affairs and Environmental Sciences)
Noriyuki Hachiya (International Affairs and Environmental Sciences)
Komyo Eto (Clinical Medicine)
Atsuhiro Nakano (Basic Medical Sciences)
Akira Yasutake (Basic Medical Sciences)
Kunihiko Nakamura (Basic Medical Sciences)
Megumi Yamamoto (Basic Medical Sciences)
Mineshi Sakamoto (Epidemiology)

Our Research Center receives visitors from developing countries such as groups of specialists who are in the training courses of JICA (Japan International Cooperation Agency). In the year of 2002, we received 21 groups composed of 184 people from China, Korea, Philippines and Brazil. Akagi H, Director of Department of International Affairs and Environmental Sciences (once), Hachiya N, Chief of Social Sciences Section (twice), Eto K, Chief Research Coordinator (12 times), Nakano A, Director of Department of Basic Medical Sciences (once), Yasutake A, Chief of the Biochemistry Section (twice), Nakamura K, Chief of Physiology Section (twice), Yamamoto M, Senior Researcher (twice) and Sakamoto M, Chief of Survey Section (5 times) corresponded. They gave lectures of “Minamata Disease”, “Mercury in the Environment and World Scale Mercury Pollution”, and “Course of Total Mercury Analysis in Fish, Soil and Hair”, and had practice of mercury measurement of trainee’s individual hair.

5. List of International Collaborative Research Work (April 2002－March 2003)

Name	Name of Organization	Position in the Organization	Inviting Period
Liu Li	Research and Designing Institute of Environmental Science	Engineer	2002.6.3 - 6.28
Li Mei	Research and Designing Institute of Environmental Science	Engineer	2002.6.3 - 6.28
Vu Duc Loi	National Centre For Science And Technology of Vietnam	Researcher	2002.6.17 –6.27
Jin Yi He	China Medical University	Association Professor	2002.9.4 – 9.26
Ricardo Bezerra	Universidade Federal do Par	Assistant Professor	2002.9.10 – 11.3
Philip W. Davidson	University of Rochester	Professor	2002.10.15 – 10.19
Gary Jay Myers	University of Rochester	Professor	2002.10.9 - 10.19
Nikolay Mashyanov	St.-Petersburg State University	Head of Laboratory	2003.2.3 – 2.24
Krzysztof Dmowski	University of Warsaw	Habilitowany	2003.2.13 – 2.28
Jean K. T. Mujumba	University of Dar es Salaam	Principal Laboratory Scientists	2003.2.21 – 3.8
Justinian Ikingura	University of Dar es Salaam	Associate Professor	2003.2.27– 3.30
Ana T. Francisco-Rivera	Occupational Health Division, Department of Health	Environmental and Occupational Health Cluster	2003.3.1 – 3.25
Nerissa M. Dando	College of Medicine, University of the Philippines	Pediatrician	2003.3.1 – 3.25
Guifang Chen	Research and Designing Institute of Environmental Science	Senior Engineer	2003.3.23– 4.8
Wei Chen	Research and Designing Institute of Environmental Science	Assistant Engineer	2003.3.23– 4.8

6. List of International Collaborative Research Work (April 2002 －March 2003) / Dispatch

Place / Organization	Name	Period
The King of Holland / Amsterdam "24th International of the International Academy of Pathology"	Director of Department of Clinical Medicine *Dr. Komyo Eto*	2002.10.4－10.12
People's Republic of China / Guizhou "Environment Science Designing and Research Institute of Province of Guizhou"	Chief of Natural Science Section / Department of International Affairs and Environmental Science *Dr. Yoshiaki Yasuda* Chief of Biochemistry Section / Department of Basic Medical Sciences *Dr. Akira Yasutake* Chief Researcher of Pathology Section / Department of Basic Medical Sciences *Dr. Ryoji Aramaki* Chief Researcher of Survey Section / Department of Epidemiology *Dr. Liu Xiaojie* Researcher of Survey Section / Department of Epidemiology *Dr. Masako Yamaguchi*	2002.10.20－11.3
United Republic of Tanzania / Dar es Salaam "University of Dar es Salaam" Republic of Seychelles / Mahe Island	Director – General *Mr. Ryo Nomura* Director of Department of International Affairs and Environmental Sciences *Dr. Hirokatsu Akagi* Chief of Natural Science Section / Department of International Affairs and Environmental Science *Dr. Yoshiaki Yasuda* Chief of Survey Section / Department of Epidemiology *Dr. Mineshi Sakamoto*	2002.11.15－11.24 2002.11.15－12.1 2002.11.15－11.26 2002.11.15－11.25
People's Republic of China / Tian jin "National Environment Protection Official, China" "Government Official of Tian jin"	Chief of Social Science Section / Department of International Affairs and Environmental Science *Dr. Noriyuki Hachiya*	2003.3.23－3.28
Brazil / Belem & Santarém "Para Federal University"	Chief of Clinical Medicine Section / Department of Clinical Medicine *Dr. Junji Wakamiya* Chief of Clinical Examination Section / Department of Clinical Medicine *Dr. Ken-ichiro Miyamoto*	2003.4.10－4.27

