PATHOLOGICAL, CLINICAL AND EPIDEMIOLOGICAL RESEARCH

ABOUT MINAMATA DISEASE, 10 YEARS AFTER. (2ND YEAR)

Kumamoto University,
Faculty of Medicine

Research Committee on Minamata Disease, after 10 years

Chairman Prof. Takeuchi

Parts I to V translated by

SCITRAN (Scientific Translation Service) of
Santa Barbara, California

for the U. S. Environmental Protection Agency

Parts VI to VIII translated by
Dr. Ryotaro Ishizaki
for Duke University, Durham, North Carolina

I. PREFACE

Tadao Takeuchi, Study Group Representative

It has been two years since this study of Minamata disease started, after
many incidents of the disease had been observed for more than 10 years. The
results of the first year's study were delineated in the study reports of
the last year. We attempted a rough analysis of the following: the
condition of mercury contamination of the Minamata district and its vicinity;
immunology of Minamata disease and its progress; detection of the outbreak
of the disease; general health hazard, etc. Clinical status of the Minamata
disease and its progress led to the finding of new symptoms and new methods
of treatment from the current viewpoints. They provide a valuable tool in
clinical diagnosis. Pathological fundamental knowledge on the dormancy of
Minamata disease, and Minamata disease which is difficult to diagnose, was
also provided by biopsies from victims of Minamata disease. Moreover,
fundamental methods of the prevention and the treatment of Minamata disease,
or methyl mercury poisoning, were attempted. Thus, during the first year,
attempts were made to study various medical problems relating to Minamata di-
sease. Moving toward a solution of these problems, the study moved on to
its second year.

We must report on the results of the second year here. When this study
originated, it was for three year's duration. However, in order to implement
the study, the prefecture authorities expressed their desire to complete
this study in two years. Thus, at the beginning of this year, the study plan
was changed for two years. Hence, efforts by study group members during this
year encompass two year's results, and the members have been constantly busy.
Also, regarding the accurate diagnoses of Minamata disease, we encountered
difficulties in communication between the area and the university medical
school researchers, in finding patients, and in examining everyone completely
concerning various non-medical problems. We had to resort occasionally to

examining a limited number of people in a limited area. However, we believe
that we have achieved enough results, as described below in each item, for
this kind of study.

With this study, the concept of Minamata disease has been clarified.
Also, it has become easier to detect the symptoms of Minamata disease and
its diagnosis. With it, the distribution of organic mercury poisoning of
the Minamata district and its vicinity has been clarified. The relationships
between mercury contamination in the human body and the outbreak of Minamata
disease, the relationship between the contamination conditions and health
hazards caused by it, and light or chronic poison symptoms have become clear.
Fundamental knowledge has been formulated in analyzing the past conditions
of the outbreak of Minamata disease, present contamination conditions, and
future prospects.

On the other hand, there will be no end to the study. We had to conclude
our two-year study, with problems still remaining for the future. The remain-
ing problems include many in the area of medicine, such as how to handle the
contaminated areas not covered by the study, how to improve the treatment of
many Minamata disease patients, how to discover new methods of treatment, and
how to prevent an outbreak among people who are contaminated. Also, the in-
vestigation of health hazards due to micro-contamination and complex genes
remains a future problem. As far as the problems within the laboratory are
concerned, the discovery of internal organization of organic mercury poisoning
still remains a problem. We would like to continue this study in some form
with some assistance. What we most desire is for the results of this study
to be fully utilized.

TABLE OF CONTENTS

1. INVESTIGATION ON MERCURY CONTAMINATION OF MINAMATA DISTRICT AND THE
SOUTHERN DISTRICT OF THE ARIAKE SEA

Masashi Fujiki, Group Member and Shizuko Tajima, Research Assistant
Kumamoto University Medical School Hygienics Department

I. INTRODUCTION

More than ten years have passed since the outbreak of Minamata disease.
As a result of the investigation of the health conditions of people who live
in the Minamata district and its vicinity, especially in the area which is
contaminated by mercury and the area where the mercury-contaminated fish
exist, it is suspected that persons who may exhibit Minamata disease-like
symptoms may extend to a fairly large range of areas [1]. It has become im-
portant to perform medical investigations to determine whether Minamata
disease-like symptoms are due to the methyl mercury contamination or not.

This study on environmental contamination by mercury has covered not
only the Yatsushiro Sea, but also wider areas. This study [2] has observed the
conditions of environmental contamination by measuring the amount of mercury
primarily in fish for the area not covered by the previous study.

II. INVESTIGATION METHOD

1. Marine Life

As illustrated in Figure 1, five specimens of marine life were collected
on November, 1972 from each of the following six places: outside of Minamata
Bay (around Awaji Island); offshore estuary of the Minamata river, fishing
grounds for scabbard fish off Minamata (in the Yatsushiro Sea); offshore

1

Shimoda, Yushima, and the estuary of the Midori river (in the Ariake Sea). Then, the amounts of methyl mercury and the total mercury contained in them were measured. Also, for the purpose of comparison, the amounts of methyl mercury and of the total mercury contained in ten frozen fish sold in the market and caught in the sea of northern Okhotsh near Hokkaido were measured.

2. Hair

The amounts of methyl mercury and of the total mercury contained in the hair of 34 persons from the Minamata district (measured on August, 1972), and of 11 persons from the Goshoura district (measured on October, 1972) were measured. They were selected from the people who complained about the occurrence of symptoms after 1966 and were considered to show Minamata disease-like symptoms, after investigations of the health conditions of people living in the Minamata and the Goshoura districts, performed by the Neuro-Psychiatry Department of Kumamoto University in 1971.

3. Analysis Method of Methyl Mercury

Approximately 50 g of the edible parts of fish and shellfish and approximately 1 g of hair were precisely measured and used as analysis subjects. Each subject was soaked in hydrochloric acid for three days at room temperature to decompose it. After collecting methyl mercury in the hydrochloric acid layer, it was extracted by the standard method and measured by the gas chromatograph method.

4. Analysis Method of Total Mercury

For hair, the remains after decomposition by hydrochloric acid and extraction of methyl mercury were treated by heating for about 48 hours using nitric acid, sulfuric acid and permanganic acid. Then, the mercury in the dissolved liquid was measured by the flameless atomic absorption photometry method [4]. This gives the amount of inorganic mercury, and the total amount of mercury is obtained by adding the amount of methyl mercury to it.

2

For fish and shellfish, approximately 5 g of the edible part were pre-
cisely measured. After drying, the mercury was collected by the quartz tube
heating absorption method [5], and then measured by the flameless atomic ab-
sorption photometry method.

III. RESULTS OF INVESTIGATION

1. Marine Life

As illustrated in Table 1, out of three places in the Minamata district
of the Yatsushiro Sea, the mercury density of fish outside of Minamata Bay
was the highest and contained 0.182-0.440 ppm of methyl mercury and 0.342-
0.530 ppm of total mercury. The fish which were offshore of the estuary
of the Minamata river contained 0.050-0.068 ppm of methyl mercury and 0.060-
0.210 ppm of total mercury. The fish in the fishing ground for scabbard fish
off Minamata contained 0.020-0.54 ppm of methyl mercury and 0.060-0.300 ppm
of total mercury.

For three places in the Ariake Sea, the fish in the offshore area of
Shimoda contained 0.018-0.080 ppm of methyl mercury and 0.58-0.320 ppm of
total mercury. The fish off Ushima contained 0.028-0.120 ppm of methyl mer-
cury and 0.45-0.205 of total mercury. The fish and shellfish which were off-
shore of the estuary of the Midori river contained 0.010-0.016 ppm of methyl
mercury and 0.022-0.085 ppm of total mercury.

The fish in the northern sea of Hokkaido contained 0.006-0.014 ppm of
methyl mercury and 0.020-1.904 ppm of total mercury.

The mercury concentration in hair is shown in Table 2. In the Minamata
district, it contained 1.35-11.37 ppm of methyl mercury and 2.10-14.82 ppm of
total mercury. In the Goshoura district, it contained 0.19-4.65 ppm of methyl
mercury and 0.39-5.15 ppm of total mercury.

IV. OBSERVATIONS

The fish in three places of the Minamata district contained 0.020-0.440
ppm of methyl mercury and 0.060-0.530 ppm of total mercury, which clearly
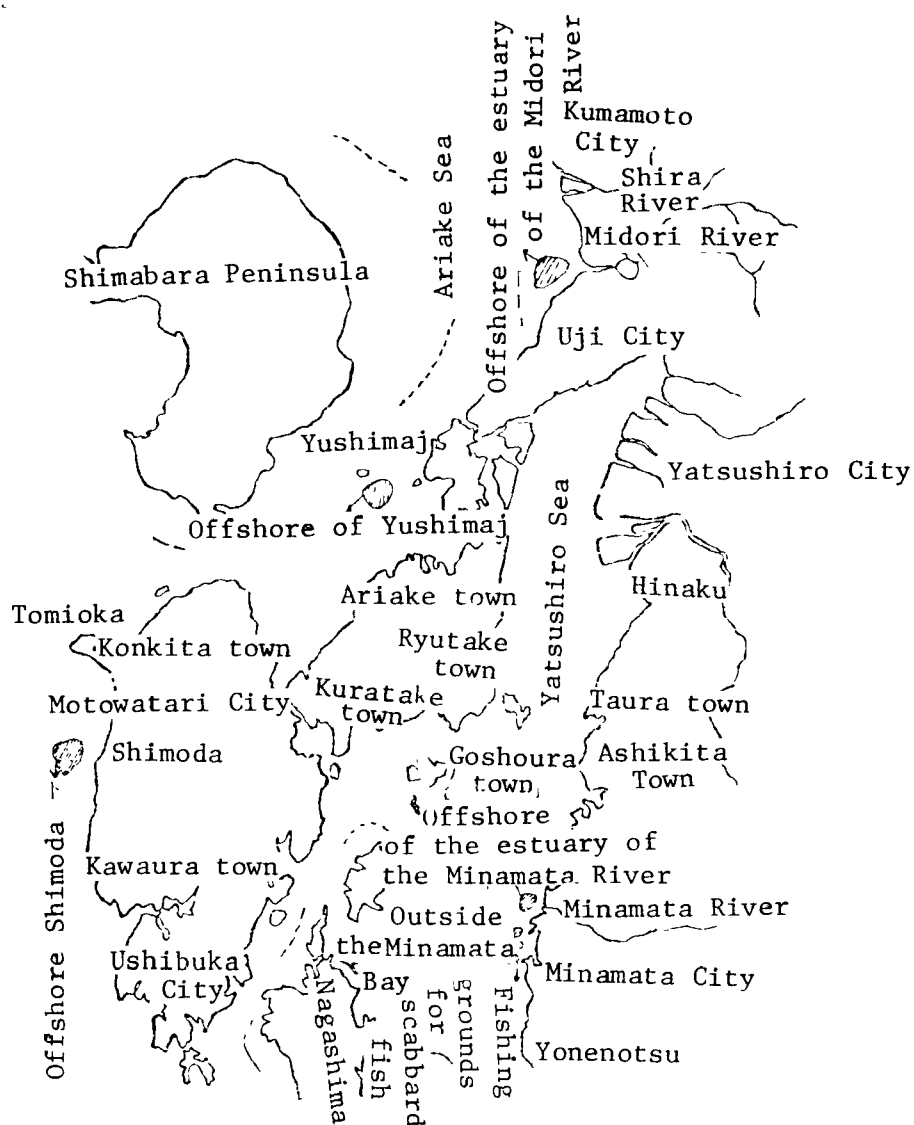
Figure 1. Fish and shellfish collection places in the Yatsushiro Sea and the Ariake Sea.

indicates a decline, compared with those in 1961 [6].

The fish in three regions of the Ariake Sea contained 0.10-0.120 ppm of methyl mercury and 0.022-0.320 ppm of total mercury. This mercury concentration was high, compared with 0.001-0.022 ppm of methyl mercury and 0.008-0.100 ppm [7] of total mercury contained in the fish of the Port of Nagasaki, which is considered as an area of comparison, or 0.006-0.014 ppm of methyl mercury and 0.020-1.904 ppm of total mercury contained in the fish of the northern sea of Hokkaido. Especially, methyl mercury concentration of the

4

TABLE 1

AMOUNTS OF MERCURY IN FISH AND SHELLFISH COLLECTED IN THE YATSUSHIRO SEA, ARIAKE SEA, AND THE NORTHERN SEA OF HOKKAIDO (COLLECTED NOVEMBER, 1972)

Collection place		Name of fish or shellfish	Methyl mercury concentration (Hg ppm/wet weight)	Total mercury concentration (Hg ppm/wet weight)
Yatsushiro Sea	outside Minamata Bay (around Awaji Island)	bera 1** bera 2 bera 3 sillago swellfish	0.402 0.182 0.255 0.110 0.202	0.510 0.312 0.172 0.528 0.530
	Offshore of the estuary of the Minamata River	sillago 1 sillago 2 flathead bera swellfish	0.040 0.065 0.062 0.060 0.068	0.060 0.110 0.085 0.210 0.130
	Fishing ground for scabbard fish offshore Minamata	scabbard fish 1 scabbard fish 2 scabbard fish 3 scabbard fish 4 scabbard fish 5	0.048 0.051 0.022 0.020 0.021	0.300 0.210 0.110 0.060 0.061
Ariake Sea	Offshore Shimoda	Kawahagi 1** Kawahagi 2 horse mackerel 1 horse mackerel 2 Kitsukori**	0.050 0.020 0.025 0.018 0.080	0.058 0.155 0.120 0.080 0.198
	Offshore Yushima	Sea bream 1 Sea bream 2 swellfish bera garakabu**	0.085 0.028 0.060 0.030 0.120	0.098 0.115 0.072 0.045 0.205
	Offshore of the estuary of the Midori River	gray mullet 1 tray mullet 2 clam short-necked clam shrimp	0.010 0.014 0.075* 0.080* 0.014	0.022 0.040 0.220* 0.240* 0.085

TABLE 1.   (Continued)

Collection place	Name of fish or shellfish	Methyl mercury concentration (Hg ppm/wet weight)	Total mercury concentration (Hg ppm/wet weight)
Northern Sea of Hokkaido	bream 1	0.014	0.145
	bream 2	0.011	0.080
	bream 3	0.008	0.053
	bream 4	0.007	0.044
	horse mackerel 1	0.010	1.904
	horse mackerel 2	0.012	0.247
	turbot 1	0.007	0.021
	turbot 2	0.006	0.020
	turbot 3	0.007	0.022
	turbot 4	0.007	0.020

Area for Comparison

* Concentration per dry weight.
** Some Japanese fish names have been romanized.

TABLE 2.   AMOUNTS OF MERCURY IN HAIR

Persons in Minamata district: collected in August, 1972			Methyl mercury concentration (Hg ppm)	Total mercury concentration (Hg ppm)
Subject No.	Sex	Age		
5	female	57	3.02	3.04
13	male	71	10.18	11.82
15	female	37	2.23	4.31
27	male	70	1.82	7.48
28	female	52	5.91	7.78
34	male	60	9.43	12.50
54	male	65	3.93	4.51
63	male	32	1.30	5.10
65	male	54	2.19	4.13
85	female	69	3.57	4.18
94	female	61	1.35	2.30
95	male	58	5.86	6.61
116	male	75	3.52	4.11
163	female	49	5.23	5.61
165	female	56	3.59	4.31
167	female	71	3.88	4.61
185	male	57	10.01	11.45
187	male	48	11.37	12.38
188	female	15	5.11	5.76
190	male	52	6.15	6.20
	male	63	4.60	4.90

6

TABLE 2. (Continued)

Persons in Minamata district: collected in August, 1972			Methyl mercury concentration (Hg ppm)	Total mercury concentration (Hg ppm)
Subject No.	Sex	Age		
	male	.2	3..7	7.70
201	male	42	7.10	8.05
208	female	73	4 10	1.56
210	female	63	2.86	4.78
214	male	59	1.79	2.10
219	female	63	9 21	10.41
229	female	51	5.58	6.58
239	female	59	4.13	4.61
245	male	71	6 90	7.87
240	female	62	6 76	7 52
254	female	46	1.98	2.53
260	female	15	4.10	4 06
272	female	43	1.31	2.11

People in the Goshoura district: collected in October, 1972			Methyl mercury concentration (Hg ppm)	Total mercury concentration (Hg ppm)
Subject No.	Sex	Age		
504	female	30	9 95	6.79
505	female	68	0 39	0.89
506	female	73	0.24	0.29
516	male	59	1 05	2.92
521	female	45	0.50	0 92
547	female	44	0 24	0.80
550	female	57	4.65	5.15
559	male	80	0 19	0.70
586	male	42	0.64	1.36
599	male	60	1.15	1.51
623	female	71	1 35	1 73

fish in Yushima is high, compared with those in the area of comparison. However, this methyl mercury concentration is equivalent to about 1/10 of 0.5 ppm methyl mercury concentration of tuna fish in the Pacific Ocean or Indian Ocean [8], and it does not reach a poisonous level [15] under normal consumption.
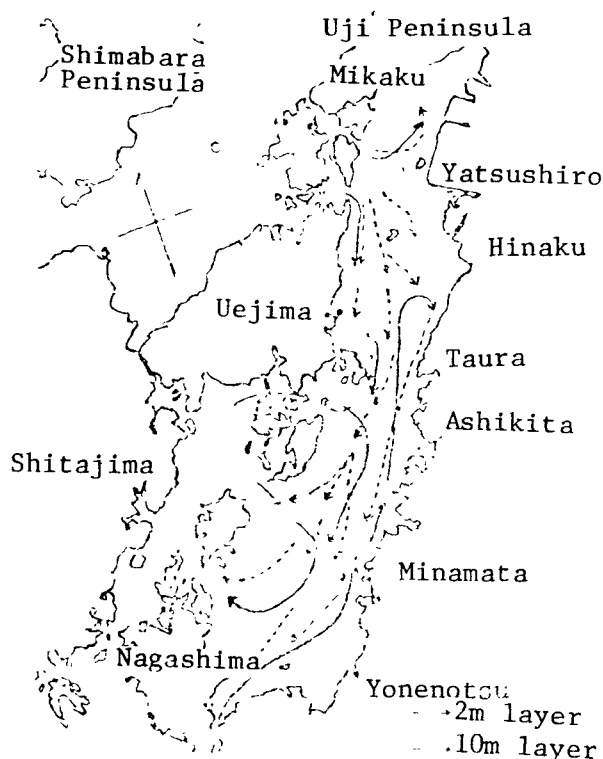
Figure 2. Normal current of the Yatu-
shiro Sea.

