Pathology of Minamata Disease*

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About the Author

Komyo Eto is a senior research coordinator at the National Institute for Minamata Disease. He began studying the pathology of Minamata disease after he graduated from Kumamoto University in 1968. He earned his M.D. in 1977. As a member of the Pathology Department at Kumamoto University, he has studied over 400 autopsy cases concerned with Minamata disease in the Kumamoto Prefecture. He has studied the pathology of Minamata disease for 30 years and published 80 papers concerning methylmercury poisoning. Recently, the problems of mercury pollution in gold mining areas has become one of worldwide concern. Preventing low-level exposure to methylmercury, especially in fetuses, neonates, and children, is important, as is distinguishing between methylmercury and inorganic mercury poisoning. Presently, Dr. Eto is studying localized lesions of the cerebrum and cerebellum in patients with Minamata disease.

ABSTRACT

Minamata disease, or methylmercury poisoning, was first discovered in 1956 around Minamata Bay, Kumamoto Prefecture, Japan. A similar epidemic occurred in 1965 along the Agano River, Niigata Prefecture, Japan. The neuropathology of Minamata disease has been well studied; this review focuses on human cases of Minamata disease in Kumamoto Prefecture. Nervous system lesions associated with Minamata disease have a characteristic distribution. In the cerebral cortex, the calcarine cortex was found to be involved in all cases of Minamata disease, particularly along the calcarine fissure. The destruction of nerve tissue was prominent in the anterior portions of the calcarine cortex. Occasionally, the centrifugal route from the visual and visual association areas (internal sagittal stratum) showed secondary degeneration in prolonged cases after acute onset. Postcentral, precentral, and temporal transverse cortices showed similar changes, though they were less severe. Intense lesions in the precentral cortex caused the development of secondary bilateral degeneration of the pyramidal tracts. In the cerebellum, the lesions occurred deeper in the hemisphere. The granule cell population was most affected. In the peripheral nerves, sensory nerves were more affected than motor nerves. Secondary degeneration of Goll’s tracts was occasionally seen in prolonged or chronic cases.

Keywords. Methylmercury poisoning; inorganic mercury poisoning; mercury histochemistry; methylmercury-induced hydrargyria

INTRODUCTION

Minamata disease was first discovered about 40 years ago in Minamata Bay, Kumamoto Prefecture, Japan. A similar epidemic occurred in 1965 along the Agano River, Niigata Prefecture, Japan. The neuropathology of Minamata disease has been well studied; this review focuses on human cases of Minamata disease in Kumamoto Prefecture. Nervous system lesions associated with Minamata disease have a characteristic distribution. In the cerebral cortex, the calcarine cortex was found to be involved in all cases of Minamata disease, particularly along the calcarine fissure. The destruction of nerve tissue was prominent in the anterior portions of the calcarine cortex. Occasionally, the centrifugal route from the visual and visual association areas (internal sagittal stratum) showed secondary degeneration in prolonged cases after acute onset. Postcentral, precentral, and temporal transverse cortices showed similar changes, though they were less severe. Intense lesions in the precentral cortex caused the development of secondary bilateral degeneration of the pyramidal tracts. In the cerebellum, the lesions occurred deeper in the hemisphere. The granule cell population was most affected. In the peripheral nerves, sensory nerves were more affected than motor nerves. Secondary degeneration of Goll’s tracts was occasionally seen in prolonged or chronic cases.

According to a report by the Kumamoto University Study Group (11), the mercury level in fish in Minamata Bay was over 10 μg/g in 1961, though the levels decreased to an average of 0.5 μg/g after 1969. A provisional regulatory standard in fish of 0.4 μg/g or less of total mercury and 0.3 μg/g or less of methylmercury was established in 1973. Kumamoto Prefecture began removing the bottom sediment of polluted water areas in Minamata Bay containing 25 μg/g of total mercury or more by dredging the bay and refilling it between 1977 and 1990. At present, only a few fish species in Minamata Bay show mercury levels exceeding the provisional regulatory standard.

The pathology of Minamata disease in animals such as...
FIG. 1.—Geographic distribution of cases of Minamata disease.

cats, rats, birds, and fish was reported by the Kumamoto University Study Group (11) in 1968. Cats were most frequently affected spontaneously. The pathology of methylmercury poisoning in cats is similar to that found in human cases of Minamata disease. Common clinical symptoms are unsteady and slow movement, ataxic gait, and paroxysmal convulsions. Convulsions followed by salivation were always observed in cats. Adult humans rarely experience convulsions associated with methylmercury poisoning, though human fetuses, neonates, and small children may experience them.