National Institute for Minamata Disease

Research Evaluation Report

December 2002

National Institute for Minamata Disease

1. Results of evaluation

(1) Summary

Fourteen research topics by the Steering Committee of the National Institute for Minamata disease are evaluated. Comprehensive evaluation was determined by averaging the evaluation points of each committee member. Overall evaluation is examined in this paper.

(2) Selection of research topics

The Institute, a part of the Ministry of the Environment concerned with mercury poisoning, is unique in the world, an organization in which each scientist is keenly aware and proud of the importance of their research. Without halting research on mercury poisoning in Japan, we have disseminated our findings throughout the world and established international cooperative research with the aim of broadening our range of activities to include the prevention of environmental pollution. It is important that research topics are selected with this goal in mind. Current research topics include

1) clinical research on Minamata disease (two studies),
2) research related to elucidation of the mechanism of manifestation of methyl mercury toxicity and development of treatments (four studies),
3) empirical studies related to toxicity tests and second-generation effects of methyl mercury (one study),
4) risk evaluation of infant and fetal exposure to methyl mercury (two studies),
5) environmental research on mercury (three studies), and 6) socioscientific research on Minamata disease (one study).

Current themes include clinical and pathological aspects of Minamata disease, mechanism of onset of mercury poisoning, and effects on fetuses. Research is broadly classified into basic, clinical, and epidemiological, as is the organization of the Institute, and the consensus is that these issues are more easily understood when the envirosociological aspects are included. It has been a quarter of a century since the founding of the Institute, and seven years since the problem of Minamata disease was politically resolved. The paramount issue before the Institute at this time is to determine the future direction of research.

(3) Research standards

This paper indicates there are apparent imbalances in research standards depending on the ability and experience of the scientist. Basic biological experiments, clinical research, and research directed toward industrialization of

mercury removal equipment point to high-level research results. In contrast, pathological and empirical research on Minamata disease has not been of comparable standards. Socioscientific research is only in the planning stage, and cannot be evaluated until future results are available. Furthermore, some research topics remain unclear as to their purpose, plan, and degree of progress. On a relative basis, however, there are no projects obviously lacking standards, and our scientists are steadily and seriously pursuing their projects. On the whole, we wish to publish more original research. Our research has been published in international journals. We realize that, in the field of science, one is evaluated solely based on the publication of original work

(4) Future research

1) The Institute is the only research facility in the world focusing on mercury, and intends to aggressively promote international research in this field. Much of our research has already established a global standard.

2) Already completed studies and studies we wish to continue to pursue have pertained to various issues ranging from the unpleasant topics to those which have had dim prospects from the very beginning. We seek a discrete discussion concerning the selection of future research topics.

3) With regard to the method of external evaluation, our opinion is that if there is a problem, it should be corrected. Evaluation of research results should be conducted for all topics, and while self-evaluation should continue within the Institute, making some part of these results available for external evaluation differs from the typical methods used by international experimental research facilities. Further investigation of this issue in particular is required. External evaluation conducted once every two to three years would require 2-day evaluations (one day for a laboratory visit, and a second day for an evaluation meeting) by a specialist in each field. If possible, evaluators from abroad should be included to obtain an international perspective that would greatly improve the evaluation as well underscore the *raison d'etre* of our Institute.

4) The Institute has seen a serious drop in the quality of its researchers. Future employees should not be divided into long-term and short-term employees, and the use of public solicitation is undesirable. In order to improve the enthusiasm of our researcher staff, we must actively promote exchanges with universities and other research facilities, both at home and abroad. Meetings will be held to discuss the specific means for accomplishing this.

5) The Institute is now a national center for research, but it may become an independently administered corporation in the future. Close observation of this issue is needed in the days to come.

2. Conclusion

The National Institute for Minamata disease, a part of the Ministry of the Environment, is providing the world with the lessons learned regarding Minamata disease as a malady caused by environmental pollution, and it is conducting research in the fields of the environment and human health. The Institute operates with a sense of international obligation. Our current scientists confirm the vitality of our efforts in regard to basic research on Minamata disease and mercury, and the prevention of mercury poisoning.