Figure 3. Lowest tide of the Yatsu-
shiro Sea.

Among the specimens from offshore of the estuary of the Midori River,
gray mullets were the only fish selected. Since the cumulative amount of
mercury differs considerably from one type of fish to another, this mercury
concentration of gray mullets cannot be compared with the mercury concentra-
tion of other types of fish in other districts. By comparing the mercury
concentration of gray mullets from offshore of the estuary of the Midori
River, with that of gray mullets examined in 1971 in the Yatsushiro district
[2] (methyl mercury 0.003-0.005 ppm, total mercury 0.005-0.009 ppm), Ushiku-
ka district [9] (methyl mercury 0.003 ppm, total mercury 0.007 ppm), Tomioka
district [9] (methyl mercury 0.002 ppm, total mercury 0.003 ppm), Nagasaki
Bay [7] (methyl mercury 0.001-0.002 ppm, total mercury 0.008-0.062 ppm), the
former exhibits a higher concentration than any others. Also, the mercury
concentration of short-necked clams in Osaki of the estuary of the Minamata
River [10] was previously established (methyl mercury 0.067 ppm, total mer-
cury 0.97 ppm; March, 1972). This concentration is based on dry weight.

8

If it is converted to the figure based on the wet weight, it will be approximately 0.016 ppm of methyl mercury and approximately 0.048 ppm of total mercury. The mercury concentration of short-necked clams from offshore of the estuary of the Midori River will not reach a poisonous level, as in the case for the fish from offshore of Yushima, if a normal amount is consumed.

Observations of the relationship between the distance from the Minamata Bay and the mercury concentration of fish reveal that for the Yatsushiro Sea, the one in the Minamata Bay shows the highest [9] (methyl mercury 0.062-0.416 ppm, total mercury 0.112-1.015 ppm), followed by that outside of Minamata Bay (around Awaji Island), offshore of the estuary of the Minamata River, the fishing ground of scabbard fish off Minamata. It was also found that the greater the distance from Minamata Bay, the lower the mercury concentration becomes. The lowest mercury concentration of fish in the Yatsushiro Sea is indicated in the fish from near Yatsushiro, measured in the previous investigation [2, 9]. However, the mercury concentration of the fish in Yushima which is further than Yatsushiro shows a higher value than that in Yatsushiro.

The tidal flow of the Yatsushiro Sea is such that during high tide the current flows in from the outside sea into the Yatsushiro Sea to the north through each channel, and during low tide it flows through the Yatsushiro Sea in a completely opposite direction to the south. Approximately 98% of the amount of flow to and from the Yatsushiro Sea is through three channels: Motonojiri, Mebuki, and Kuro. The amount of flow to and from the Ariake Sea through two northern channels of the Yatsushiro Sea, Mikaku and Yanagi, is extremely small. Since the average water level of the northern part is higher than that of the southern part, the normal flow is in the direction of the south for the surface level (2m layer). Even for the 10 m level, the flow of the northern part and close to the islands of Tengusa is in the direction of the south, similar to the surface level. It has been reported that only the flow through the Kuro channel can go to the north offshore of Minamata and reach the offshore of Hinaku [11] (see Figure 2 and Figure 3). Since this south-bound normal current prevails, it is impossible that the mercury flowing into the Minamata Bay flows into the Ariake Sea through

9

Yatsushiro by the tidal flow. The following facts [12] prove that the mercury flowing out to the Minamata Bay does not reach the Ariake Sea offshore of Yatsushiro: the mercury concentration in scabbard fish in the estuary of the Minamata river was 10.6 ppm, whereas the one in Yatsushiro was 0.4-0.08 ppm, as reported by Kitamura, et al. in 1959, and the mercury concentration of fish in the Yatsushiro district indicates a lower value than that of the Ariake Sea, as shown in 1971 [2, 9] and the present investigations.

During this investigation, the mercury concentration of the fish off-shore of Yushima was higher than that in the Port of Nagasaki [7] and was comparable or higher than that in the Goshoura and the Kuratake districts of the 1971 investigation [2, 9]. Therefore, the reason for the mercury in fish offshore of Yushima must be considered. In order for the mercury to accumulate in fish, it is necessary that one of the following conditions be met: the area is mercury contaminated, fish swim from an area which is contaminated by mercury, or the fish are on the top of the feed chain and easily accumulate mercury. As mentioned previously, Yushima offshore is not contaminated by mercury flowing out from Minamata Bay. Also, except sea breams, wellfish, bera, and garakabu, all three stay in fixed places. According to the report by the Seikai district marine laboratory, [11] almost all of the marked fish released in the southern part of the Yatsushiro Sea moved to the south, and the only fish which moved northward were Konoshiro, released in the middle part of the Yatsushiro Sea. Although the fact that the fish run from the outside sea into Minamata Bay, stay there until the mercury is accumulated, move into the Yatsushiro Sea northward, go out to the Ariake Sea, and reach to offshore Yashima cannot be denied, the proportion of such fish among fish offshore Yushima is extremely small and also the mercury is not accumulated from the chain of eating fish offshore Yushima. Therefore, it can be assumed that the Yushima area contains a relatively high concentration of mercury and the mercury does not come from that discharged from the Minamata Bay, but from some other sources. The information on whether the Yushima offshore was contaminated by mercury in the past is only available through the results of measuring the mercury concentration in the hair of people who lived in the three districts along the Ariake Sea in

1961 [13]: Nagasu (average, 9.7 ppm), Kawauchi (average, 5.2 ppm), and Kawaguchi (average 10.1 ppm), and the 1959 report [12] that intestines of a cat showed a relatively high concentration of mercury.

Some people in the Minamata district had more than 100 ppm of mercury concentration in their hair in 1960 [12, 13]. However, the mercury concentration gradually declined to 2.6-73.8 ppm, average, 9.2 ppm (65 persons) in 1968, 1.2-18.3 ppm, average 5.5 ppm (44 persons) in 1969, and 1.2-9.5 ppm, average 3.7 ppm (26 persons) in 1970 [6]. This decline matches well with the decline of the mercury concentration in fish and shellfish in Minamata Bay [6]. In this investigation, the mercury concentration in hair in the Minamata district was 2.14-14.82 ppm; the average was 6.16 ppm (34 persons). This value is slightly higher than the mercury concentration in hair in general at the present time, but is not exceptionally high. Sixteen out of 34 subjects in the Minamata district have families who were diagnosed as having Minamata disease, and are considered to have been exposed to high mercury contamination in the past.

The mercury contamination in hairs of people in the Goshoura district was 0.39-5.15 ppm and an average of 1.54 ppm (11 persons). This value is lower than the value in general cases. Since the investigation of the health conditions of people started there, they have begun not eating fish so often. This is considered to be the reason for the low value of mercury in hair.

In general, the mercury in hair relates to the amount of mercury intake. The more the mercury intake, the more the mercury in hair. According to the report of the hygienic laboratory of Kagoshima prefecture [14], a person whose hair contained 228 ppm of mercury in 1960 reduced the mercury concentration to 33.1 ppm after 22 months; for a person with 144 ppm of mercury it was reduced to 14 ppm after 20 months. For a person with 208 ppm it was reduced to 18.8 ppm after 21 months, and for a person with 153 ppm it was reduced to 15.8 ppm after 23 months by controlling the eating of fish and shellfish under the prefecture's guidance. Therefore, the mercury contained in hair is effective in determining the current contamination by mercury, but

does not provide important information for observing the past contamination of the mercury. In this investigation, people in the Goshoura district showed a low value of mercury concentration in their hair. However, judging from the fact that 13.4% of the subjects in 1960 (1160 persons) had 50-920 ppm [13], it cannot be denied that the people in the Goshoura district were contaminated in the past.

TABLE 3. AMOUNTS OF MERCURY IN SHELLFISH FROM THE MINAMATA BAY AND THE ESTUARY OF THE MINAMATA RIVER (µg/g dry weight)

Type of shellfish Collection place	1960 1	1960 4	1960 8	1961 1	1961 4	1961 12	1962 1	1963 10	1965 5	1965 10	1965 12	1966 12	1967 4	1967 6	1967 8	1967 10	1967 12
Midori (Tsukinoura) igai*	85	50	31	56	30	9											
Midori (Tsukinoura) short-necked clam							12	12				8	8	15	26	24	20
Myojin "							28	28	33	84			7	8	3	16	13
Awaji Island "							43	40	16	21			60	19	48	32	14
Osaki "							5	5	5	81			6	3	6	5	9

Note: * igai, hibarigaimodoki

(CONTINUATION OF TABLE 3.) Quoted from Japan Public Health Journal 19, 25, 1972

12

TABLE 4. AMOUNTS OF MERCURY CONTAINED IN FISH FROM THE MINAMATA BAY AND ITS VICINITY

(μg/g wet weight)

Collection date	March,1961		Oct.,1963		May,1965		Oct.,1966		May, 1968		June, 1968		July, 1968;	
Type of fish (FT) and Total mercury (TM)	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM
Collection place														
Inside the Minamata Bay	Pike	23Δ	Gray mullet	0.4Δ	Gray mullet	0.4 Δ	Swell-fish	0.4Δ			Spar-row swell-fish	0.4		
	Hanta	12Δ	Sil-lago	4Δ	Sil-lago	12 Δ	Sil-lago	0.2 Δ		Sil-lago 0.5	Sil-lago	0.5	Sil-lago	0.7
	Kuchizoko	9 Δ	Crab	4~6Δ	Crab	5Δ							Watari crab	1.0
	Shijugo	7Δ	Sole	4Δ	Sole	3Δ					Goby	0.4		
			Mebari	0.8~3Δ	Mebari	2 Δ					Tiger goby	0.4	Tiger goby	0.2
			Ray	5Δ	Guchi	8Δ			Gara-kabu	Gara-kabu 2.5	Gara-kabu	2.0	Gara-kabu	0.9
			Tur-bot	4Δ									Red ray	0.4
			Kono-shiro	0.4~1Δ									Kusabi	0.7
													Eel	0.4
													Scab-bard fish	0.1

13

TABLE 4 (CONTINUED)

Quoted from Japan Public Health Journal
19, 25, 1972

Collection date	November 1968			August 1969			August 1970		
	Fish type	Total Merc.	$\frac{M\%}{T}$	Fish type	Total Merc.	$\frac{M\%}{T}$	Fish type	Total Merc.	$\frac{M\%}{T}$
	Gray mullet (2)	0.1~0.2	5	Swell fish (2)	0.1	40	Swell fish (8)	0.2~0.4	20
	Sillago (10)	0.2~0.7	20	Sillago (2)	0.3~0.5	50	Sillago (10)	0.4~0.7	15~20
							Sweat bream (1)	0.4	6
	Eel (1)	0.6		Kawahagi (2)	0.2	2	Sea bream (14)	0.1~0.3	5
	Sea bream (3)	0.2~0.5	25	Takaba (7)	0.1~0.4		Tiger goby (2)	0.4	20
	Swell fish (3)	0.3~1.1	6~20	Garakabu (5)	0.4~1.3	24~54	Tiger goby (3)	0.4~1.3	10~20
	Kawahagi (3)	0.1~0.2	15				Kawahagi (6)	0.2	10-15
	Kusabi (6)	0.3~0.7	30	Kusabi (5)	0.2~0.5	30~40	Kusabi (8)	0.2~0.8	5~10
	Chinu (2)	0.2~0.5	30~60				Ashata (3)	0.2~0.6	5~10
	Konoshiro (11)	0.1~0.2	1~40				Nominokuchi (1)	0.2	24
	Horse mackerel (11)	0.1~0.3	8~40						
	Octopus	0.1	5	Octopus (3)	0.9~1.6	1~2			

13 a

TABLE 4 (CONTINUED)

Collection date	March,1961		Oct.,1963		May,1965		Oct.,1966		May,1968		June,1968		July,1968;	
Type of fish (FT) and Total mercury (TM)	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM	FT	TM
Collection place														
Offshore Yawata					Chinu 47Δ									
Offshore Yunoko					Scab-bard fish 2Δ				Scab-bard fish 0.5Δ				Sea urchin 1.5 Nabe-gyoro 1.2	

Note) Δ – Amount of mercury per wet weight is obtained by amount of mercury per dry weight x $\frac{1}{2.5}$.

* Number in parenthesis indicates the number of fish examined, T/M%: methyl mercury/total mercury (%).

14

TABLE 5. COMPARISON OF MERCURY CONCENTRATION IN FISH

FROM VARIOUS DISTRICTS

Collection place	Name of fish or shellfish	Methyl mercury Hg ppm/wet wgt.	Total mercury Hg ppm/wet weight
(1) Inside the Minamata Bay	Kusabi	0.217	0.494
	Sea bream	0.186	0.465
	Kawahagi	0.062	0.112
	Sillago	0.312	1.015
	Sillago	0.374	0.915
	Bera	0.236	0.910
	Bera	0.416	0.636
	Swellfish	0.258	0.308
	Swellfish	0.173	0.219
	Tiger goby	0.251	0.579
	Short-necked clam	*	*
	(Awaji Island)	0.081	2.400
(2) Outside the Minamata Bay	Bera	0.402	0.510
	Bera	0.182	0.342
	Bera	0.255	0.472
	Sillago	0.440	0.528
	Swellfish	0.202	0.530
(2) Offshore the estuary of the Minamata River	Sillago	0.050	0.060
	Sillago	0.065	0.110
	Flathead	0.062	0.085
	Bera	0.060	0.210
	Swellfish	0.068	0.130
	Short-necked clam	*	*
	(Osaki)	0.067	0.970
	Scabbard fish	0.048	0.300
(2) Fishing ground for scabbard fish off Minamata	Scabbard fish	0.054	0.240
	Scabbard fish	0.022	0.110
	Scabbard fish	0.020	0.060
	Scabbard fish	0.024	0.064
(3) Goshoura	Hantai	0.009	0.027
	Yano	0.004	0.014
	Itsusaki	0.019	0.141
	Horse-mackerel	0.009	0.039
	Sea bream	0.011	0.040

TABLE 5 (CONTINUED

Collection place	Name of fish or shellfish	Methyl mercury Hg ppm/wet wgt.	Total mercury Hg ppm/wet weight
(3) Kuratake	Sea carp	0.008	0.074
	Takaba	0.006	0.023
	Itsusago	0.094	0.442
	Anaki	0.073	0.244
	Huehuki bream	0.007	0.075
(1) Ushihuka	Perch	0.049	0.231
	Sea bream	0.024	0.181
	Sole	0.008	0.041
	Gray mullet	0.003	0.007
	Itsusaki	0.015	0.140
	Kosho bream	0.016	0.100
	Urazurahagi	0.009	0.075
	Kawahagi	0.046	0.134
	Gray mullet	0.005	0.009
(3) Near Yabushiro	Gray mullet	0.003	0.005
	Gray mullet	0.004	0.008
	Perch	0.021	0.049
	Konoshiro	0.007	0.015
(1) Tomioka	Kuro	0.004	0.011
	Perch	0.056	0.223
	Chinu	0.013	0.031
	Turbot	0.006	0.039
	Konoshiro	0.008	0.037
	Mebaru	0.008	0.020
	Gray mullet	0.002	0.003
	Aigo	0.003	0.005
	Kaokaki bream	0.008	0.031
	Shark	0.015	0.041
(2) Offshore Shimoda	Kawahagi	0.050	0.058
	Kawahagi	0.020	0.155
	Horse mackerel	0.025	0.320
	Horse mackerel	0.018	0.080
	Kitsukori	0.080	0.198

TABLE 5 (CONTINUED)

Collection place	Name of fish or shellfish	Methyl mercury Hg ppm/wet wgt.	Total mercury Hg ppm/wet weight
(2) Offshore Yushima	Sea bream Sea bream Swellfish Bera	0.085 0.028 0.060 0.030	0.098 0.115 0.072 0.045
(2) Offshore the estuary of the Midor River	Garakabu Gray mullet Gray mullet Clam Short-necked clam Prawn	0.120 0.010 0.014 * 0.075 * 0.080 0.014	0.205 0.022 0.040 * 0.220 * 0.240 0.085
(2) Northern Sea of Hokkaido	Bream Bream Bream Bream Horse mackerel Horse mackerel Turbot Turbot Turbot Turbot	0.014 0.011 0.008 0.007 0.010 0.012 0.007 0.006 0.007 0.007	0.145 0.080 0.053 0.044 1.904 0.247 0.021 0.020 0.022 0.020
(4) Offshore the Port of Nagasaki	Scabbard fish Gray mullet Gray mullet Horse mackerel Black bream Turbot Short-necked clam	0.004 0.002 0.001 0.007 0.022 0.005 * 0.001	0.046 0.062 0.008 0.067 0.100 0.035 * 0.030

(1) Investigation of Environmental Contamination by Mercury, collected in November, 1971 (Analyzed by Fujiki): compiled by Japan Public Health Society.