Miyakawa et al (15-17) reported the pathology of methylmercury poisoning as it affects peripheral nerves in rats. Shaw et al (23, 24) examined mercury intoxication in the rhesus macaque; Kawasaki et al (9) reported a long-term toxicity study of methylmercury chloride in monkeys.

Recently, the problem of mercury pollution has reappeared. In Brazil, mercury pollution has occurred as the result of dumping a large quantity of inorganic mercury along the Amazon River (1, 2, 10). Investigations are under way to determine whether gold miners suffer inorganic mercury poisoning and whether residents who eat fish from the Amazon River suffer organic mercury poisoning.

The pathology of Minamata disease has been described in detail in earlier reports (7, 31, 35, 43-47, 49) and reviews (26, 27, 32, 34, 36-39, 41). This paper discusses organic mercury poisoning and suggests ways to differentiate between organic and inorganic mercury poisoning.
in humans. In addition, the methodology of histochemical determination is discussed in detail, as understanding the histochemistry of mercury in organs is essential to making a diagnosis of mercury poisoning.

**ACUTE AND CHRONIC CASES OF MINAMATA DISEASE**

**Clinical Signs and Symptoms**

Typical clinical signs and symptoms of methylmercury poisoning, known as Hunter-Russell trias, consist of ataxia, impairment of speech, and constriction of the visual field (8, 20). A committee established by the Japanese government proposed official guidelines for the diagnosis of Minamata disease in July 1977. According to these guidelines, Minamata disease syndrome has the following signs and symptoms: sensory disturbance in the distal parts of the extremities followed by ataxia, disequilibrium, concentric constriction of the visual field, impairment of gait and speech, muscle weakness, tremor, abnormal eye movement, and hearing impairment. These are occasionally accompanied by mental disorders and disturbances of the senses of taste and smell.

**Pathologic Changes in Minamata Disease**

**Cerebrum.** In the more than 200 autopsy cases of Minamata disease on file at Kumamoto University School of Medicine, lesions characterizing the disease were found mainly in the nervous system and predominantly in the brain cortex (7, 11, 26, 31, 34, 39, 49). In acute cases, histopathologic findings were those of acute edema in the perivascular space, with occasional perivascular extravasation and perivascular demyelination. Neurons in the cerebral cortex were swollen; there were severe cellular changes with neuronophagia in both acute and subacute cases. Acute neuronal shrinkage and ischemic changes were also apparent. Neuronal loss was found in the brains of patients who had died less than 1 mo after the onset of symptoms. The surviving neurons were often atrophic and sclerotic. Severe neuronal damage often resulted in cortical atrophy. Individual nerve cell necrosis occurred focally or diffusely with or without glial cell proliferation. Proliferation of hypertrophic astrocytes was frequent in the affected areas of the cerebral cortex in patients who died a few months after onset (38, 41).

These changes occurred predominantly in selective areas, including the calcareous region, the postcentral and precentral gyri, and the temporal transverse gyrus. Acute onset and prolonged cases always showed secondary degeneration of the pyramidal tracts, internal sagittal stratum, and central parts of the cerebral white matter.

Takeuchi and Eto (39) described a 6-grade system in an attempt to classify the distribution and severity of the cerebral lesions. The grades are as follows: grade 1, a decrease in the number of neurons by \(\geq 30\%\); grade 2, a decrease in the number of neurons by 30–50%; grade 3, a \(\geq 50\%\) loss of neurons (Fig. 2); grade 4, an increasing loss of neurons, progressing towards a spongy state; grade 5, microscopic spongy state; and grade 6, macroscopic spongy state.

**Cerebellum.** The characteristic cerebellar alteration caused by methylmercury poisoning was loss of granule cells with the presence of relatively well-preserved Purkinje cells. The lesions occurred in relatively deeper portions of the cerebellar hemisphere.

Takeuchi and Eto (39) classified the degree of severity of the cerebellar lesions into 6 grades. Grade 1, the mildest change, also called apical scar formation (33, 34, 36–41), is characterized by granule cells that disappear immediately underneath the Purkinje-cell layer at the crest of the folia, accompanied by a mild loss of Purkinje cells and a proliferation of Bergmann glial cells (Fig. 3). Grade 2 is characterized by a slight loss of granular cells (30–50%); grade 3 is \(>50\%\) loss of granule cells (Fig. 4); grade 4 is complete loss of granule cells with Purkinje-cell preservation; grade 5 is complete loss of both granular cells and Purkinje cells; and grade 6 is characterized by microscopic spongiosis of the granular cell layer.