It is essential that our research topics are selected very carefully, that both short- and long-term plans be formulated, discussion of experiments held within the Institute, and more active research be undertaken. Developments can be expected in the future thanks to the enthusiasm and quality of our staff. While asking for the diligent cooperation and deeper understanding of all concerned, we express our heartfelt gratitude for the evaluations of our present research and to those in charge of them.

International Cooperation Between NIMD and Foreign Countries

March, 2003

Contents

Annex:

1. ⅩⅩⅣth International Congress of the International Academy of Pathology

5-10 October 2002

Amsterdam

Symposium 19: Environmental Pathology

Chairpersons: F.G. Mullick (USA), K. Eto (Japan)

Pathogenesis of methylmercury poisoning in the primates.

Komyo Eto

National Institute for Minamata Disease, Ministry of the Environment,

4058-18, Hama, Minamata City, Kumamoto 867-0008, Japan

INTRODUCTION

Minamata disease (methylmercury poisoning) was discovered in 1956 around Minamata Bay, Kumamoto Prefecture, Japan. It is now well established that the source of the methylmercury responsible for the Minamata outbreak was mercuric sulfate, a catalyst used for the industrial production of acetaldehyde between 1932 and 1968. Recently Nishimura et. al [20) 21)] reported that the large amount of methylmercury compounds generated in the Chisso Minamata Plant. was due to a change in a procedural step. In August 1951, manganese dioxide, which was used as a promoter for maintaining the activity of mercury catalyst, was changed to ferric sulfide. Ferrous iron was reduced in the reaction mother liquor and then oxidized with nitric acid. As a result, the amount of methylmercury degenerated in the process was rapidly increased. From 1953 onward, patients appeared with neurological signs and symptoms around Minamata Bay due to methylmercury pollution into Minamata Bay. .In 1968, the plant stopped dumping the wastewater into the Bay. During these 17 years of pollution, fish and shellfish were contaminated with methylmercury through the gills or through the intestinal tracts and the chemical had accumulated at high concentrations in the food chain. Fishermen and cats suffered from methylmercury poisoning after consumption of contaminated fish or shellfish. The amount of methylmercury in the fish and shellfish rose sharply in 1952 but abruptly dropped in 1968. No new patients have been reported since 1975, however cases of chronic Minamata disease have been reported as a result of methylmercury poisoning during the 17 years from 1951 to 1968.

Many chemicals, such as Se, Tl, Mn, etc, have been implicated as the cause of the disease. In 1968, the national government recognized methylmercury as a possible cause. Takeuchi et al.,[26)] Second Department of Pathology, Kumamoto University School of Medicine reported on July 14, 1959 its view that organic mercury was most likely the cause of Minamata disease. One week later, on July 21, 1959, Hosokawa et al. initiated an experiment in order to assess the toxicity of industrial waste from the acetaldehyde plant. [8)]

The initial neuropathological studies of methylmercury poisoning by Eto et al. [4)] , demonstrated edema, especially surrounding the deep sulci using common marmosets. The results were an explanation for

the concentric constriction of the visual fields.[7] The glove-and-stocking type of sensory disturbances is due to toxic effects on peripheral nerves. This effect should improve after nerve regeneration following the last exposure to methylmercury in 1968.

Takeuchi and Eto [25] published the book that are summarized the all autopsy cases concerned Minamata disease from 1956 to 1995.

This paper reports the pathogenesis of the methylmercury poisoning (Minamata disease) based on the recent knowledge and the experiments from the common marmosets.

PATHOGENESIS

Selective vulnerability of the cerebrum

Pathological changes predominantly occur in selective areas, including the calcarine region, the post-central and pre-central gyri, and the temporal transverse gyrus. These areas are localized near deep sulci, that is, the Calcarine fissure, Central sulci (Roland's fissure) and Sylvian's fissure. Ischemia may result from the compression of arteries by edema in the adjacent tissues. In studies of acute methylmercury poisoning in common marmosets, which have two deep sulci except the central sulcus, there is edema in the white matter of occipital lobes.[4] In acute cases of methylmercury poisoning, neuronal loss with gliosis is found in the all layers of the cortex, and the second and third layers of cortices are damaged in moderate or mild cases. As a result of the location of the pathological changes, there is resultant bilateral concentric constriction of the visual fields and impairment of visual acuity. Similarly, there is sensory disturbance due to the damage of the sensory center (post-central gyrus), motor disturbance due to the damage of motor center (pre-central gyrus) and hearing impairment.