(2) This investigation, collected in November, 1972.

(3) Fujiki, Tajima, Omori: Kumamoto Med. Journal, Vol. 46, 1972, p. 635 collected in November, 1971

(4) Fujiki, Tajima, Omori: Unpublished, collected in February, 1971.

*Hg ppm/dry weight

**Collected March 1971

5. Conclusion

In this investigation, mercury contamination in fish of every district did not exceed 1 ppm and was contained within the general concentration of the natural environment stated in the "Measures for Environmental Contamination by Mercury" issued by the Ministry of Public Welfare. Presently, there are no areas contaminated environmentally by mercury which may cause problems.

Relatively high concentrations of mercury in fish in some districts in the Ariake Sea have been observed, compared with other areas. The reason for the accumulated mercury in the fish is not related to the mercury flowing out from Minamata Bay, but is considered to be due to the mercury from other sources. Also, since there is no previous investigation ranging over the entire Ariake Sea, we cannot say whether this district was contaminated by mercury in the past or not . However, from the results of this investigation, a suspicion has arisen that there might have been many more mercury contaminated areas in the past. This will be an important subject in the further study of mercury contamination.

REFERENCES

1. Department of Medicine, Kumamoto University. Minamata Disease at the End of 10 Years Research. Epidemiological, Clinical-Medical and Pathological Study of Minamata Disease at the end of 10 Years, 1972, p. 41.

2. Fujiki, Motoshi, Shizuko Tajima and Akiko Omori. Surveys and Studies on Mercury Contamination in the Surrounding Areas of Minamata District and Its Transition. Kumamoto Medical Assn. Journal, Vol. 46, 1972, p. 635.

3. Irukayama, Katsuo, Motoshi Fujiki, Shizuko Tajima and Akiko Omori. Changes in Mercury Contamination Conditions in Fish, Shellfish and Sea Floor Sediments in the Minimata Region. Japan Public Health Journal, Vol. 19, 1972, p. 25.

4. Yamaguchi, S and H. Matsumoto. Ultramicrodetermination of Mercury
    in Biological Materials by Atomic Absorption Photometry. Industrial
    Medicine, Vol. 10, 1968, p. 125.

5. Japan Public Health Society. Biological Contamination Survey of Water
    Contaminated Areas; Guidelines on Metallic Analysis Method of
    Examining Animals and Plants (Report No. 1), 1970, p. 14.

6. Fujiki, M. The Transitional Condition of Minimata Bay and the Neigh-
    boring Sea Polluted by Factory Waste Water Containing Mercury.
    Proceedings of the Sixth International Water Pollution Research
    Conference, 1972, pp. C-6-12.

7. Fujiki, Motoshi, Shizuko Tajima and Akiko Omori. Unpublished.

8. Yamanaka, Sumie, Mie Suzuki, Takeshi Kondo and Kiichi Ueda. Mercury
    Level in Tuna and in Hair of Tuna Fishermen (Report No. 2). Japan
    Hygiene Journal, Vol. 27, 1972, p. 117.

9. Japan Public Health Society. Survey on Environmental Contamination
    by Mercury; Detailed Survey on Minimata Bay. 1973, p. 1.

10. Pollution Bureau, Kumamoto Prefecture. Report on Surveys and Studies
    Concerning Mercury Environmental Contamination of Minimata Coastal
    Area. 1972.

11. Western Sea Region Marine Research Center. Surveys and Studies of
    Marine Life Relative to Minamata Disease. 1964.

12. Kitamura, Seiji, Kyoji Ueda, Jitsuko Niino, Irei Ujioka, Hikoji Misumi
    and Toshiyuki Kakita. Findings on Chemical Toxins Relative to Mina-
    mata Disease (Report No. 5). Kumamoto Medical Association Journal,
    Vol. 34 (Supplement 3), 1960, p. 593.

13. Matsushima, Giichi. Survey of Mercury Level in Hair Relative to
    Minamata Disease. Kumamoto Prefectural Hygiene Research Center
    Report for 1970. 1972, p. 26.

14. Sakata, Akira, Takeo Orita, Muneyasu Koriyama, Kin Nakahara and Teiko
    Hozumi. Survey of Mercury Level in Hair Relative to Minamata
    Disease. Kanaito Prefectural Hygiene Research Center Report, No. 2.
    1962.

15. Kitamura, Seiji. Extremely Small Amount of Heavy Metals in Food.
    Food Hygiene Research, Vol. 21 (6), 1971, p. 19.

2. STUDIES ON TRANSFORMATION OF INORGANIC MERCURY FOUND IN MINAMATA BAY SEDIMENTS INTO METHYL MERCURY

Hygiene Seminar, Department of Medicine
Kumamoto University

Team member: Motoshi Fujiki
Research assistant: Shizuko Tajima

I. PREFACE

More than ten years have passed since the outbreak of Minamata disease in Minamata Bay and the surrounding areas. When one observes the transition of the mercury contamination situation [1, 2, 3, 4, 5] in the area of Minamata Bay during this period, it can be seen that there were no changes in the mercury level in sediments in Minamata Bay, but that there was a decrease of mercury in fish and shellfish. At present, almost none of the fish and shellfish contain mercury in excess of 1 ppm. As for mercury in the hair of Minamata residents, its level occasionally exceeded 100 ppm up until 1960 [1], but gradually declined to an average 9.23 ppm around 1968, 5.36 ppm in 1970, and is presently 3.75 ppm. Moreover, the acetaldehyde manufacturing facility of the Minamata plant of the Chisso Corporation modified its mercury utilizing process in June, 1966, to that of a complete circulation-type and suspended its manufacture in May, 1968. Further, at the vinyl chloride facility, a mercury removal device was installed in July, 1969, and the use of mercury was dropped by revising manufacturing methods in March, 1971. No mew mercury contamination has since been reported from that plant.

Jensen and his associates [6] claimed that anaerobes in sediments on the floor of lakes transformed inorganic mercury into methyl mercury, while Wood and his associates [7] held that methyl mercury was formed by the reaction of methyl cobalamine found in anaerobes with inorganic mercury. Tajima [8]

20

established the formation of methyl mercury through the reaction of acetaldehyde, acetic acid, and peracetic acid with inorganic mercury, as well as by directing ultraviolet rays on acetic acid and inorganic mercury. Ukita and his associates [9] formed methyl mercury through a reaction of inorganic mercury in the liver of tuna. Sotomura and his associates [10] reported that aerobes decomposed and transformed mercury compounds into metallic mercury, whereas anaerobes decomposed methyl mercury and transformed it into inorganic mercury, with a part of the anaerobes transforming inorganic mercury into methyl mercury. Kitamura and his associates [11] contended that both aerobes and anaerobes converted inorganic mercury into methyl mercury, followed by reconversion of methyl mercury into inorganic mercury. Matsumura and his associates [12] collected 35 kinds of microorganisms from sediments on the bottom of a lake and cultured them in a culture containing phenyl mercury acetate, but were unable to find any trace of methyl mercury. Tajima [8] administered inorganic mercury in mice in an attempt to learn whether there was transformation into methyl mercury in vivo, only to find that there was no such transformation. There are various reports, as can be seen above. Many researchers have confirmed, and it is widely accepted, that methyl mercury can be formed by the reaction of methyl cobalamine, found in anaerobes, with inorganic mercury and by the reaction of acetaldehyde and acetic acid with inorganic mercury. However, there still is disagreement as to the transformation of inorganic mercury into methyl mercury in microorganisms or in vivo.

As explained earlier, the contamination of the biological environment by mercury in the Minamata district has been alleviated to a great extent, although it is still incomparable with that of the non-contaminated areas. However, a large amount of inorganic mercury (thought to be primarily mercury sulfide) exists even now in sediments in Minamata Bay. Based on numerous reports on the formation of methyl mercury, it cannot be categorically stated that inorganic mercury contained in sediments in Minamata Bay will not naturally be transformed into methyl mercury. In our belief that further studies were warranted, we conducted the following experiment.

II. EXPERIMENTAL MATERIALS AND METHOD OF
ANALYSIS OF MERCURY

1. Experimental Results

1) Minamata Bay sediments

Minamata Bay sediments (containing 100 ppm of total mercury and 0.01 ppm of methyl mercury) were obtained from a point offshore the southern coast of Myojin-misaki in the northern sector of Minamata Bay (see Sketch).

2) Waste water

The following five types were used as waste water for our experiment:

A: 1.5% acetic acid solution;

B: City sewage (from Kumamoto city limits);

C: Waste water from sorbic acid plant of Minamata plant of Chisso Corporation;

D: Waste water from glyoxal plant of above plant;

E. Waste water from triacetate plant of same plant.

2. Method of Mercury Analysis

Analysis of the reactant and of methyl mercury in sediments was conducted as follows:

The liquid layer was adjusted to pH 1 by adding hydrochloric acid; following treatment with cuprous chloride, the methyl mercury was extracted with benzene, treated with glutathione, re-extracted with benzene and measured by the gas chromatograph method [13].

The sediments were adjusted to pH 1 by adding hydrochloric acid, with the liquid level at about 100 ml. Cuprous chloride was added and allowed to stand at room temperature for three days, after which it was suction-filtered, and methyl mercury contained in the filtrate was extracted by the usual method. Measurement was by gas chromatography [13].
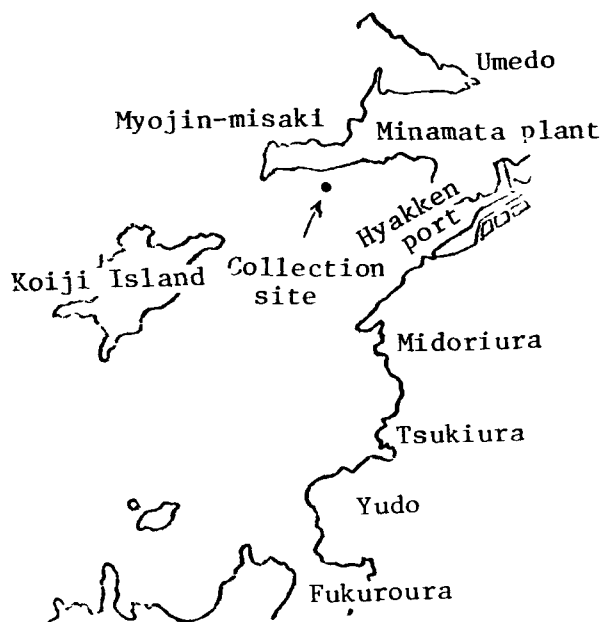

Figure 1. Site of collection of Minamata bay sediments

The total mercury in the sediments was measured by the dithizone colorimetric method by adding nitric acid to the sediments and letting it stand for three days, after which it was suction-filtered. An appropriate amount of filtrate was then taken, heated, and reduced with sulfuric acid and potassium permanganate.

III. EXPERIMENTAL METHOD AND FINDINGS

1. Reaction of Reagent with Inorganic Mercury, Acetic Acid, City Sewage, and Industrial Waste Water

Three mg of mercury (II) nitrate and 2 mg of mercury (II) oxide were added as reagent into each of the five 500 ml beakers containing waste water A - E. Further, 300 ml of 1.5% acetic acid solution was added to A, 300 ml of city sewage (from Kumamoto city limits) to B, 300 ml of waste water from the sorbic acid plant of Minamata plant to C, 300 ml of waste water from the glyoxal plant of the same plant to D, and 300 ml of waste water from the triacetate plant of the same plant to E. The beakers were then capped with a

film of saran wrap and left outdoors from October 24 to December 18, 1972, exposing them to sunlight for a total of 200 hours.

Table 1 reflects the results of measurements of methyl mercury formed in the reactant upon reaction. All of the reactions produced methyl mercury.

TABLE 1. METHYL MERCURY FORMED FROM MINAMATA BAY SEDIMENTS, INORGANIC MERCURY AND WASTE WATER (Hg μg)

Source of inorganic mercury / Type of waste water	A	B	C	D	E
3 mg of mercury (II) nitrate and 2 mg of mercury (II) oxide	0.90	0.36	4.14	2.34	0.27
50 g of Minamata Bay sediments (wet sediments, includes about 5 mg of total mercury and about 0.5 μg of methyl mercury)					
Liquid layer portion	0.00	0.00	0.00	0.00	0.00
Sediment portion	0.52	0.42	0.50	0.45	0.48
Minamata bay sediments to which 3 mg of mercury (II) nitrate and 2 mg of mercury (II) oxide have been added					
Liquid layer portion	–	–	0.00	0.00	0.00
Sediment portion	–	–	0.52	0.51	0.49

2. Reaction of Minamata Bay Sediments with Acetic Acid, City Sewage, and Industrial Waste Water

As in experiment 1, above, 50 g of Minamata bay sediments (including about 5 mg of total mercury and 0.5 μg of methyl mercury) were put into each of the five 500 ml beakers containing waste waters A - E. Then, 300 ml of 1.5% acetic acid solution was added to A, 300 ml of city sewage (from Kumamoto city limits) to B, 300 ml of waste water from the sorbic acid plant of Minamata plant to C, 300 ml of waste water from the glyoxal plant of the

above plant to D, and 300 ml of waste water from the triacetate plant of the same plant to E. The beakers were then exposed to sunlight for 200 hours.

Following the reaction, they were suction-filtered, and methyl mercury in the liquid layer and sediments was measured, with results as shown in Table 1. No methyl mercury was detected in any of the liquid layers. In the sediments, methyl mercury corresponding to a level of methyl mercury existing prior to the reaction was noted, but no new methyl mercury was found.

3. Reaction of Minamata Bay Sediments, in Which the Reagents Mercury (II) Nitrate and Mercury (II) Oxide were Added, with Industrial Waste Water

Into each of the three 500 ml beakers containing waste waters C - E was added 50 g of Minamata Bay sediments (including about 5 mg of total mercury and 0.5 μg of methyl mercury), 3 mg of mercury (II) nitrate and 2 mg of mercury (II) oxide as reagents. Then 300 ml of waste water from the sorbic acid plant of Minamata plant was added to C, 300 ml of waste water from the glyoxal plant of the same plant was added to D, and 300 ml of waste water from the triacetate plant of the same plant was added to E. And, as in the experiment 1, above, they were exposed to sunlight for 200 hours.

Following the reaction, they were suction-filtered, and the methyl mercury in the liquid layer and sediments was measured, with the results shown in Table 1. No methyl mercury was found in any of the liquid layers, and no newly formed methyl mercury was found in the sediments.

4. Effects of Aeration of Inorganic Mercury Source on Methyl Mercury Formation

Fifty grams of Minamata Bay sediments from which water had been removed by suction and 300 ml of deionized water were put into each of the three 500 ml triangular flasks and mixed well. One of these was immediately suction-filtered, and methyl mercury in the liquid layer and sediments was measured.

The remaining two were aerated for 40 hours at a permeability of 150 ml per minute. Following aeration, one of the two was immediately suction-filtered and methyl mercury in the liquid layer and sediments was measured. The last flask was added with acetic acid to make a 1.5% solution, its contents transferred to a 500 ml beaker, and exposed to sunlight for 200 hours, as in experiment 1, above. Following the reaction, it was suction-filtered, and methyl mercury in the liquid layer and sediments was measured.

To the 500 ml triangular flask was added 5 mg of the reagent mercury sulfide and 300 ml of artificial sewage [14] seeded with 2 ml of city sewage, the contents being aerated for 40 hours at a permeability of 150 ml per minute. Following aeration, acetic acid was added to make a 1.5% solution, the contents were transferred to a 500 ml beaker, and exposed to sunlight for 200 hours, as in experiment 1, above. After the reaction, it was filtered and measured for methyl mercury.

The above results are shown in Table 2. Methyl mercury in Minamata Bay sediments increased slightly as a result of aeration. Also, when the reaction was effected with 1.5% solution of acetic acid under exposure to sunlight following aeration, methyl mercury was formed, being detected in the liquid layer and in the sediments.

When reaction was effected with 1.5% solution of acetic acid under exposure to sunlight following aeration with the reagent mercury sulfide, methyl mercury was found in the reactant.

5. Adhesiveness of Methyl Mercury in Minamata
   Bay Sediments

To each of the three separating funnels were added 50 g of Minamata Bay sediments from which had been removed by suction 300 ml of deionized water, and 0 μg, 1 μg, and 5 μg of methyl mercury, respectively. After they were shaken and mixed for 3 hours, they were suction-filtered and methyl mercury in their liquid layer and in sediments was measured.