**Spinal Cord.** No primary lesion occurred in the spinal cord. Secondary Wallerian degeneration of the pyramidal tracts was found in patients with acute onset and long-term survival. Secondary degeneration of the posterior columns, particularly of Goll’s tracts, was occasionally seen in prolonged or chronic cases, probably as a result of ganglion cell involvement and posterior root nerve fiber damage. This change was observed more in the caudal portion of the spinal cord than in the rostral part. Involvement of ganglion cells in the spinal ganglia was observed occasionally. In general, the spinal ganglion cells were less afflicted than cortical neuronal cells (39).

**Peripheral Nerves.** Biopsy of the sural nerve was performed on 6 chronic cases (6) and 2 fetuses (44) and was studied by light and electron microscopy. In the spinal
radix, the posterior roots were affected in almost all of the prolonged and chronic cases autopsied. The anterior roots were less involved and appeared normal in moderate and mild cases. Sensory nerve fibers tended to be destroyed more easily. In adult cases, the morphological changes in the sural nerve revealed an incomplete regeneration, including abnormally small nerve fibers and sprouting fibers, partial and complete demyelination, abnormal regeneration with irregular branching, and deformation of regenerated fibers, along with formation of regeneration units. The Schwann cells and collagen fibers increased in number in order to repair the damage of the sural nerves (Fig. 5).

Other Organs. The Kumamoto University Study Group (11) reported that pathologic changes in other organs include erosive inflammation of the digestive tract, particularly the duodenum, hypoplasia of the bone marrow, lymph node atrophy, and fatty degeneration of the liver and kidney. Later, disturbance of pancreatic islet cells was found in fetal, child, and adult cases of Minamata disease.

Though mercury deposits were detected in the kidney and liver, no functional disorder was clinically recognized.

Fetal Cases of Minamata Disease

The existence of fetal methylmercury poisoning in humans was first announced by Takeuchi et al. (48), who reported pathologic findings in 2 cases in which fetuses were exposed to methylmercury in utero. In these cases, the fetuses were also clinically diagnosed with cerebral
Fig. 6.—Histochemical distribution of mercury deposits in the kidney of a 66-yr-old female (Kumamoto University no. 8085). Mercury deposits in the epithelial cells of Henle’s loop are indicated by arrows. Photoemulsion method of histochemistry for mercury. ×300.

Fig. 7.—Deposition of mercury granules of the microglial cells (phagocytes) in the calcarine area of the occipital lobe of a 23-yr-old female (Kumamoto University no. 6383). Photoemulsion method of histochemistry for mercury. ×300.

Fig. 8.—Deposition of mercury granules in neurons of pontine nuclei. The granules are presumably deposited in the lysosome system of the neurons. The patient is the same as that in Fig. 7. Photoemulsion method of histochemistry for mercury. ×300.

Fig. 9.—Mercury granules are present in granular cells, Purkinje cells, and Bergmann's glia. The patient is the same as that in Fig. 7. Photoemulsion method of histochemistry for mercury. ×300.
palsy. Despite this report, skepticism as to the presence of Minamata disease in fetuses persisted until subsequent cases regarding in utero exposure to methylmercury were reported. Six autopsy cases of Minamata disease in fetuses are on file at Kumamoto University School of Medicine. In addition, Snyder (28) reported an infant exposed in utero to methylmercury who also had cerebral palsy, and Choi et al (4) discussed a fetus from Iraq damaged by methylmercury. Pathologic findings in these and related cases indicate that Minamata disease in fetuses is characterized by neuron loss and hypoplasia of neurons in parts of the cerebral cortex (35). In the case reported by Choi et al (4), hypoplasia of neurons without neuronal damage was described.

Hypoplastic changes are also found in the cerebellum. Purkinje cells show arrest of migration and persist in the granule cell layer or show disarray in the Purkinje-cell layer. Loss of granule cells is difficult to assess by light microscopy (4).

In one of the cases, electron microscopy indicated that synapses between parallel fibers and Purkinje cells were well formed in the cerebellum (5). Biopsy of the sural nerve in fetal cases (44) revealed similar findings, characterized particularly by aplastic and hypoplastic myelination of small nerve fibers with abnormal, whirled myelin degeneration, which are also characteristic of adult cases.