Concentric constriction of the visual fields

One of the typical clinical signs of Minamata disease is bilateral concentric constriction of visual fields. The pathological changes are more prominent in the anterior areas of the calcarine cortex compared with the posterior pole.[23) 24)] It is well known that the anterior area of the calcarine cortex relates to the peripheral parts, and the posterior areas to the central parts.[19)] Edema surrounding the anterior sulcus of the occipital lobe may compress the anterior calcarine cortex greater than the posterior cortex, since the anterior sulcus is deeper than that of the posterior. The posterior part of the occipital cortex is spared from the pathological changes of methylmercury poisoning.[7)]

Cerebellar ataxia

Minamata disease patients show cerebellar ataxia, disequilibrium, impairment of gait and speech.[23) 24)] Purkinje cells are well preserved compared with the smaller granule cell neurons. Granule cell loss beneath the Purkinje cell layer is a characteristic finding of Minamata disease. Cerebellar edema occurs in

methylmercury poisoning of common marmosets in the acute stage, similar to the cerebrum. Cerebellar sulci were found compressed by edema, and Purkinje cells were lost in severe cases of methylmercury poisoning.[4)]

Sensory disturbance

Typical signs and symptoms of the sensory disturbance occur initially in the distal parts of the extremities. The post-central cortex, the sensory center is always damaged in entire area of the cortex. Therefore it is very difficult to explain the glove-and-stocking sensory disturbance due to damage of the sensory center alone.

The initial lesions of the peripheral nerve in the methylmercury poisoning of common marmosets show axonal injury of the peripheral nerves.[5)] It is not clear from autopsy cases of Minamata disease, there was no study of peripheral nerves.[24)] Axonal change and destruction of myelin sheath of the sciatic nerves are recognized in the severe cases of methylmercury poisoning of the common marmosets.[5)] Immunohistochemical study of the sciatic nerves of common marmosets show the degeneration of the peripheral nerves and infiltration of the macrophages.[6)] Long-term, over 2.5 years, methylmercury-treated common marmosets show regeneration of the axons and myelinated nerve fibers. The neurons of the posterior ganglion are usually well preserved in the patients of Minamata disease. Therefore the peripheral nerves might regenerate over the long term. It is important to consider the period of pollution of the methylmercury around Minamata Bay to understand patient's sensory disturbance. Axonal changes are recognized in the initial stage of the degeneration of the peripheral nerves of methylmercury poisoning. The peripheral nerves of acute onset of Minamata disease patients have not been studied in detail. Sural nerve biopsy was performed in three patients of Minamata disease in 1976. Eight years had passed since the end of the methylmercury pollution into Minamata Bay. Eto et al.[3)] described incomplete regeneration of the sural nerves by electron microscopy. Within 10 years, the patients of Minamata disease might show complete regeneration of sural nerves. Nagaki[18)] reported in 1999 the sural nerve biopsy findings of eight patients of Minamata disease with eight controls. Sural nerve biopsies of the patients were performed before 1983, 20 years after the onset of Minamata disease. He found no statistical difference between the both groups using pathological and electrophysiological studies. The conclusion was that the Minamata disease patients had no peripheral nerve pathology. He hypothesized that the cause of the sensory disturbance might be the damage of sensory center of post-central cortex. He also believes that the lack of the controls in our paper mistook the judgments of pathological changes of the sural nerves. It is reasonable to suspect that regeneration of peripheral nerve over twenty years after the onset of Minamata disease may appear as within normal limits clinically and pathologically. The report probably demonstrates the complete regeneration of the peripheral nerves over 20 years after the onset of Minamata disease. Tokuomi et al.[27)] reported similar results that typical cases of the Minamata disease patients surviving over twenty years suggested the disturbance of the sensory center rather than peripheral nerve injury. These results

demonstrate the recovery of the peripheral nerves of Minamata disease.

SUMMARY

After 1995 the political problems of Minamata disease had resolved in Japan and new facts were gradually revealed. It was discovered that large quantities of methylmercury were produced inside the Chisso Minamata Plant and were dumped into Minamata Bay directly. Later it was revealed that fish and shellfish were contaminated with methylmercury through gills or through the intestinal tracts for about 17 years. These facts contribute to understanding of chronic Minamata disease who survived since the last exposure in 1968.

Study of methylmercury poisoning in common marmosets could provide a good model of Minamata disease in the severe, acute and chronic stages. Many theories of direct methylmercury intoxication to the neurons have been proposed.[1) 2) 11)-14)] The initial change of edema of the methylmercury poisoning in the cerebrum and cerebellum was a very important finding in the understanding of the pathogenesis of the disease. The hypothesis that edema was important in the pathogenesis of methylmercury poisoning, by Takeuchi and Shaw over twenty years ago, have now been demonstrated pathologically.