TABLE 2. EFFECTS OF AERATION OF SOURCE OF INORGANIC MERCURY UPON
METHYL MERCURY FORMATION (Hg μg)

Source of inorganic mercury		Pre-aeration	Post-aeration*	Reactant after aeration with 1.5% solution of acetic acid
50 g Minamata Bay sediments (water removed by suction)	Liquid layer portion	0.00	0.00	0.24
	Sediment portion	0.80	1.05	3.20
5 mg mercury sulfide	Liquid layer portion	–	–	1.43

*
Conditions of aeration: 150 ml/min of air
over 40 hours.

TABLE 3. ADHESIVENESS OF METHYL MERCURY IN MINAMATA BAY SEDIMENTS
(Hg- μg)

Amount of methyl mercury added (Hg- μg)	0	1.0	5.0
300 ml of deionized water to which methyl mercury was added was shaken with 50 g Minamata Bay sediments (wet sediments)			
Liquid layer portion	0.00	0.00	0.00
Sediment portion	0.44	1.41	5.64

The results are shown in Table 3. Methyl mercury, in 1 μg and 5 μg
levels, was found only in the sediments, and not in the liquid layers.

6. Effects of Exposure Time to Sunlight on Methyl
Mercury Level

The standard aqueous solution of methyl mercury — 100 ml of 0.05 ppm,
0.5 ppm, and 2.5 ppm — was poured into 200 ml beakers and exposed to sunlight
for 200 hours, as in experiment 1, in order to study the effects of exposure

time on the level of methyl mercury. As shown in Table 4, the level of methyl mercury declined as time progressed.

TABLE 4. EFFECTS OF EXPOSURE TIME TO SUNLIGHT ON METHYL MERCURY LEVEL (WITH 100 AS THE METHYL MERCURY LEVEL PRIOR TO SUNLIGHT EXPOSURE)

Sunlight exposure time / Mercury density	0 hrs	150 hrs	200 hrs
0.05ppm, 100ml	100	65	4
0.5 ppm, 100ml	100	89	79
2.5 ppm, 100ml	100	89	81

IV. COMMENTS

It is widely known [15] that various inorganic mercury compounds other than mercury sulfide form methyl mercury [8] as a result of reaction with compounds having active methyl groups, such as acetaldehyde, acetic acid, and peracetic acid. However, no experiment has been conducted so far to determine whether this type of reaction occurs in nature. As the first step in our experiment to ascertain whether methyl mercury is formed in nature, the authors initially studied whether methyl mercury can be formed as a result of reaction between industrial waste water and inorganic mercury [16]. We learned as a result that a methyl mercury yield of from 0.6% to 2.4% (yield relative to the level of mercury used) can be obtained as a result of reaction of 300 mg mercury (II) nitrate and 200 mg mercury (II) oxide with waste water from a sorbic acid plant, from a glyoxal plant, and from a tri-acetate plant, and that methyl mercury is not formed when these waste waters were treated with a cyclator or when the source of mercury was mercury sulfide. As the amount of mercury used in this experiment was relatively large, we could not consider the situation to be identical in nature. For this reason in our latest experiments, we utilized a level of reagents — inorganic mercury and Minamata Bay sediments — equivalent to the density of

inorganic mercury in Minamata Bay sediments, in order to ascertain whether methyl mercury was formed in nature.

The reaction of the reagent inorganic mercury containing a level of mercury identical to that in Minamata Bay sediments with industrial waste water resulted in a low methyl mercury yield (relative to the amount of mercury used) of from 0.005 to 0.08%, as compared to earlier experiments. The reason for the low yield is believed to be due to the loss of newly formed methyl mercury because of inclement weather during the experiment period, requiring about 40 days to acquire 200 hours of exposure to the Sun's rays. In the sunlight exposure experiment with a standard aqueous solution of methyl mercury, a distinct decrease of methyl mercury was noted after 200 hours. Thus, it is possible that in our latest experiment the reactionconditions for formation of methyl mercury were unsuitable.

Through the reaction of inorganic mercury with city sewage, a 0.07% yield of methyl mercury was obtained. This was significant, in that it was an indication of the dangers of methyl mercury formation, not only in industrial waste water, but in nature as well.

In the Minamata Bay sediments, about 5 mg of mercury was found in the form of total mercury. No methyl mercury was formed as a result of a reaction between Minamata Bay sediments and acetic acid, city sewage, and industrial waste water. Although methyl mercury was formed when the source of mercury was the reagents mercury (II) nitrate and mercury (II) oxide, it was not formed when the source of mercury was inorganic mercury in the sediments. The reason probably is the abundance of sulfides in Minamata Bay sediments [17], with inorganic mercury in the sediments assuming reactive inactivity in the form of mercury sulfide. The fact that methyl mercury was not formed when mercury (II) nitrate and mercury (II) oxide were newly added as reagents to Minimata Bay sediments to effect a reaction with acetic acid, city sewage, and industrial waste water probably indicates that sulfides in the sediments made the newly added reagents of mercury (II) nitrate and mercury (II) oxide inactive.

As indicated by the results of the foregoing experiments, methyl mercury will not form in Minamata Bay under existing conditions, despite the presence of inorganic mercury in the sediments and inflow of acetic acid, city sewage, and industrial waste water. Even if there were an additional inflow of inorganic mercury in an active form as mercury (II) nitrate and mercury (II) oxide, it would change into an inactive form of inorganic mercury in the sediments, provided the level of inorganic mercury was then equal to or less than that in the sediments, without any possibility of methyl mercury being formed in nature. However, methyl mercury could be formed under ideal conditions, such as aeration of Minamata Bay sediments. As indicated in experiment 4, should Minamata Bay sediments be aerated, inorganic mercury found in the sediments will probably change into an active form with respect to the formation of methyl mercury. As explained earlier, mercury sulfide is a stable form and will not form methyl mercury in reaction with acetic acid and industrial waste water [16]. However, Westöö [18] theorized, while lacking any experimental evidence, that mercury sulfide was oxidized in nature by aerobes and changed into mercury (II) sulfate, active in the formation of methyl mercury. In order to verify this theory, the authors applied artificial sewage seeded with aerobe-containing city sewage, aerated the reagent mercury sulfide and, following aeration, effected a reaction with acetic acid in an attempt to ascertain whether methyl mercury would form. It was found that a greater amount of methyl mercury was formed than when mercury (II) nitrate and mercury (II) oxide were used.

Following aeration of Minamata sediments and reaction with acetic acid under sunlight exposure, methyl mercury thus formed was greater in the liquid layer than in the sediments. This was an indication of the adhesive nature of the sediments toward methyl mercury. In an experiment to determine the adhesive property of the sediments on methyl mercury, 5 μg of methyl mercury was completely adhered to 50 g of sediments from which water had been removed by suction. Perhaps methyl mercury is not eluted, being bonded with a SH group of humus in the sediments and surviving therein. The appearance of a trace of methyl mercury in the liquid layer when methyl mercury was formed

as a result of reaction following aeration of sediments was probably due to a decrease of adhesivity of methyl mercury toward sediments, because of partial decomposition of humus in the sediments under aerobic conditions.

The possibility of new dangers posed by the elution of methyl mercury in sea water caused by the disturbance of Minamata Bay sediments containing very small amounts of methyl mercury or by ingestion of methyl mercury containing sedimentary particles by fish and shellfish has been pointed out. However, it was found that, notwithstanding disturbance of Minamata Bay sediments and elution of some methyl mercury, the latter tended to adhere to the sediments. In 1959, when Minamata Bay was considered to be at peak contamination [19], Kitamura and his associates [1] conducted a breeding experiment using Minamata Bay sediments on lubworms, short-necked clams and small fishes, and measured the mercury level in their bodies. Despite the fact that the sediments during the breeding experiment were sufficiently disturbed, the deposit of mercury in their bodies was very small. Such a deposit was negligible when compared to the time when the Kumamoto Prefectural Marine Experimentation Station, in an oyster breeding experiment in Minamata Bay, found that oysters from a control district which were suspended in waters of Minamata Bay had as high a mercury density (50 ppm) as local fish and shellfish. Based on such findings, it is thought that the high level of methyl mercury deposit in fish and shell- fish is largely due to methyl mercury existing in sea water, and that methyl mercury adhering to sediments hardly affects them. Thus, even if sediments in Minamata Bay were to be disturbed by dredging, safety can probably be assured if aerobic conditions were avoided.

V.   CONCLUSION

It has become clear that inorganic mercury, if present, will react with city sewage and industrial water containing compounds with active methyl groups, and form methyl mercury in nature. Methyl mercury will not be formed in this instance if the mercury is in the form of mercury sulfide. However, under aerobic conditions, mercury sulfide changes into inorganic mercury in this reaction, and forms methyl mercury.

Even now, sediments in Minamata Bay contain a large quantity of inorganic mercury with the potential of forming methyl mercury. However, those inorganic mercury compounds were of the inactive type, and did not form methyl mercury during our experiment.

REFERENCES

1. Kitamura. Seiji, Kyoji Ueda, Jitsuko Niino, Irei Ujioka, Hikoji Misumi and Toshiyuki Kakita. Findings on Chemical Toxins Relative to Minamata Disease (Report No. 5). Kumamoto Medical Association Journal, Vol. 34 (Supplement 3), 1960, p. 593.

2. Irukayama, Katsuo, Fumio Dai, Motoshi Fujiki, Takako Kondo, Shizuko Tajima, Tsutomu Den, Hiroyuki Sasa, Masanori Hashiguchi and Sadao Ushigusa. Mercury in Minamata Bay Sediments. Japan Public Health Journal, Vol. 11 (9), 1964, p. 645.

3. Irukayama, Katsuo, Motoshi Fujiki, Shizuko Tajima, Akiko Omori, Hiroshi Nakamura and Shunsuke Kuwahara. Mercury Contamination at the Time of Suspension of Acetaldehyde Production at the Minimata Plant. Kumamoto Medical Association Journal, Vol. 43 (11), 1969, p. 946.

4. Irukayama, Katsuo, Motoshi Fujiki, Shizuko Tajima and Akiko Omori. Transition of Mercury Contamination Conditions of Fish, Shellfish, and Sea Floor Sediments of Minamata Region. Japan Public Health Journal, Vol. 19 (1), 1972, p. 25.

5. Fujiki, M. The Transitional Conditions of Minamata Bay and the Neighboring Sea Polluted by Factory Waste Water Containing Mercury. Proceedings of the Sixth International Water Pollution Research Conference, 1972, p. C-6-12.

6. Jensen, S. and A. Jernelöv. Biosynthesis of Mono- and Dimethyl Mercury. Nordforsk Biocidinformation, No. 10. 1967, p. 4.

7. Wood, M. J., F. Scott Kenedy and C. F. Rosen. Synthesis of Methyl Mercury Compounds by Extracts of a Methanogenic Bacterium. Nature, Vol. 220, 1968, p. 178.

8. Tajima, Shizuko. Studies on the Formation of Methyl Mercury Compounds. Kumamoto Medical Association Journal, Vol. 44 (10), 1970, p. 873.

9. Imura, Nobumasa, Hidemitsu Bo and Tadanoshin Ukita. Studies on Organic Transformation of Inorganic Mercury; II. Reaction Transforming Inorganic Mercury into Methyl During Tests in Animals. Collection of Addresses before the 92nd Year Meeting of Japan Pharmacological Society, Supplement No. 3., 1972, p. 196.

10. Tonomura, K., T. Nakagami, F. Futai and K. Maeda. Study on the Action of Mercury-Resistant Microorganisms on Mercurials (1). The Isolation of Mercury-Resistant Bacterium and the Binding of Mercurials to the Cells. J. Ferment. Technol., Vol. 46, 1968, p. 506.

    Yamada, M. and K. Tonomura. Formation of Methylmercury Compounds from Inorganic Mercury by Clostridium cochlearium. J. Ferment. Technol., Vol. 50, 1972, p. 159.

11. Kitamura, Masaji, Kimiaki Sano, and Masahiko Hirayoshi. Synthesis and Decomposition of Organic Mercury Compounds by Micro-Organisms. Japan Hygiene Journal, Vol. 24, 1969, p. 76.

12. Matsumura, F.,Y. Gotoh, and G. M. Boush. Phenylmercuric Acetate: Metabolic Conversion by Microorganisms. Science, Vol 173, 1971, p. 49.

13. Fujiki, Motoshi, Shizuko Tajima and Akiko Omori. Surveys and Studies on Mercury Contamination in the Surrounding Area of Minamata District. Kumamoto Medical Association Journal, Vol. 46 (11), 1972, p. 635.

14. McKinney, R. E. et al. Biocatalysts and Waste Disposal. II. Effect on Activated Sludge. Sew. and Ind. Wastes, Vol. 25 (11), 1953, p. 1268.

15. Imura, Nobumasa. Changes in Mercury Compounds in Nature. Hygiene Chemical, Vol. 18 (5), 1972, p. 291.

16. Pollution Bureau, Kumamoto Prefecture. Report on Surveys and Studies Concerning Mercury Environmental Contamination of Minamata Coastal Area. 1972.

17. Motosato, Yoshiaki. Report on Countermeasures Against Sludge off Yatsushiro and in Mizunashi River.

18. Westloo, G. Personal communication, 1972.

19. Fujiki, Motoshi, Shizuko Tajima, and Akiko Omori. Transition of Contamination by Mercury in the Minamata Region. Japan Hygiene Journal, Vol. 27 (1), 1972, p. 115.

3. PROGRESS ON MINAMATA DISEASE AND ITS EPIDEMIOLOGICAL STUDY (Part 2)

Chair of Public Hygiene, Medical Department, Kumamoto University

Staff: Shigeru Nomura
Asst. Staff: Toshio Matsushita, Shin Nizuka, Noriki Arimatsu,
                Atsushi Ueda, Junichi Misumi, Toshie Tomio,
                Hiroyuki Teruya, Tadako Ueda

We already reported the results of a pilot study in the previous report
in regard to the environmental contamination due to organic mercury in
Minamata Bay, including its adjacent areas and along the Shiranui Sea, and
also in regard to the health damage to inhabitants in the seacoast area. In
the study, we conducted the health examination for the inhabitants of Tsukiura,
Idetsuki and Yudo of Minamata City, areas in which multi-breakouts of the disease
occurred and for the inhabitants of Goshoura-machi, Amakusagun, which are
areas susceptible to contamination.

In this report, first we investigated several health indicators that show
how the dynamic populations of these objective districts have been changing
for years due to the health damage to its inhabitants caused by the organic
mercury contamination along the coast area. Second, we investigated the way
that the inhabitants were examined in districts and the significance of the
examinations. Third, we investigated the actual conditions of the fisherman's
work and their dietary habits on the Shiranui Sea coast which area is extremely
susceptible to direct contamination by organic mercury from the sea. This is
why the Minamata Disease has broken out so frequently there. We also tried to
trace down the actual conditions of the Minamata disease in our investigations,
the results of which are described below.

1. Influence of the Environmental Contamination as Viewed from the
    Local Health Index

It is assumed that the environmental contamination due to organic mercury, etc. has caused a number of Minamata disease cases, and has clearly affected the health of the inhabitants who live in the contaminated districts; therefore, the health standard of the districts has declined. To clarify this decline, last year we objectively interviewed families in the contaminated districts. We checked the average age and causes of death of anyone in the interviewed families who died between 1945 and 1971. The results showed that the mercury contamination directly influenced the age and cause of death [1].

There are various health indicators to measure a health standard of a district; however, an expert group of WHO particularly adopts the PMI (proportional mortality indicator) [2], rough mortality rate and average remainder of life based on the mortality statistics as the health indices. These are considered to be reliable sources to use for the general health indices [3]. Furthermore, there is an index that gives average death rates [4, 5] which displays the same characteristics as the indices described above, and its applicability is being investigated [6].

Therefore, by using these indices, we investigated the influence of organic mercury, etc. on the health standard of the contaminated districts, and also tried to analyze its influence on the cause of death.

I. OBJECTIVE METHOD OF INVESTIGATION

We selected the districts of Tsukiura and Fukuro of Minamata City which are heavily contaminated districts, and the district of Arashiguchi in Goshouramachi which is on the opposite seashore of Minamata City as a susceptible district of contamination for our objective investigation. We compared them with the results of the Akazaki, Suko and Ohura districts of Ariakemachi that faces the Shimabara Bay, which were considered districts

free of the contamination. The characteristics of these districts were described in a previous report. The investigation dealt with the people who had been living in these districts during the period from January 1, 1948 to December 31, 1971. As an investigation reference, we used the death certificates of the inhabitants in the said districts obtained from each respective town office for this 24-year period, and the official items were copied and summarized for analysis.

The total number of deaths in the districts during this period were as follows: male 468, female 374 in Minamata district, male 330, female 280 in Goshoura district; and male 620, female 682 in Ariake district (these figures excluded deaths due to war).

In addition, in order to obtain the mortality rate by age groups, the corrected PMI and the corrected average death age for the Kumamoto prefecture in 1960, we standardized the age figures of the dynamic population statistics by placing them in categories of 5-year differences. Furthermore, the classification of the cause of death is based on a "50-Item Classification of Death Causes (Table B)" of the "International Classification".

II. INVESTIGATION RESULTS

1. Index of Mortality Statistics

The actual condition of mortality in the districts investigated during the 24 years from 1948 to 1971 was examined according to the indices of mortality rate by age group and PMI.