### Table I

<table>
<thead>
<tr>
<th>Neuronal type (N)</th>
<th>MMC</th>
<th>P</th>
<th>N (P: disappeared)</th>
<th>MMC</th>
<th>P</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagocytic type (P)</td>
<td>MMC</td>
<td>P</td>
<td>N (necrosis)</td>
<td>MMC</td>
<td>P</td>
<td></td>
</tr>
</tbody>
</table>

** MMC: Methylmercury chloride
** P: Phagocyte
** N: Neuron

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### Table II

<table>
<thead>
<tr>
<th>Cerebrum</th>
<th>White matter</th>
</tr>
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<tbody>
<tr>
<td>6403</td>
<td>++ (N)</td>
</tr>
<tr>
<td>7572</td>
<td>47 M</td>
</tr>
<tr>
<td>7553</td>
<td>79 M</td>
</tr>
<tr>
<td>7568</td>
<td>50 M</td>
</tr>
<tr>
<td>7272</td>
<td>73 F</td>
</tr>
<tr>
<td>8177</td>
<td>77 M</td>
</tr>
<tr>
<td>8309</td>
<td>50 M</td>
</tr>
</tbody>
</table>

** Abbreviations: N = neuron; ++ = moderate; + = mild; - = negative.

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### Histochemistry of Mercury in Organs

#### Methodology of Determination

Methods of mercury demonstration by histochemistry are reported as diphenylthiocarbazide, dithizone [Okamoto (18) and Okamoto et al (19)], and mercury sulfide [Voight (53) and Timm and Arnold (50)]. Localization of mercury in the tissues was studied histochemically by our modified method of Sakai et al (21) with a time of development of 6 min. A modification of this method by Tokunaga et al (51) increased sensitivity 5- to 10-fold.

Pieces of excised brain, liver, and kidney were fixed in 10–20% neutral formalin. These tissue blocks were processed through ethanol and xylol and embedded in paraffin. Six-micrometer-thick sections were cut, completely deparaffinized using fresh xylol and ethanol, rinsed with distilled water, incubated in Kardaessch’s mixture (100 parts of 70% ethanol and 1–5 parts of 28% ammonia) for 2 hr at room temperature. After washing in water for 5 min, the sections were dehydrated with a graded ethanol series from 70 to 100%, dried, and mounted on glass slides. The slides were then transferred to photographic emulsion (40–50% NR-M 2 [Konika-Medical Co. Ltd., Tokyo, Japan] mixed with distilled water) at 40–50°C in utter darkness. The slides were blotted with filter paper to remove excess emulsion. Slides were then allowed to stand perpendicularly overnight at room temperature in utter darkness. They were developed with FD-111 (4 g monomethyl-p-amonophenol sulfate; 60 g sodium sulfate anhydrous; 10 g hydroquinone; 53 g sodium carbonate; 2.5 g potassium bromide dissolved in 1,000 ml distilled water) for 6 min at 20°C. Next, the sections were fixed with photographic fixative (Fuji-Fix®) for 4 min at 20°C. These operations were performed in utter darkness. After washing in water for 10 min, the sections were stained with hematoxylin and eosin, washed thoroughly with distilled water, dehydrated with ethanol, treated with xylol, and mounted with resin or Bioleit® (Ohkenshoji Co. Ltd., Tokyo, Japan).

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### Distribution of Mercury in Organs

Experimentally, Suda et al (29) demonstrated that the appearance of mercury granules in tissues corresponded...
TABLE III.—Differential diagnoses of organic and inorganic mercury poisoning.

<table>
<thead>
<tr>
<th>Organic mercury poisoning</th>
<th>Inorganic mercury poisoning</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃HgCl</td>
<td>HgCl₂</td>
</tr>
<tr>
<td>CH₃HgSCH₃</td>
<td>Hg²⁺</td>
</tr>
<tr>
<td>BBB</td>
<td>BBB</td>
</tr>
<tr>
<td>Brain</td>
<td>Kidney</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Localization of pathologic changes in the brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minamata disease (Hunter-Russell's trias)</td>
</tr>
<tr>
<td>Histochemistry of mercury biotransformation into inorganic mercury is positive</td>
</tr>
<tr>
<td>EM: rER, microtubules destruction</td>
</tr>
<tr>
<td>mitochondria; normal cytoplasmic dense inclusions; rare</td>
</tr>
</tbody>
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Abbreviations: EM = electron microscope; rER = rough endoplasmic reticulum.

to the levels of inorganic mercury in tissues but not to those of organic mercury. In human autopsy cases involving Minamata disease, mercury granules were observed in brain tissue containing >0.2–0.3 µg/g of total mercury. When the amount of inorganic mercury in these brain samples was evaluated by subtracting the amount of mercury, more than 80% of total mercury was found to be in the form of inorganic mercury (41).