The toxic effects of methylmercury differ among the species. The peripheral nerves of the rodents are sensitive to the toxicant.[15)-17)] Primates show different sensitivities to methylmercury poisoning. There are no lesions of peripheral nerves in rhesus monkey[10) 22)] or squirrel monkey,[9)] in contrast to the common marmoset. New techniques such as immunohistochemistry may help characterize the pathology of the peripheral nerves in common marmosets, as well as human autopsy cases.[6)]

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2. The 2nd China - Japan Seminar on Minamata Disease in Tianjin

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The seminar on Minamata disease has been held in developing countries since 1996 by Ministry of the Environment of Japan (MOE). The aim is dissemination of health, social and economic impacts of environmental pollution that have been experienced through Minamata disease affairs in Japan. It has been conducted as an international cooperation program following the statement of Prime Minister on solving the problem of Minamata disease in 1995. This seminar has provided an opportunity to understand the serious effects of environmental pollution on several aspects of community and society and the importance of environmental protection for people of developing countries, where environmental destruction is worried about under the economic growth, by the presentation and discussion about Minamata disease experiences and lessons from different standpoints including of scientist, government administrators, and the patients and victims.

The seventh seminar was held under the joint sponsorship of State Environmental Protection Administration of China and MOE with cooperation of Tianjin Environmental Bureau; Earth, Water and Green Foundation; and Embassy of Japan in China on March 26, 2003 at Tian Yu Hotel in Tianjin, China. Thirteen members dispatched to the Tianjin seminar from Japan included three victims and a citizen in Minamata and Niigata, two local government administrators who were sent by governments of Niigata Prefecture and Minamata City, Director Kamiya of Special Environmental Disease Office of MOE, Dr. Hachiya of NIMD and Prof. Yanagi of Meikai University.

Nearly three hundreds audiences attended the Tianjin seminar. Government administrators, scientists, students and physicians of research or medical institutes and universities were among them. The guidance video of Minamata disease was shown prior to the presentation and panel display on the Minamata disease was also exhibited.

The title of the presentation was the followings:

In suffering Minamata disease	T. Matsusaki
Minamata disease that attacked on my family	S. Kaneko
Minamata and Japan, as a mirror of human development	J. Kanasashi
A message from a patient of Minamata disease in Niigata for the children‘s future	H. Gonpei
Non-government organization activity for victim support	K. Kudo
The outbreak of Minamata disease and issues in the early stage	N. Hachiya
Minamata disease problems and measures of Minamata City	T. Shimizu

Niigata Minamata disease and measures of Niigata Prefecture	A. Eguchi
Minamata disease measures and environmental policy of Japan	K. Kamiya
Environmental lows policy of Japan	K. Yanagi
Study on the source and protection of mercury pollution in the process of chemical materials production	L Qu *
Current status and measures on heavy metals pollution in Hangu area in Tianjin	L. Lu *

*Presentation from China

3. The Mission on Agreement about Investigation into the Actual Condition of Mercury Pollution and Health Effects

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On April 10, 2002, Wakamiya and Miyamoto who are members of NIMD departed from Narita to Brazil with a member of JICA. The object of this mission was to agree on investigation into the actual condition of mercury pollution and sign the minute. We intended to held workshop participating the inhabitants with high hair mercury concentration.

The following is the brief working schedule.

April 11: Arrived in Belem, Brazil
Discussed on methods of workshop with Para University and Evandoro Chagas Institute.
April 14: Visited the collaborative organizations.
(the Health Bureau, the Environmental Bureau and the Mining Bureau of Para State.)
Discussed and agreed on collaboration.
April 15: Went to Itaituba (where district office is located).
Discussed collaboration with officers in the Health Bureau.
April 16: Visited Sao Luis do Tapajos and Barreiras where the inhabitants showed high hair mercury concentration lived.
Discussed collaborates with the inhabitants and heard their requests.
April 18: Returned to Belen.
April 19: Had meeting on workshop with JICA.
April 21: Held workshop with Para University and Evandro Chagas Institute using Project Cycle Managemant (PCM).
This method can make the problems and causes in the investigation clear, and make interests of the relative organization coincide.
At first, the organization had different opinions.
April 23: Found the common recognition on target, results and action, agrees on investigation among the collaborative organization.
Sign the minute.

April 24: Went to Bazilia.

Reported on this investigation at JICA and the Japanese Embassy in Brazil.

Went to Sao Paulo.

April 25: Visited a researcher in the University in Campinas.

Confirmed that there was no methylmercury-polluted area.

April 27: Came back to Japan.