(1) Mortality rate by age group

The mortality rate by age group in each district is obtained by calculating the total deaths during the 24 years as indicated in Figure 1. In every district, the mortality rate of school children, young and middle-aged is comparatively low, and high in infants and the old, which is a

34

tendency that is present throughout the country. However, when comparing 3
districts, there is some difference in the mortality rate by age group. Namely,
the mortality rate in the Minamata district is slightly higher for infants
and for those up to 59 years and less in the old as compared to the Ariake
district. This tendency is quite apparent in females. On the other hand,
the Goshoura district is very similar to the Ariake district except that the
mortality rate of baby girls and those in the age bracket of 45 to 49 is
slightly higher than in the Ariake district. When these are standardized
by the age grouping according to the dynamic population statistics of the
Kumamoto Prefecture in 1960, some different tendencies are observed. That is,
the mortality rate in the Minamata district is slightly lower than in the
Ariake district in infants and the young of both sexes and higher in
school children.   It is slightly higher in the middle and old age brackets,
which indicates that both districts are similar. Furthermore, the Goshoura
district exhibits no particular differences in males but the rate is higher in
school children, and lower in the old, as far as females are concerned.

(2) Mortality of infants and stillbirths

The death rate in infants is often used as the index which sharply
reflects a local health standard. Using that index, we analyzed those dis-
tricts as indicated in Table 1. The death rate in infants for the 24 year
period is as follows: 26.3 in the Minamata district, 36.1 in the Goshoura
district and 32.8 in the Ariake district. When we check the yearly changes
for the 5-year periods, the death rate in the Ariake district markedly
declines year after year. In the Minamata district, it gradually declines
except it slightly increases from 1958 to 1962. On the other hand, in the
Goshoura District, the decline in the number of births does not parallel
the decline in the number of deaths, that is, the death rate in infants tends
to increase. The results of the existing statistics of the districts are
shown in Table 2. The death rate in infants in Minamata City and Ariakemachi
gradually declines, whereas the decline is slow in the Goshoura district,
which substantiates the results of the districts.

Although a record of stillbirths is not dependable because each one has not always been reported, we checked the stillbirth rate in the districts from the existing references as shown in Table 2. The stillbirth rate in Minamata City is slightly higher than in Goshouramachi and Ariakemachi. However, we must take into consideration the discrepancy in notification of stillbirths between the city and the remote village.

(3) PMI and death rate of the old

Good health can be maintained by eliminating the damaging factors and a long life can thus be promised. Therefore, as the index to measure the local health and the sanitation standards, the PMI and death rate of the old are used frequently. With these standards, we investigated the mortalities in the districts and indicated the results in Table 3. If we summed up the total deaths in 24 years for each district, the PMI, as the index of population structure, is 62.4% for males and 64.5% for females in the Minamata district, which percentages are significantly lower than the statistics of 70.3% for males and 76.0% for females in the Ariake district. On the contrary, in the Goshoura district, the PMI is significantly lower than in the Ariake district only for females.

The death rate for the over 65-year bracket, is the same as the PMI, i.e., 41.9% for males and 46.1% for females in the Minamata district, whereas the PMI is 51.9% and 62.3%, respectively, in the Ariake district. The death rate for the old in the Minamata district is significantly (statistically) lower than in the Ariake district for both sexes, which indicates a low health standard. On the other hand, no noticeable difference is observed in the Goshoura district.

In order to obtain the corrected PMI, we standardized the ages based on the dynamic population statistics of the Kumamoto Prefecture in 1960. As you can see in Table 4, all the districts show that the corrected PMI for 5-year-periods gradually increased for both sexes, which complies with a yearly change. When we compared these between two districts, the value in
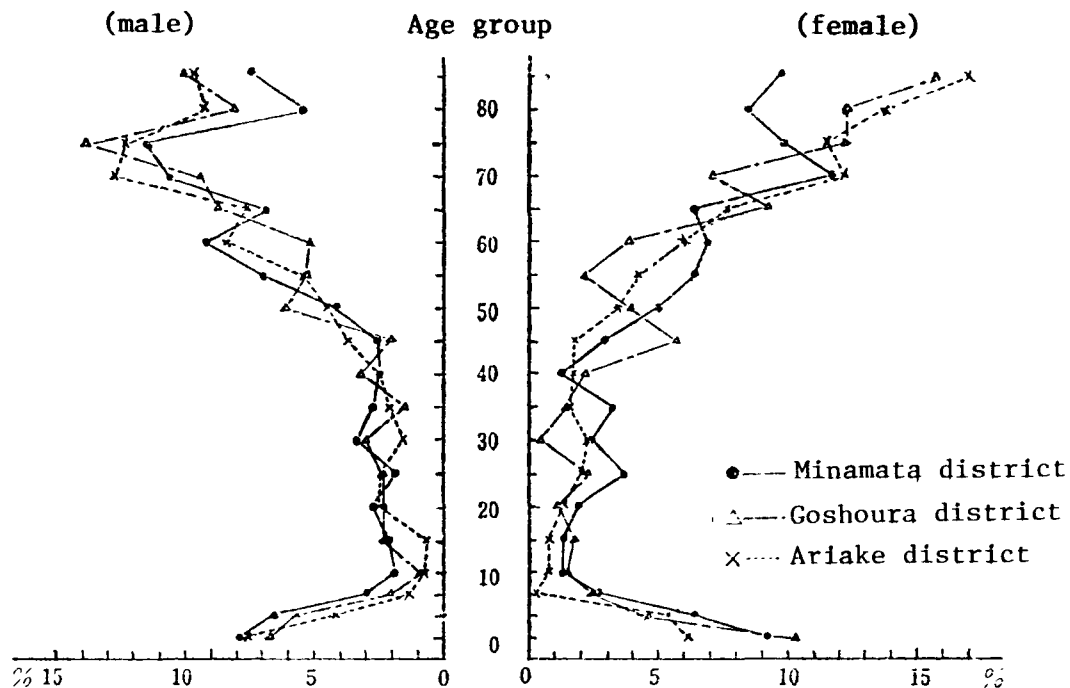
36

Figure 1. Mortality frequency classified by age group.

TABLE 1. LAPSE OF INFANT DEATH RATE

Years	Minamata			Goshoura			Ariake		
	No. births	No.inf. deaths	Inf. dth rate	No. bths.	No. inf. deaths	Inf. dth. rate	No. bths.	No.inf. deaths	Infant death rate
1948-1952	770	20	26.8	518	13	25.1	660	31	47.2
1953-1957	605	14	23.1	356	13	46.5	512	35	33.1
1958-1962	166	19	40.5	258	9	34.9	780	18	22.9
1963-1967	420	9	21.1	153	8	52.3	791	3	5.1
1968-1971	300	6	19.4	126	5	63.5	105	0	0.0
1948-1971	2832	68	24.3	1411	51	36.1	2748	91	32.5

Note: 1) The number of births was obtained by the birth certificates
(Family register) of inhabitants in the districts.

2) Infant death rate per 1000 births.

37

the Minamata district is generally lower than in the Ariake district, which is quite apparent in females. Furthermore, the PMI value for females in the Goshoura district is slightly higher than in the Minamata district. In particular, we can say that local factors are recognized which make health standards for females in the Minamata district inferior.

(4) Average age at death

We calculated the average death age by each 5 year period in regard to the total deaths in 24 years for each district. As indicated in Table 5, the average death age of the total deaths in 24 years is, 50.36 ± 28.91 years for males and 52.50 ± 29.49 years for females in the Minamata district, which is apparently lower than in the Ariake district, i.e., 56.56 ± 27.74 years for males and 61.22 ± 28.06 years for females. This tendency is also observed in the yearly change for each 5-year-period. On the other hand, the total deaths in 24 years in the Goshoura district are significantly lower than in the Ariake district for females and when the yearly changes are checked each 5-year period, the death average for both sexes between 1963 - 1967 are lower in the Ariake district. From the above figures, we calculated the corrected average death age by standardizing the ages according to the population structure of Kumamoto Prefecture in 1960 as shown in Table 6. When observing the yearly changes for each 5-year-period, the death ages in both sexes for the respective districts are notably prolonged in proportion to the passage of time. In addition, if the districts as a group are compared, no noticeable differences are observed for males whereas the life expectancy for females in both the Minamata and Goshoura districts are lower than in the Ariake district.

2. Causes of Death

As regarding the cause of death written on a medical death certificate, its preciseness has a certain limit; therefore, its dependability differs according to every disease. Considering this factor, we investigated the causes of death of the inhabitants in the Minamata district (which is a contaminated district) and in the Goshoura district (which is a district

susceptible to contamination) and checked if any epidemiological factors could be observed. In this case, as the population is small, we analyzed the causes of all the deaths for the 24 year period from 1948 to 1971.

(1) Death rate classified by causes of death

The total deaths for the 24 years in each district were divided into 5 groups, classified according to sex and causes of death, i.e., Group A (bacterial infection), Group B (adult disease), Group C (diseases of pregnant women and infants), Group D (non-disease) and Group E (others), and investigated their characteristics in each district. The results are shown in Figure 2. When the Minamata district is compared with the Ariake district, deaths occur less from adult diseases and more from non-diseases (P < 0.05) in males, and deaths occur more (P < 0.05) from bacterial infections and less (P < 0.01) from adult diseases in females. In the Goshoura district, deaths occur less from adult diseases and more (P < 0.01) from non-diseases in females. It is noted that the deaths from adult diseases in the Minamata district are comparatively lower in both sexes.

(2) Comparison of main causes of death between the districts

The total deaths in 24 years between the districts according to the "50-Item Classification of Causes of Death (Table B)" are shown in Table 7. When we compared males in the Minamata district with males in the Ariake district, deaths by inflammation of the intestines and other laxative diseases, car accidents and other unexpected accidents were apparently higher, and deaths from senility (not accompanied by mental disease), non-inflammatory diseases of the nerve center system were lower. Most of the deaths from other unexpected accidents were caused by drowning in children and adults. When we consider females, deaths from tuberculosis, meningitis and other unexpected accidents (mostly by drowning) are higher, and deaths from cardiac diseases and senility (not accompanied by mental disease) are lower. In other words, for both sexes in the Minamata district, it is very rare for anyone to complete the natural span of life and many deaths were attributed to inflammatory diseases and accidents.

39

TABLE 2. HEALTH STATISTICS IN THE DISTRICTS

Years	Districts	Population	Birth rate (per population of 1,000)	Death rate (per pop. of 1,000)	Infant death rate (per birth of 1,000)	Stillbirth rate (per birth of 1000)
1960	Minamata City	48,312	17.1	7.0	29.0	90.5
	Goshoura-mura	8,551	23.9	10.3	39.2	53.9
	Ariake-machi	10,925	17.7	12.1	51.5	82.9
	Kumamoto Pref.	1,856,192	17.9	8.6	35.4	97.6
1965	Minamata City	45,577	15.6	7.3	19.7	90.9
	Goshoura-machi	7,653	20.1	11.8	32.5	77.8
	Ariake-machi	9,864	14.7	12.4	20.7	52.3
	Kumamoto Pref.	1,770,736	16.9	8.6	23.2	87.1
1975	Minamata City	38,109	15.0	8.6	7.0	69.9
	Goshoura-machi	6,549	15.0	11.8	30.6	48.5
	Ariake-machi	8,171	10.4	13.2	11.8	65.9
	Kumamoto Pref.	1,700,229	14.8	8.8	16.2	76.3

Note: 1) Before becoming a town (Organized as a town in 1963).
2) Sources: Annual Hygienic Report, Kumamoto Prefecture

40

TABLE 3. PMI AND DEATH RATE OF THE OLD

	Male			Male		- Female
	Minamata	Goshoura	Ariake	Minamata	Goshoura	Ariake
PMI[1)] (%)	62.4**	67.3	70.3	64.5**	66.4**	76.0
Death rate (%) of those over 65 years	41.9**	50.6	51.9	46.1**	56.4$^\Delta$	62.3
Total number of deaths	468	330	620	374	280	682

Note: 1) PMI: Proportional Mortality Indicator

(Death rate of those over 50 years in total deaths)

2) $^\Delta$ P < 0.1 *P < 0.05 **P < 0.01

Indicated the significant differences against the values of the Ariake district according to the x^2-examination.

TABLE 4. COMPARISONS OF CORRECTED PMI BETWEEN THE DISTRICTS (Unit:%)

Years	Male			Female		
	Minamata	Goshoura	Ariake	Minamata	Goshoura	Ariake
1948-1952						
1953-1957						
1958-1962						
1963-1967						
1968-1971						

Note: Age correction was made by using the dynamic population statistics of the Kumamoto Prefecture in 1960.

TABLE 5. YEARLY CHANGE OF AVERAGE DEATH AGE   (M ± σ, Units: Years)

Years	Minamata		Goshoura		Ariake	
	Male	Female	Male	Female	Male	Female
1948-1952	37.32±30.45* (97)	41.82±31.43⁴ (80)	45.37±33.22 (70)	43.78±31.35 (60)	46.71±30.94 (151)	50.03±31.60 (189)
1953-1957	45.73±29.30 (99)	49.64±30.13 (72)	55.97±29.02⁴ (59)	52.10±31.93 (38)	51.42±28.35 (177)	56.27±31.25 (162)
1958-1962	49.32±28.80⁴ (95)	52.22±28.84** (90)	53.41±25.71 (70)	60.93±29.03 (61)	56.25±26.76 (126)	66.82±23.41 (158)
1963-1967	62.32±23.12* (82)	57.10±27.52** (77)	58.35±26.24** (78)	60.22±30.33** (46)	69.02±18.16 (89)	71.67±19.24 (100)
1968-1971	59.26±24.40** (95)	65.51±21.60* (55)	57.45±26.76** (53)	65.67±26.52⁴ (55)	73.93±9.43 (67)	72.63±16.43 (73)
Totals	50.36±28.91** (468)	52.59±29.49** (374)	54.51±28.80 (330)	56.30±30.92* (260)	56.56±27.71 (620)	61.22±25.06 (682)

Note: 1) The numbers in brackets indicate the number of people.

2) $^\Delta$p < 0.1   *P < 0.05   ** P < 0.01

(These are the significant differences against the values of the Ariake district according to t-examination)

42

TABLE 6.   CORRECTED AVERAGE DEATH AGE   (Unit: Years)

	Male	Female		Male	Female
Years	Minamata	Goshoura	Ariake	Minamata	Goshoura	Ariake
1948-1952						
1953-1957						
1958-1962						
1963-1967						
1968-1971						

Note: Corrected ages by each 5 year group according to the dynamic
population statistics of the Kumamoto Prefecture in 1960.

(1948-1971)

Note: 1) Group A (bacterial infection)   Group B (adult disease)   Group C (diseases of pregnant women and infants)

Group D (non-disease)   Group E (others)

2) *P < 0.05   **P < 0.01 (indicated the significant
differences against the values of the Ariake district
according to x^2 examination)
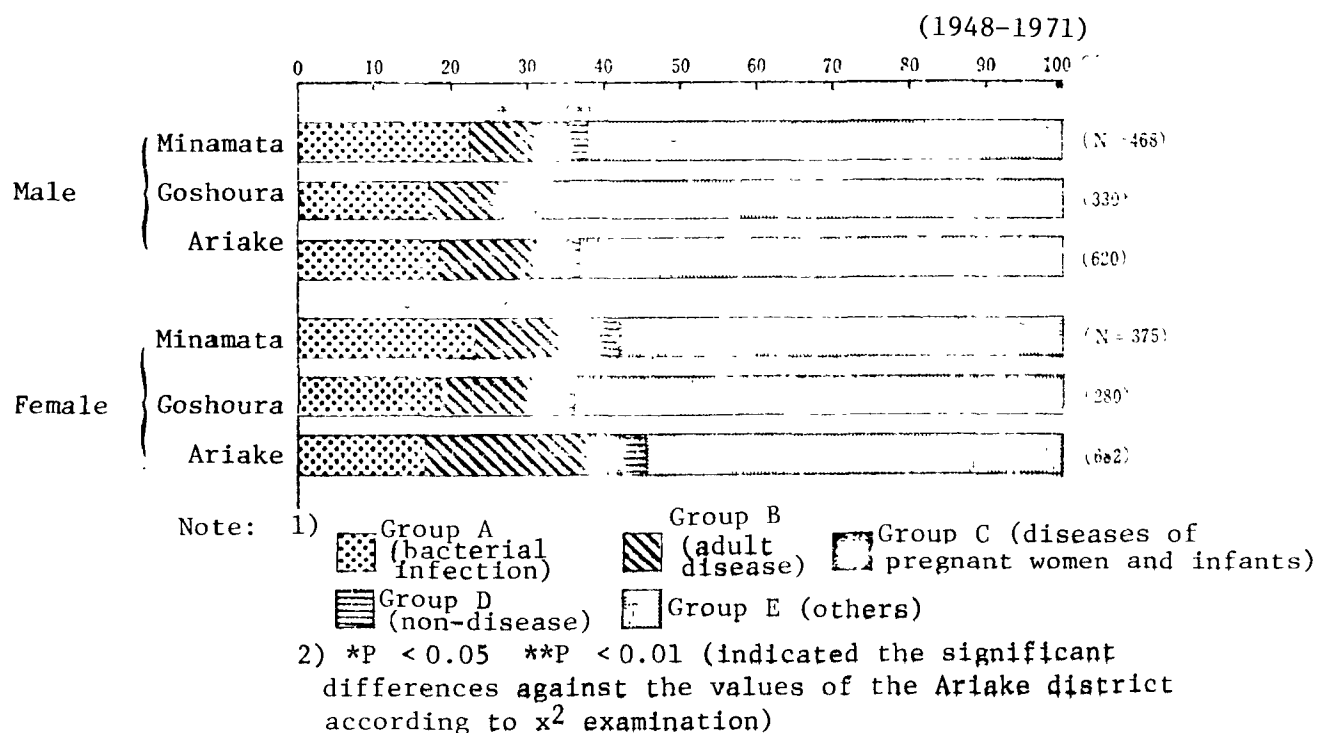
Figure 2.   Death rate classified by causes of deaths.