With the use of a photoemulsion method (21, 51), mercury deposits were detected mainly in the kidney, liver, and brain. In the kidney, significant mercury deposits were found in the epithelial cells of proximal convoluted tubules (Fig. 6); no demonstrable precipitate was found in the glomerulus. In the liver, mercury granules were found in the parenchymal and Kupffer cells. In the brain, mercury deposits occurred in neurons and glial cells or in perivascular spaces (Figs. 7–9). Four types of mercury deposits were found in brains affected by Minamata disease: neuronal, phagocytic, mixed, and perivascular. Electron microscopy revealed that mercury granules were deposited mainly in lysosomes. Moreover, mercury concentration in subcellular organelles was always accompanied by selenium deposits, which were found by electronmicroscopic X-ray microanalysis (25).

METHYLMERCUry-INDUCED HYDARGYRIA

Even after a prolonged clinical course, we were able to demonstrate the presence of widespread mercury deposits in patients severely affected with Minamata disease. In mild or chronic cases, the persistence of mercury can still be found using similar methods, but in these cases, the mercury is localized in neurons or phagocytes cells.

In the neuronal type of mercury deposits in the brain affected by Minamata disease, 3 routes were considered. First, methylmercury entered a phagocyte. The life span of a phagocyte is 2–3 mo; therefore, mercury granules were found in neurons after the phagocyte died. Second, the methylmercury biotransformed to Hg²⁺, allowing mercury to enter the neurons. Third, methylmercury entered the neurons directly.

In the phagocytic type of mercury deposits, 2 routes were considered. First, mercury granules were found in phagocytes after methylmercury entered a phagocyte directly. Second, phagocytosis occurred after necrosis of...
neurons as a result of methylmercury poisoning. These mechanisms are hypothesized from the evidence of the mercury deposits (Table I).

In 7 out of 221 autopsy cases, mercury deposits were found in the brain by use of a photoemulsion histochemical method, but no clinical signs or symptoms of Minamata disease were found, nor were pathological changes associated with Minamata disease found. All cases came from mercury-polluted areas. Because mercury deposits in the brain were thought to be abnormal, Takeuchi et al. (45) referred to these cases as “methylmercury-induced hydrargyria” (Table II).

**DIFFERENTIAL DIAGNOSIS OF ORGANIC AND INORGANIC MERCURY POISONING**

Methylmercury and the vapor of inorganic mercury (Hg⁰) easily pass through the blood–brain barrier and enter the brain tissue. Inorganic mercury poisoning causes gingivitis, tremor, or erethism (3). Mercury chloride, a form of inorganic mercury, affects the epithelial cells of proximal convoluted tubules in the kidney. In the nervous system, the histopathologic changes associated with organic mercury poisoning are localized. However, in the central nervous system, histopathologic changes associated with inorganic mercury poisoning cannot be demonstrated by routine light microscopy. The detection of methylmercury is possible only when it transforms into inorganic mercury in some organs.

In organic mercury poisoning, the involvement of the endoplasmic reticulum (3) and microtubules in neurons (13, 14) and the preservation of mitochondria are demonstrated by electron microscopy (5) (Fig. 10). In contrast, organelles were well preserved in the case of inorganic mercury poisoning, but numerous cytoplasmic dense inclusions were found (30) (Table III; Fig. 11).

**PATHENOGENESIS**

The mechanism of methylmercury intoxication remains unclear. Methylmercury enters the central nervous system through the blood–brain barrier; deposits of inorganic mercury are present diffusely. The lesions of Minamata disease, however, are localized, and the relationship between mercury deposition and lesion distribution is unknown.

Takeuchi (37) showed that nerve damage by methylmercury in cases of Minamata disease predominantly affected smaller neurons. Cavanaugh (3) suggested that a low ribosomal content in small neurons may increase...
their vulnerability to methylmercury poisoning. Furthermore, the ability of these elements to recover from early inhibition of protein synthesis may be selectively impaired. Anoxic conditions related to disturbances in both the erythrocytic and blood–brain barrier functions (40) may also play a role in the pathogenesis of nervous system lesions in Minamata disease.

The distribution of lesions associated with Minamata disease in the nervous system is shown in Fig. 12. This figure shows the summary of the lesions of moderate cases of Minamata disease. The lesions of Minamata disease always show systematically in the nervous system. The moderate lesions are found in calcarine areas of occipital lobes, pre- and postcentral lobes, and temporal transverse gyri; mild, diffuse lesions are also found in the cerebrum with the secondary degeneration of central parts of white matter and internal sagittal stratum due to primary damage of neurons. Granular cells are decreased in number in the central portion of the cerebellum. In the spinal cord, there is no primary change, but the secondary degeneration is found on pyramidal tracts due to the damage of motor neurons of precentral cortices. Also, the secondary degeneration of Goll’s tract is found due to the primary degeneration of sensory nerve fibers including ganglion cells, sciatic nerves, and sural nerves.

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