TABLE 7. COMPARISON OF THE MAIN CAUSE OF DEATHS BETWEEN DISTRICTS

1948 – 1971

Cause of deaths	Male			Female		
	Minamata	Goshoura	Ariake	Minamata	Goshoura	Ariake
Inflam. of intestines and other laxative diseases	18 (4.03)*	3 (0.94)	9 (1.55)	7 (1.92)	5 (1.85)	20 (3.00)
Tuberculosis	36 (8.05)	15 (4.72)	36 (6.22)	26 (7.12)*	7 (2.58)	25 (3.75)
Other infectious diseases and parasitic diseases	7 (1.57)	4 (1.26)	6 (1.04)	7 (1.92)	2 (0.74)	8 (1.20)
Malignant new-growth	51 (11.41)	35 (11.01)	70 (12.09)	40 (10.96)	25 (9.23)	59 (8.86)
Meningitis	4 (0.89)	0 (0.00)	2 (0.35)	6 (1.64)*	1 (0.37)	2 (0.30)
High blood pressure disease	2 (0.45)	1 (0.31)	3 (0.52)	2 (0.55)△	3 (1.11)	13 (1.95)
Cardiac disease	53 (11.86)	56 (17.61)	80 (13.82)	34 (9.32)**	33 (12.18)	105 (15.77)
Cerebral disease	71 (15.88)	46 (14.47)	92 (15.89)	59 (16.16)	59 (21.77)**	112 (16.82)
Pneumonia and bronchitis	36 (8.05)	33 (10.38)	46 (7.94)	28 (7.67)	27 (9.96)△	43 (6.46)
Vesicular emphysema and asthma	13 (2.91)	5 (1.57)	16 (2.76)	13 (3.56)△	0 (0.00)*	12 (1.80)
Liver cirrhosis	10 (2.24)	9 (2.83)*	6 (1.04)	3 (0.82)	5 (1.85)	7 (1.05)
Nephritis and nephrosis	8 (1.79)	13 (4.09)**	6 (1.04)	8 (2.19)	12 (4.43)**	10 (1.50)
Congenital abnormality	2 (0.45)△	4 (1.26)	9 (1.55)	5 (1.37)	6 (2.21)*	4 (0.60)
Senility not accomp. by mental disease	37 (8.28)*	22 (6.92)*	70 (12.09)	43 (11.78)**	31 (11.44)**	136 (20.42)
Other ill-defined diseases	0 (0.00)	0 (0.00)	1 (0.17)	0 (0.00)	2 (0.74)	0 (0.00)
Mental disorder	3 (0.67)	0 (0.00)	1 (0.17)	2 (0.55)	1 (0.37)	4 (0.60)
Non-inflammative disease of nerve center system	1 (0.22)*	2 (0.63)	9 (1.55)	0 (0.00)	0 (0.00)	2 (0.30)
Gastritis, duodenitis and chronic gastroenteritis	10 (2.24)	3 (0.94)*	19 (3.28)	16 (4.38)	11 (4.06)	17 (2.55)
Other diseases	59 (13.20)	46 (14.47)	86 (14.85)	53 (14.52)	34 (12.55)	76 (11.11)
Car accident	7 (1.57)*	1 (0.31)	2 (0.35)	3 (0.82)	2 (0.74)	1 (0.15)
Other unexpected accidents	13 (2.91)**	17 (5.35)**	3 (0.52)	4 (1.10)**	4 (1.48)**	0 (0.00)
Non-disease and other outer causes	6 (1.34)	3 (0.94)	7 (1.21)	6 (1.64)	1 (0.37)	10 (1.50)
Totals	447(100.00)	318(100.00)	579(100.00)	365(100.00)	271(100.00)	666(100.00)

Note: 1) Classified by the 50-Item Classification of Death Causes (Table B).

2) The numbers in the brackets indicate the death rate (%)

3) △p < 0.1   * P < 0.05   ** P < 0.01 (Compared with the Ariake district according to
x^2 examination)

44

On the other hand, in the Goshoura district, deaths from cirrhosis of the liver, nephritis, nephrosis and other unexpected accidents were higher, whereas deaths from senility (not accompanied by mental disease), gastritis, duodenitis and chronic gastroenteritis were lower in males. In females, deaths from cerebral disease, nephritis, nephrosis, congenital abnormalities and other unexpected accidents were higher, whereas the deaths from vesicular emphysema, asthma and senility (not accompanied by mental disease) were lower, which shows a reverse tendency as compared to the Minamata district.

III. GENERAL CONSIDERATIONS

This time we tried to investigate if the health standards of the inhabitants of the contaminated districts have been affected by the waste water from the factories which contains organic mercury, by using the health indicators related to mortality. However, for the analysis of the references obtained, we could not avoid a considerable extent of bias because each district is so small and is divided into a limited district as a city or town, and the population was also sparse; besides the collection of those references was limited as the investigation required references from the past.

The PMI, rough mortality rate and average remainder of life, are regarded to be very significant as health indicators to measure a local health standard. However, their high applicabilities are better suited for large population groups, such as a state. These are not appropriate indices to measure the health standard of small local districts. It is thought, therefore, that the indices can be used effectively by correcting the PMI or the average age of mortality which are obtained by correcting and grouping the ages [6,7].

In order to correct the bias of the references due to the changes in the population structure, we standardized the population and the age structure of mortality in each district by using the 1960's dynamic population statistics of the Kumamoto Prefecture. The year 1960 was just prior to the changes in the population structures of the districts had started, attributed to the influence

of a high economic growth policy. According to the corrected PMI thus obtained, the values for the Minamata district are lower than for the Ariake district for both sexes and they are especially very low for females. The corrected average age of mortality for females is slightly lower in the Minamata district than in the Ariake district. Moreover, the stillbirth rate is slightly higher in the Minamata district. In general we can assume that the health standard in the Minamata district is inferior to that in the Ariake district.

In regard to the local differences in the causes of death, we must take into consideration such differences as a concentration of medical care or the social and economic conditions of the localities. As for the concentration of medical care, the number of medical institutions in the districts are 2 internal disease clinics and 1 child and maternal center in the Goshoura district, 2 internal disease clinics in the Ariake district, which indicates the appropriation of medical care in the Goshoura district is slightly inferior compared to the 2 other districts.

Comparing the causes of death in the districts classified according to groups for causes of deaths, deaths from adult diseases are comparatively lower in the Minamata district and deaths from non-disease or infectious cases are higher than in the Ariake district. Acknowledging that adult diseases have increased after the infectious cases have been put under control, by which the national health trend in Japan has been transformed, we can assume there is a mortality problem in the Minamata district.

2.   Considerations of the Health Examination of Inhabitants who
     Suffered from the Minamata Disease

Organic mercury affects human health in various ways which results not only in a subclinical patient but also a non-subclinical or forme fruste patient [8]. In regard to examining inhabitants for the Minamata disease, the examination is conducted by questionnaires in order to screen the patient; it is controversial how effectively this system is utilized. In recent years,

a discussion has been centered on disease screening [9].

Then, in order to examine the health of inhabitants in the contaminated districts from the organic mercury, and particularly to screen the people for the disease, we examined adults (above 6 years), and investigated the obtained information.

I. OBJECTIVE METHOD OF INVESTIGATION

For the districts, as in the previous report [1], we selected Tsukiura, Idetsuki and Yudo of Minamata City, which are highly prone to the disease, and Arashiguchi of Goshouramachi on the opposite seashore of Minamata City, which are districts susceptible to contamination, and compared them with the results of Akazaki, Suko and Ohura of Ariakemachi facing the Shimabara Bay which have almost no seafood contamination.

We collected the investigation information similarly to the way it was collected in the previous report, and used both along with the references of previous investigations. Furthermore, we checked the results of clinical diagnosis of the people with the Minamata disease; the diagnosis was conducted by the Neuro-Psychiatry Department, Kumamoto University, and we investigated the relationship between complaints and the inspection of clinical diagnosis.

II. INVESTIGATION RESULTS

(1) Relationship between clinical diagnosis and subjective symptoms

As reported previously, when we compared the Minamata district with the other investigated districts, we observed a statistical significant difference in the complaints of inhabitants in the over-30 age group who lived in heavily contaminated areas. Then, we checked the relationship between the main complaints and the clinical diagnosis for inhabitants over the age of 30 in the Minamata district as shown in Table 8. We naturally counted many complaints among the patients definitely having the Minamata disease, the

patients afflicted by the Minamata disease and the patients susceptible to the
Minamata Disease, as compared to inhabitants of other areas. Particularly,
several complaints peculiar to the Minamata disease were observed. However, even
though some show typical symptoms of the Minamata disease, not all the
Minamata patients or the patients susceptible to the disease have reported com-
plaints when we check their symptoms individually. In other words, we en-
counter some similar complaints from patients who are not suffering from the
Minamata disease. It indicates that all the complaints are important just like
in the Hunter-Russel syndrome in the clinical diagnosis.

In this connection, we investigated how effectively we could use some
combinations of several complaints, which have been considered important for the
diagnosis of the Minamata disease, by the Minamata patients or the susceptible
patients who were screened for complaints. Therefore, we divided combinations
of complaints into 4 groups, i.e., disorder of perception, stricture of visual
field, disorder of movement and hearing disturbance; regarding the disorder of
perception as the main symptom, we then observed the frequency of the com-
plaints from the districts. As shown in Table 9, among those who exhibited
the disease symptoms, the frequency that their symptoms corresponded to the
4-item groups are 170 for males (49.4%) and 251 for females (52.1%) in the
Minamata district, which figures are significantly (and statistically) higher
than the 81 for males (29.2%) and the 77 for females (23.5%) in the Ariake
district. Furthermore, when the frequency of these complaints are analyzed
by placing them in several of the 4-group combinations, the frequency of
correspondence to the combination of disorder of perception [hereafter
referred to as (P), stricture of visual field (V), disorder of movement (M)
and hearing disturbance (H)]showed to be 11.3% for males and 9.1% for females
in the Minamata district, which are figures significantly higher than the
2.2% for males and the 1.2% for females in the Ariake district, and the
frequency of the combination of (P) + (M) or (H) is also high. On the other
hand, no significant statistical differences were observed between the
Goshoura and Ariake districts for both sexes.

Comparisons of the relationship between the combinations of complaints
and the results of the diagnosis are indicated in Table 10. The symptoms of

the patients in the Minamata district nearly corresponded to any of the 4-item groups of the Minamata disease for both sexes. More than 60% of the symptoms of the patients, including the susceptible patients in the Goshoura and Ariake districts, correspond to any of the 4-item groups. On the other hand, approximately 20-30% of the symptoms of the patients in the Goshoura and Ariake districts do not correspond to the Minamata disease, but nevertheless correspond to any of the 4-item group of the disease. Moreover, such a case is highly observed in the Minamata district, that is, the approximate value of 40% is statistically more significant than in the other districts, which is noteworthy as it is quite possible the patients or the susceptible patients are included among them.

We investigated the relationship between the combination of the subjective symptoms and the clinical diagnosis in the Minamata district for both sexes. As you can see in Table 11, when the frequencies of complaints correspond to any of the combinations of (P), (V), (M) and (H), the percentages of the patients diagnosed to have the Minamata disease or patients susceptible to the disease were found to be 48.2% for males and 40.2% for females. On the other hand, for those diagnosed as having the disease, or those susceptible to the disease, the percentages of frequencies of their complaints corresponding to any of the combinations of 4-item groups were found to be 76.7% for males and 82.1% for females, which figures are quite high.

The technique of screening the disease, which means the ability to distinguish between those having the disease from those not having the disease is very important and is called "validity". The validity of a general screening examination is indicated in Table 12. In this examination method, the ability to classify those as infected patients who are the positive symptom bearers is called "sensitivity" and the ability to classify the non-infected patients who are the negative symptom bearers is called "specificity".

If the above combinations of complaints are adopted as the screening method for the Minamata disease, the validity is as follows: sensitivity 77% and specificity 63% for males, and 82% and 58%, respectively, for females. The

49

TABLE 8.   RELATION BETWEEN CLINICAL DIAGNOSIS AND SUBJECTIVE SYMPTOMS
(Minamata district, those over 30 years)

Sex / Group — No. of patients or others / Subjective symptoms	Male				Female			
	Infected patient	Patient	Susceptible patient	Others	Infected patient	Patient	Susceptible patient	Others
	17 (100.0)	42 (100.0)	46 (100.0)	154 (100.0)	16 (100.0)	47 (100.0)	52 (100.0)	246 (100.0)
Disorder of perception Present	9** (52.9)	22** (52.4)	16** (34.8)	17 (11.0)	11** (68.8)	32** (63.1)	20** (38.5)	31 (12.6)
Past	11** (64.7)	24** (57.1)	23** (50.0)	38 (24.7)	13** (81.3)	29** (61.7)	24 (46.2)	65 (26.4)
Disorder of perception of the mouth or tongue	8** (47.1)	16** (38.1)	3ᐃ (65.2)	7 (4.5)	8** (50.0)	14** (29.8)	7** (13.5)	7 (2.8)
Stricture of visual field	8** (47.1)	21** (50.0)	5 (10.9)	8 (5.2)	10** (62.5)	17** (36.2)	8* (15.4)	15 (6.1)
Hearing disturbance	8** (47.1)	24** (57.1)	15** (32.6)	20 (13.0)	9** (56.3)	23** (48.9)	13* (25.0)	32 (13.0)
Smelling disturbance	5ᐃ (29.4)	15** (35.7)	5 (10.9)	23 (13.0)	7** (43.8)	17** (36.2)	8ᐃ (15.4)	19 (7.7)
Tasting disturbance (morbid appetite)	6** (35.3)	17** (40.5)	5 (10.9)	7 (4.5)	6** (37.5)	10** (21.3)	5 (9.6)	11 (4.5)
Walking disturbance	4ᐃ (23.5)	16** (38.1)	12** (26.1)	15 (9.7)	7* (43.8)	29** (61.7)	22** (42.3)	47 (19.1)
Shaking	9** (52.9)	21** (50.0)	12** (26.1)	8 (5.2)	11** (68.5)	15** (31.9)	9** (17.3)	6 (2.4)
Muscular atrophy	8** (47.1)	27** (64.3)	10* (21.7)	15 (9.7)	12** (75.0)	21** (44.7)	13ᐃ (25.0)	37 (15.0)
Tremor	8** (47.1)	17** (40.5)	7 (15.2)	25 (16.2)	10** (62.5)	15** (31.9)	5 (9.6)	21 (8.5)
Muscular atrophy	8** (47.1)	17** (40.5)	10** (21.7)	6 (3.9)	7** (43.8)	11** (23.4)	7 (13.5)	24 (9.8)
Muscular weakness	11** (64.7)	26** (61.9)	20** (43.5)	29 (18.8)	13** (81.3)	33** (70.2)	20** (38.5)	47 (19.1)
Articular pain	7ᐃ (41.2)	22** (52.4)	15 (32.6)	33 (21.4)	8** (50.0)	26** (55.3)	22** (42.3)	45 (18.3)
Amnesia	10** (58.8)	33** (78.6)	23** (50.0)	37 (24.0)	12** (75.0)	38** (80.9)	26* (50.0)	84 (34.1)
Decline in activity	9ᐃ (52.9)	25** (59.5)	26** (56.5)	43 (27.9)	12** (75.0)	28** (59.6)	31** (59.6)	71 (28.9)
Headache or heavy feeling in the head	9ᐃ (52.9)	24** (57.1)	22ᐃ (47.8)	50 (32.5)	8** (50.0)	36** (76.6)	36** (69.2)	53 (13.4)
Autonomic nerve impediment	8** (47.1)	28** (66.7)	22** (47.8)	19 (12.3)	10** (62.5)	20** (42.6)	16* (30.8)	41 (16.7)
Vesical excretion disorder	8** (47.1)	17** (40.5)	9** (19.6)	9 (5.8)	7** (43.8)	11** (23.4)	3 (5.8)	18 (7.3)
General languor or simple weariness	11 (64.7)	34** (80.1)	31* (67.4)	76 (49.4)	12ᐃ (75.0)	36** (76.6)	42** (80.8)	130 (52.8)

Note: 1)   Classifications of "patients" and "susceptible patients" in the clinical diagnosis is based on the diagnosis (for the first year) done by the Neuro-Psychiatric Department of Kumamoto University.

2)   ᐃP < 0.1   * P < 0.05   ** P < 0.01

Indicated the significant differences against the values of "others" according to x^2- examination.

TABLE 9. COMPARISON OF THE COMBINATIONS OF COMPLAINTS BY
DISTRICT GROUPS

(Those above 6-years)

Combinations of complaints	Male			Female		
	Minamata	Goshoura	Ariake	Minamata	Goshoura	Ariake
(P) + (V) + (M) + (H)	39 (11.3)**	4 (0.9)	6 (2.2)	44 (9.1)**	9 (1.7)	4 (1.2)
(P) + (V) + [(M) or (H)]	13 (3.8)	10 (2.3)	8 (2.9)	25 (5.2)**	6 (1.1)ᐞ	0 (0.0)
(P) + [(M) or (H)]	50 (14.5)**	30 (7.0)	17 (6.1)	73 (15.1)ᐞ	35 (6.5)	23 (7.0)
(V) + [(M) or (H)]	4 (1.2)	2 (0.5)	4 (1.4)	5 (1.0)	2 (0.4)	3 (0.9)
(P) + [any of (V) or (M) or (H)]	6 (1.7)	3 (0.7)	2 (0.7)	9 (1.9)*	5 (0.9)	1 (0.3)
Only (P)	58 (16.9)	67 (15.6)	44 (15.9)	95 (19.7)*	83 (15.3)	46 (14.0)
Subtotals	170 (49.4)**	116 (27.0)	81 (29.2)	251 (52.1)**	140 (25.9)	77 (23.5)
Other subjective symptoms	174 (50.6)	313 (73.0)	196 (70.8)	231 (47.9)	401 (74.1)	251 (76.5)
Totals	344(100.0)	429(100.0)	277(100.0)	482(100.0)	541(100.0)	328(100.0)

Note: 1)   Classification of complaints

(P)... Disorder of perception

(V)... Stricture of visual field

(M)... Disorder of movement

(H)... Hearing disturbance

2)   The numbers in the brackets indicate the percentages

3)   ΔP< 0.1   * P < 0.05   ** P < 0.01

(Indicated the significant differences against the respective
values of the Ariake district according to x^2-examination).

TABLE 10.   RELATION BETWEEN THE COMBINATIONS OF COMPLAINTS AND
THE RESULTS OF DIAGNOSIS -   PART 1

(Those above 6 years)

Sex	Combinations of complaints	Minamata			
		Infected patients	Patients	Susceptible patients	Others
Male	(P) + (V) + (M) + (H)	9	16	1	13
	(P) + (V) + [(M) or (H)]	2	4	3	4
	(P) + [(M) or (H)]	2	16	14	23
	(V) + [(M) or (H)]	0	1	0	3
	(P) + [any of (V) or (M) or (H)]	1	1	1	3
	Only (P)	2	3	12	41
	Subtotals	16 (88.9)	35 (83.3)	31 (66.0)	88* (67.1)
	Other subjective symptoms	2	7	16	119
	Totals	18 (100.0)	42 (100.0)	47 (100.0)	237 (100.0)
Female	(P) + (V) + (M) + (H)	10	12	4	18
	(P) + (V) + [(M) or (H)]	4	6	1	14
	(P) + [(M) or(H)]	2	14	11	46
	(V) + [(M) or (H)]	0	2	1	2
	(P) + [any of (V) of (M) or (H)]	0	2	2	5
	Only (P)	1	11	13	63
	Subtotals	17 (100.0)	47 (88.7)	37 (69.8)	140** (19.1)
	Other subjective symptoms	0	6	16	209
	Totals	17 (100.0)	53 (100.0)	53 (100.0)	349 (100.0)

Note: 1)   The clinical diagnosis here is based on the diagnosis (for the
first years) done by the Neuro-Psychiatric Department of
Kumamoto University.

2)   Classification of complaints
(P) ... Disorder of perception
(V) ... Stricture of visual field
(M) ... Disorder of movement
(H) ... Hearing disturbance

3)   "No subjective symptom" is included in "Others".

4)   The numbers in the brackets indicate the percentages of the totals
of each item.

5)   *P < 0.05   ** P < 0.01
(Indicated the significant differences against the values of the
Ariake district according to x^2-examination) .

TABLE 10 (CONTINUED)

	Goshoura			Ariake	
Patients	Susceptible patients	Others	Patients	Susceptible patients	Others
0	3	1	0	1	5
0	4	6	0	0	8
0	6	24	0	1	16
0	0	2	0	0	4
0	0	3	0	0	2
1	6	60	0	1	43
1	19	96	0	3	78
(50.0)	(61.3)	(21.2)	(0.0)	(60.0)	(28.7)
1	12	300	0	2	194
2	31	396	0	5	272
(100.0)	(100.0)	(100.0)	(0.0)	(100.0)	(100.0)
0	3	6	0	0	4
0	1	5	0	0	0
0	8	27	0	1	22
0	0	2	0	0	3
0	0	5	0	0	1
0	5	78	0	3	13
0	17	123	0	4	73
(0.0)	(56.7)	(24.1)	(0.0)	(57.1)	(22.7)
1	13	387	0	3	248
1	30	510	0	7	321
(100.0)	(100.0)	(100.0)	(0.0)	(100.0)	(100.0)

TABLE 11. RELATION BETWEEN THE COMBINATIONS OF COMPLAINTS AND THE RESULTS OF DIAGNOSIS - PART 2

(Minamata district, those above 6-years)

Combinations of complaints	Infected patients	Patients	Susceptible patients	Subtotals	Insanity	Mental deficiency
Male						
(P) + (V) + (M) + (H)	9	16	1	26(48.7)	1	0
		(28.1)	(2.1)		(50.0)	
(P) + (V) + [(M) or (H)]	2	1	5	8(42.2)	0	0
	(11.1)	(9.5)	(16.1)			
(P) + [(M) or (H)]	2	10	11	23(52.0)	0	0
	(11.1)	(23.8)	(29.8)			
(V) + [(M) or (H)]	0	1	0	1(25.0)	0	0
		(2.4)				
(P) + [any of (V) or (M) or (H)]	1	1	1	3(50.0)	0	0
	(5.6)	(2.4)	(2.1)			
Only (P)	2	3	12	17(29.3)	0	1
	(11.1)	(7.1)	(25.6)			(100.0)
Subtotals	16	35	31	82(48.2)	1	1
	(88.9)	(83.3)	(66.0)	(76.7)	(50.0)	(100.0)
Other subjective symptoms	2	7	16	25(51.4)	1	0
	(11.1)	(16.7)	(24.0)	(23.3)	(50.0)	
Totals	18	42	47	107(51.1)	2	1
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)
Female						
(P) + (V) + (M) + (H)	10	12	4	26(59.1)	0	0
	(58.8)	(22.6)	(7.5)			
(P) + (V) + [(M) or (H)]	4	6	1	11(61.0)	0	0
	(23.5)	(11.3)	(1.9)			
(P) + [(M) or (H)]	2	14	11	27(37.0)	0	1
	(11.8)	(26.4)	(20.8)			(12.5)
(V) + [(M) or (H)]	0	2	1	3(60.0)	0	1
		(3.8)	(1.9)			(12.5)
(P) + [any of (V) or (M) or (H)]	0	2	2	4(44.4)	0	1
		(3.8)	(3.8)			(12.5)
Only (P)	1	11	18	30(31.6)	2	2
	(5.9)	(20.8)	(34.0)		(66.7)	(25.0)
Subtotals	17	47	37	101(40.2)	2	5
	(100.0)	(88.7)	(69.8)	(82.1)	(66.7)	(62.5)
Other subjective symptoms	0	6	16	22(9.5)	1	3
		(11.3)	(30.2)	(17.9)	(33.3)	(37.5)
Totals	17	53	53	123(25.5)	3	8
	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)

Note: 1) Subtotals in brackets indicate the percentages of the horizontal totals.
2) The clinical diagnosis here is based on that (for the first year) done by the Neuro-Psychiatric Dept. of Kumamoto University.
3) Classification of complaints:
(P) ... Disorder of perception,   (V) ... Stricture of visual field
(M) ... Disorder of movement,   (H) ... Hearing disturbance
4) The validity of the screening technique designed to cope with the combinations of complaints:

Male $\left\{\begin{array}{l}\text{Sensitivity } \frac{82}{107} \times 100 = 77\% \\ \\ \text{Specificity } \frac{149}{237} \times 100 = 63\%\end{array}\right.$

Female $\left\{\begin{array}{l}\text{Sensitivity } \frac{101}{123} \times 100 = 82\% \\ \\ \text{Specificity } \frac{209}{359} \times 100 = 58\%\end{array}\right.$

54

TABLE 11 (CONTINUED)

Parkinson's disease	High blood pressure	Spinal disorder	Schizophrenia	Depression	Nerve disturbances	Others	No subjective symptom	Totals (%)	
1 (33.3)	6 (8.5)	0	0	1 (50.0)	0	4 (20.0)	0	35 (11.3)	(100.0)
1 (33.3)	3 (1.2)	·	0	0	0	0	0	13 (3.8)	(100.0)
0	11 (15.5)	1 (50.0)	0	1 (50.0)	2 (50.0)	2 (10.0)	7 (5.4)	50 (11.5)	(100.0)
0	2 (2.8)	0	0	0	0	1 (5.0)	0	4 (1.2)	(100.0)
0	0	0	0	0	0	0	3 (2.3)	6 (1.7)	(100.0)
0	17 (23.9)	0	1 (33.0)	0	1 (25.0)	5 (25.9)	16 (12.4)	58 (16.9)	(100.0)
2 (66.7)	39 (54.9)	1 (50.0)	1 (33.3)	2 (100.0)	3 (75.0)	12 (60.9)	26 (20.2)	170 (49.1)	(100.0)
1 (33.3)	32 (45.1)	1 (50.0)	2 (66.7)	0	1 (25.0)	8 (40.0)	103 (79.8)	171 (50.6)	(100.0)
3 (100.0)	71 (100.0)	2 (100.0)	3 (100.0)	2 (100.0)	4 (100.0)	20 (100.0)	129 (100.0)	341 (100.0)	(100.0)
0	13 (10.7)	1 (25.0)	0	0	0	3 (8.1)	1 (0.6)	41 (9.1)	(100.0)
1 (100.0)	8 (6.6)	0	0	0	0	3 (8.1)	2 (1.1)	25 (5.2)	(100.0)
0	32 (26.4)	0	0	0	0	4 (10.8)	9 (5.0)	75 (15.2)	(100.0)
0	0	0	0	0	0	0	1 (0.6)	5 (1.0)	(100.0)
0	1 (8.3)	0	0	0	0	1 (2.7)	3 (1.1)	9 (1.9)	(100.0)
0	18 (14.9)	1 (25.0)	0	0	1 (50.0)	11 (29.7)	30 (16.5)	94 (19.7)	(100.0)
1 (100.0)	72 (59.5)	2 (50.0)	0	0	1 (50.0)	22 (59.5)	45 (24.7)	251 (52.1)	(100.0)
0	49 (40.5)	2 (50.0)	1 (100.0)	0	1 (50.0)	15 (40.5)	147 (75.3)	231 (47.9)	(100.0)
1 (100.0)	121 (100.0)	4 (100.0)	1 (100.0)	0	2 (100.0)	37 (100.0)	182 (100.0)	482 (100.0)	(100.0)

55

TABLE 12. VALIDITY OF THE SCREENING EXAMINATION

(Remein & Wilkerson) [9]

Screening results	Classification of diseases according to groups of people who look healthy in appearance	
	Patients	Non-patients
Positive	Has the disease and the examination result is positive (True-positive)	Has no disease but the examination result is positive (Pseudo-positive)
Negative	Has disease but the examination result is negative (pseudo-negative)	Has no disease and the examination result is negative (true negative)
Totals	Total patients included people in whom the disease is still undetected.	Total of non-disease

$$\text{Sensitivity} = \frac{\text{The number of those having the disease and positive results of the examination}}{\text{Total number of sufferers in the group}}$$

$$\text{Specificity} = \frac{\text{The number of those not having the disease and negative results of the examination}}{\text{Total number of non-sufferers in the group}}$$

56

TABLE 13. RELATIONSHIP BETWEEN THE EXAMINATION OF OBJECTIVE SYMPTOMS
AND THE CLINICAL DIAGNOSIS

(Minamata district, those
above 10 years)

	Have both the subjective symptoms and the abnormal recognition by the objective symptoms examination							
	Disorder of perception		Stricture of visual field		Hearing disturbance		Disorder of synergic movement	
Sex	M	F	M	F	M	F	M	F
No. of people	15	23	21	19	78	76	15	44
(%)	(5.5)	(5.9)	(6.6)	(4.4)	(13.2)	(9.3)	(3.6)	(8.1)
Total male and female (%)	38 (5.7)		40 (5.3)		154 (10.9)		59 (6.1)	

	Have the subjective symptoms but no abnormal recognition by the objective symptoms examination							
	Disorder of perception		Stricture of visual field		Hearing disturbance		Disorder of synergic movement	
Sex	M	F	M	F	M	F	M	F
No. of people	32	58	14	21	44	56	18	54
(%)	(11.8)	(14.8)	(4.4)	(4.8)	(7.4)	(6.8)	(4.3)	(9.9)
Total male and female (%)	90 (13.6)		35 (4.7)		100 (7.1)		72 (7.5)	

Note: 1) Criteria for the abnormal value in the objective symptoms examination.
Disorder of perception ... Pain sensation in the fingers at the finger
periphery part when over 3 g were applied in the examination.
Stricture of visual field ... Less than 80° by the Forster peri-
meter (stricture of visual field)

Hearing disturbance ... Loss of over 30 db measured by the audiometer
for the 6-division method.

Disturbance of synergic movement ... Over (—σ) deviation from the
mean value of the respective age group in the Ariake district
according to the matchboard test.

2) "Not tested as no measurement is required according to the perimeter"
is included in "No objective symptoms" in the "Stricture of visual
field".

57

TABLE 13 (CONTINUED)

	Have no subjective symptoms but abnormal recognition by the objective symptoms examination							
	Disorder of perception		Stricture of visual field		Hearing disturbance		Disorder of synergic movement	
Sex	M	F	M	F	M	F	M	F
No. of people	17	13	16	38	48	108	59	72
(%)	(6.3)	(3.3)	(4.1)	(8.7)	(8.1)	(13.2)	(14.1)	(13.3)
Tot. M and F	30		54		156		131	
(%)	(4.5)		(7.2)		(11.0)		(13.6)	

	Have neither the subjective symptoms nor the abnormal recognition by the objective symptoms examination							
	Disorder of perception		Stricture of visual field		Hearing disturbance		Disorder of synergic movement	
	M	F	M	F	M	F	M	F
No.of people	208	298	266	357	422	581	328	373
(%)	(76.5)	(76.0)	(83.9)	(82.1)	(71.3)	(70.8)	(78.1)	(68.7)
Total male and female	506		623		1003		701	
(%)	(76.2)		(82.8)		(71.0)		(72.8)	

Note (Continued):

3) For the "Hearing disturbance", the total number of both ears is indicated.

4) Frequencies (%) indicate the rate of the total number of the respective items.

TABLE 14. RELATIONSHIP BETWEEN THE EXAMINATION OF OBJECTIVE SYMPTOMS
AND THE CLINICAL DIAGNOSIS

(Minamata district,
those over 10 years)

Investigation item	Division	Male			Female		
		Infected patients susceptible	Others	Totals	Infected patients susceptical	Others	Totals
Finger pain sensation	Abnormal	26 (8.9)	5 (1.7)		22 (5.6)	5 (1.3)	
	Borderline	33 (11.3)	31 (10.6)	292 (100.0)	22 (5.6)	32 (8.2)	392 (100.0)
	Normal	31 (10.6)	166 (55.8)		17 (12.0)	231 (61.5)	
Stricture of visual field	Abnormal	13 (14.5)	1 (1.4)	296 (100.0)	56 (14.0)	5 (1.3)	392 (100.0)
	Normal	19 (16.6)	290 (67.6)		15 (11.5)	283 (75.0)	
Hearing disturbance	Abnormal	19 (17.0)	25 (8.7)	289 (100.0)	5 (11.7)	18 (12.8)	389 (100.0)
	Normal	11 (11.2)	174 (60.2)		45 (11.6)	239 (61.4)	
Disorder of synergic movement (Matchboard)	Abnormal	21 (7.1)	7 (2.1)	291 (100.0)	18 (5.0)	22 (6.1)	360 (100.0)
	Borderline	22 (7.5)	26 (8.8)		12 (3.3)	36 (10.0)	
	Normal	47 (16.6)	171 (58.2)		39 (10.8)	233 (64.7)	

Note: 1) Criteria of the abnormal value in the objective symptoms examination.

Disorder of perception ... Pain sensation in the fingers at the finger periphery part when over 3 g were applied in the examination.

(Included the abnormality of a finger in one hand). "Borderline" is above 1 g less than 3 g.

Stricture of visual field ... Less than 80° by the Förster perimeter (those not required to take the test are judged as "normal" according to the confrontation check).

Hearing disturbance ... Loss of over 30 db by the audiometer of 6-division method (Includes the abnormality in one year)

Disorder of synergic movement ... Over (-2 σ) deviation from the mean value of the respective age group in the Ariake district according to the matchboard test. "Borderline" is less than (M-σ) ∿(M - 2 σ).

2) Division of clinical diagnosis
Infected ... Infected patients of the Minamata disease
Patients ... Diagnosed as Minamata disease
Susceptible... Susceptible patients of the Minamata disease

59

Note for Table 14 (Continued):

3) The numbers in the brackets indicate the frequencies (%).

4) The validity as a screening technique in the respective examinations (in case "borderline" is included in "normal").

Investigation item	M Sensitivity	F Specificity	M Sensitivity	F Specificity
Finger pain sensation (a)	29%	98%	22%	98%
Stricture of visual field	47	98	55	98
Hearing disturbance	54	87	56	83
Disorder of synergic movement (b)	23	97	26	92

a) When screening level is over 1 g,

(66%, 82% respectively, in males

(53%, 87% respectively, in females.

b) When screening level is over 2 σ,
(48%, 84% respectively, in males
(43%, 80% respectively, in females.
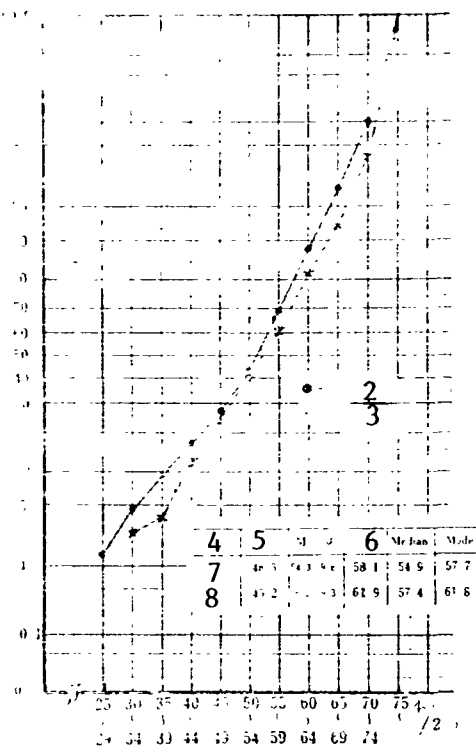
Note: Males over 30 years

Figure 3. Regular probability curve of matchboard test values.

1- Cumulative relative frequency;
2- Minamata district; 3- Ariake district; 4- district; 5- 25%-ile value;
6- 75%-ile value; 7- Minamata;
8- Ariake.
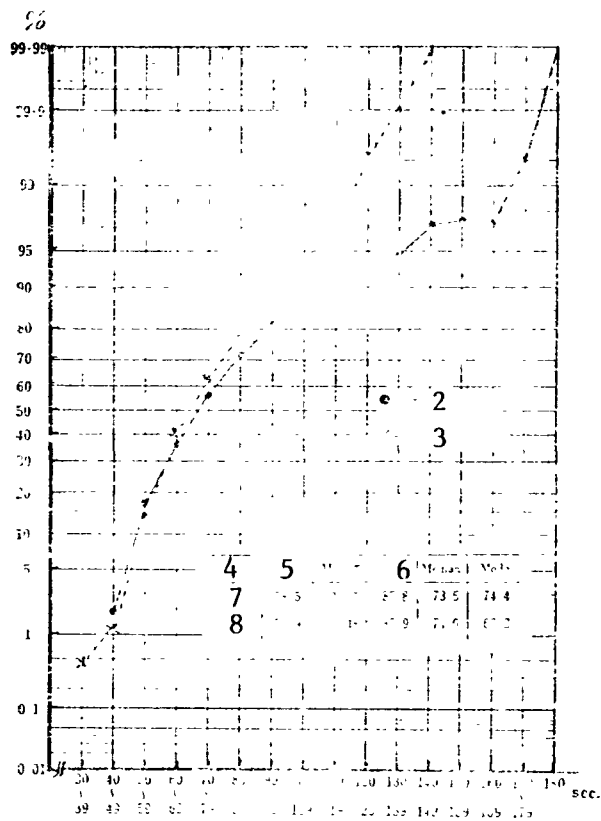
Note: Males over 30 years

Figure 4. Regular probability curve of blocking test values.

1- Cumulative relative frequency;
2- Minamata district; 3-Ariake district;
4- district; 5- 25%-ile value;
6- 75%-ile value; 7- Minamata;
8- Ariake.

61

Figure 5. Patients' examination system of the Minamata disease.

1- Inhabitants susceptible to environmental contamination;   2- First
examination;   3- Investigation of examination items and criteria;
4- (Questionnaire, several examinations);   5- Those without abnormal diagnosis;
6- (Re-examination at a certain interval);   7- Those with abnormal diagnosis;
8- Second examination;   9-(Close examination, discrimination of diagnosis);
10- Investigation of examination items and criteria;   11- Surveillance;
12- Those as either normal or abnormal;   13- (Borderline group);
14- Minamata disease patients;   15- Patients of other diseases;   16- (Diagnosis
distinguished);   17- Treatment of diseases, living patterns advised;
18- or;   19- Observation required (pre-disease condition);   20- Prevention
of diseases;   21- Development of a cure;   22- Rehabilitation;   23- Observation
and study required;   24- Close examination at a certain interval;   25- Those
who require observation;   26- Criteria problem;   27- Establishment of a
disease picture and a diagnosis method.

degree of false-negative is rather small, while the degree of false-positive is relatively large. That is, approximately 20% of the patients having the Minamata disease are dropped while approximately 40% of the patients not having the Minamata disease are picked. Therefore, we must consider the above point if we use this method for screening the Minamata disease.

(2) Relationship between the examination of objective symptoms and the clinical diagnosis

We observed the relationship between the patients' complaints and the examinations of objective symptoms in regard to some symptoms which are considered the important characteristics of Minamata disease. As shown in Table 13, 5-10% have both the subjective symptoms and the abnormal recognition in the objective symptoms examination, and 70-80% have neither the subjective symptoms nor the abnormal recognition in the objective symptoms examination, although there is some difference depending on the examination items. Furthermore, approximately 10% have the subjective symptoms but no abnormal recognition in the objective symptom examination, whereas approximately 10% have no subjective symptoms but the abnormal recognition in objective symptom examination. Such results should be considered for the analysis.

Next, we observed the relationship between these examination results and the clinical diagnosis as shown in Table 14. In examining the finger pain sensation, 8.9% of the males and 5.6% of the females were found to show abnormal signs of those recognized as having the disease or those susceptible to the disease, whereas 21.9% and 20.2%,respectively,were found to exhibit normal signs to the examination of those who either recognized patients as having the disease or those susceptible to the disease, whereas, 21.9% and 20.2%,respectively,were found to exhibit normal signs to the examination of those who either recognized patients, as having the disease, or those susceptible to the disease. When we consider these results for "validity" as the screening examination of the Minamata disease, the sensitivity is a low 29% for males and 22% for females; on the other hand, the specificity is a high 98% for both sexes. A similar investigation was done for stricture in the visual field,

63

hearing decline and disorder of synergic movement (matchboard test), and the results are as follows: the sensitivity was approximately 50% and specificity was over 80% in all cases. That is to say, false-negative was large while false-positive was relatively small, from these results we noticed the problem of this existing method for the screening of the Minamata disease.

(3) Problem of examination items and screening level

The screening level which we used in Table 14 showed that the specificity is high while the sensitivity is low in general. From a public hygiene standpoint, however, the examination items and the screening level application should be chosen so as to produce less false-negative than false-positive.

In this regard, we described the results of the matchboard test in a regular probability paper as shown in Figure 3, in order to see the disorder of the synergic movement. The Ariake district shows a relatively straight normal curve while the Minamata district shows a slight inclination to the left as a whole, (besides a skewness to the left in the middle is recognized). This indicates not only a group of slightly low values but it also includes an apparent abnormal group as compared with the values of the Ariake district. (This is also assumed by 25%-ile value, 75%-ile value, M ± σ, median value and mode value.

When we plotted the measured values of the blocking test on a regular probability paper in Figure 4, the skewness has increased and no straight lines we observed. Namely, the Ariake district shows neither a normal distribution nor straight line, and the Minamata district shows extreme skewness. Therefore, in screening the Minamata disease by using examination items of such results, it is very important which value is to be adopted as a screening level, and the degrees of false-negative and false-positive are variable depending on the the values used.

Regarding the matchboard test, we calculated the mean values and the standard errors of the respective age groups in the Ariake district, by

64

judging the deviation of (-2σ) or more from the mean value as an abnormal value. According to this measure, the specificity is as high as 95%, and the sensitivity is as low as 25%. This indicates that the false-negative is so large that this measure is not suitable for the screening for that condition. Then, judging the abnormal value when the deviation is (-σ) or more from the mean value, we obtained a specificity of approximately 45% and a sensitivity of approximately 80%. Although they decrease or increase respectively, the false-negative is high, so that the patients would not pass the screening test which makes it difficult to apply them to the screening of the Minamata disease.

Now, we established this criterion of abnormal values for examining finger pain sensations, that is, if pain is felt in any finger (of hand or both hands) other than the thumb at or above the second knuckle when more than 3g of metal weight is applied, the pain is regarded as abnormal. According to this criterion, the sensitivity is approximately 25% and the specificity is 98%. Furthermore, when we relaxed this criterion of abnormal values by applying just over the 1g of metal weight, the results are approximately 60% for sensitivity and approximately 85% for specificity. The specificity slightly decreases and the sensitivity increases; that means, from a public hygiene standpoint, the percentages of patients dropped by the screening method is small. Therefore, it seems to be preferable to use. However, the percentages of patients not having the disease would increase as the objectives of the second examination becomes large.

In the examination method for screening the disease, a dependability (reappearance, accuracy) and a practicality (expense, hours needed, etc.) are just as important as the validity. The examination items and their screening level should be decided upon the above matters. In this case, as in the combinations of complaints, several combinations of examination methods, or the combinations of complaints and examination methods for some functions are considered effective to raise the validity of the screening examination of the Minamata disease in the infected patients; this, however, depends highly upon further investigations.

(4) Inhabitants examination system of the Minamata disease

We charted the procedures of the examination of patients in the district susceptible to contamination as described in Figure 5. To start, we conducted the first examination of all the patients in the district susceptible to contamination, and separated them according to abnormal views and normal views. For this we selected examination items according to the screening principle of the disease, in which a validity or dependability should be high and economical, as well as practicable. Furthermore, a screening level of the applied examinations should be positive with at least a false-negative, and the items and the criteria are to be adopted with as high a specificity as possible. Here, although the questionnaire investigation is highly significant, it has its limits; therefore, we must recognize a risk in which the abnormal cases without subjective symptoms should be dropped from the screening procedure [22].

If the abnormal views or traces of the Minamata disease are recognized in the first examination, we conduct the second examination. Mainly clinical doctors participate in this stage, and the close examination is given to those who have been selected by the screening process and each symptom is distinguished by referring the items for epidemiological examinations. Again, the same considerations as in the first examination are given to each examination item and its criterion. If the abnormal views are recognized in the second examination, we can distinguish the Minamata disease (including complications) from non-Minamata disease. However, we still cannot disclose the normal views, nor judge them as normal or abnormal. The latter case is called "The borderline group" about which we must disclose the disease pictures and the diagnostic methods from future observations and investigations, and the criteria for the diagnoses of diseases should be investigated. If their clinical diagnoses are disclosed, the medical treatment of the disease and the life pattern should be given, and as a matter of course, we must expedite the rehabilitation process as quickly as possible. Furthermore, some exhibit symptoms of the pre-disease condition, the diagnoses of which are already known; therefore, preventive measures for this disease are necessary.

Moreover, if patients need to be further observed, thorough examinations should
be given regularly in order to discover the disease in the early stage, as well
as to give medical treatment as early as possible. The significance of
surveillance is especially important in the Minamata disease due to the fact
that the crisis may happen after several years, or as a possible influence from
the antenatal conditions.

III. CONSIDERATIONS

In considering an approach to the disease, the ordinary medical treatment
involves the going of patients with various complaints to the hospital. On
the contrary, the screening of diseases in the public hygiene sense deals
with people who seem to be healthy in appearance when going to the hospital,
or at least who have complaints but not related to the diseases or disease groups,
that is, the objects of screening. On that account, there is a difference
between the two as mentioned above. Therefore, when dealing with the
Manamata disease, basically we must keep in mind that such a difference in the
treatment method has existed. A so-called "inhabitant" examination belongs to
the latter approach.

For the clinical diagnosis of the Minamata disease, the efforts of
systematization were made by Tokuomi [10] in the early stage, and further by
Tsubaki, et al. [11], and Tatetsu, et al [12]. In the Minamata disease, the
Hunter-Russel syndrome is unanimously regarded as the main constituent in
the advanced stages. However, recently in the disease picture of the Minamata
disease, another noted symptom appears [12], so that the difficult problem
arises in its screening, for which a disease picture has not yet been
established. If a new disease picture is established as a significant one,
a screening method should be adopted, based upon that disease picture.
Therefore, even if a certain screening method for the Minamata disease has
been presented, it must not be accepted unquestioningly, but should be im-
proved by continuous efforts that parallel the establishment of disease pic-
tures and the development of new methods.

In this study, we first investigated the significance of the complaints examination in the screening of the Minamata disease. We focused on the 4-item complaints, such as disorder of perception, stricture of visual field, disorder of movement and hearing disturbance which have been considered as typically important symptoms of the Minamata disease. Recognizing the disorder of perception as a main symptom, we investigated the validity of the screening of the Minamata disease by the extraction method depending on the combinations of those complaints. As a result, approximately 80% sensitivity and approximately 60% specificity, which is such a high false-negative pattern that the patients would be dropped from the screen. On one hand, to screen the Minamata disease based on complaints from the questionnaire examination is advantageous, as it can be done promptly and economically, but by the same token, it has certain limits in its applications. Secondly, as regards some function examinations in the health examination of the patients in the Minamata district, we investigated its validity for screening the disease. As a result, the specificity is relatively high; however, the degree of false-negative is large if the screening is done by using these examinations, according to the criteria we adopted. Therefore, if only the first item of the respective examination is used for screening, there is a considerable risk that the patients would be dropped in the screening process. Furthermore, for the problem of a screening level applied to this case, we analyzed the results of a few examinations given to inhabitants of the Minamata district. There were many cases which did not indicate a normal distribution as a group, besides they deviate from the results in the Ariake district as a whole. Therefore, a thorough investigation is required for the adoption of the criteria.

To control the health level of the inhabitants who have been contaminated by waste water (with organic mercury) from the factories, serious consideration must be given not only to the disease control of the organic mercury poisoning in patients, but also to the health standard of the whole local area. Thus, even though it has not been determined that the diseases or the symptoms have been caused by water contamination due to its organic mercury content,

it must not be neglected and the proper measures should be taken. A so-called "multiphasic" or "multiple mass screening examination" should be introduced which is used to distinguish not only the patients of the specific disease, but also the susceptible patients of various kinds of diseases. Furthermore, a so-called surveillance of the health conditions of inhabitants shall be undertaken for an extended time by using a proper screening procedure which should be definitely conducted in the contaminated district due to the contaminated waste water from the factories.

3. Actual Conditions Relating to the Fisheries and Fishermen's Diets (Fishing Seasons of Katakuchi-sardine and Young Ayu) along the Shiranui Seacoast.

One of the aspects underlying the outbreak of the Minamata disease is considered to be connected to the living area of the fishermen along the Shiranui Seacoast. However, few results concerning the actual conditions of the fishermen's life have been accumulated. We now explain the purpose of introducing the above new investigation subject. First, it is to investigate the background of the outbreak of the Minamata disease relating to the fishermen's life from a public hygiene standpoint. The basic references (based on medical labor matters) in regard to the fishing industry along the Shiranui Seacoast are collected, and the factors contributing to the health impediment are sought through references such as aspects of the fishermen's labor, labor conditions or labor environment. To collect references about the characteristics of the fishermen's diets; especially, concrete references about the intake of fish products is a principal subject for investigating dosages of methyl mercury in the living body. Secondly, the outbreak of the Minamata disease is considered to relate to the fishermen's life itself, if so, then the basic references in regard to the fishermen's life should be collected for the investigations on the contamination caused by methyl mercury, the subsequent outbreak of Minamata disease and its progress afterwards.

However, there are two phases in general when observing the local conditions at the present. Namely, one still remains nearly the same

(such as, construction of life hours) as in the years from 1955 - 1965 during which the Minamata disease frequently broke out, and the other has seen changes (such as technical renovations in fishery labor, different diet, etc.) as compared to those times.

Therefore, in regard to the objectives and assuming that their whole lives completely depend on the fish industry, we investigated the actual conditions of their lives from a public hygiene standpoint.

I. OBJECTIVE METHOD OF INVESTIGATION

We selected a fisherman's family from the Tsunaki-machi, Ashikita-gun and Kumamoto Prefecture as the sources of this investigation. Thirty-three families in this district consist of fishermen and farmers (citrus-culture, etc.) except one merchant. The main focus is put on one of three fishermen's bosses in this district. The family structure is as shown in Figure 6. Among seven members of the family (including the lodgers), two are patients who have the Minamata disease (one of which is a patient who has the congenital Minamata disease). The fish labor is usually operated by the family members, as husband, wife, eldest son, eldest daughter and second daughter, and three netters are added during the busy fishing season.

As shown in Figure 7, the busy fishing season which is mainly net fishing, along the Shiranui inshore, is from June to September whereas January and February are relatively slack. We conducted the investigation in the first part of August of 1972 for Katakuchi-sardine fishing and in the last part of February of 1973 for young Ayu fishing. Our investigation was as follows. We checked the working hours of the fisherman's wife, eldest son, and eldest daughter for three consecutive days, in which the investigation sheets were marked by the investigators. The hours were divided in seconds. During the fishing operation, we also used a stop-watch in order to obtain more accurate results. In the three consecutive days, the nutritional value of five members of this family, i.e., husband, wife, eldest son, eldest daughter
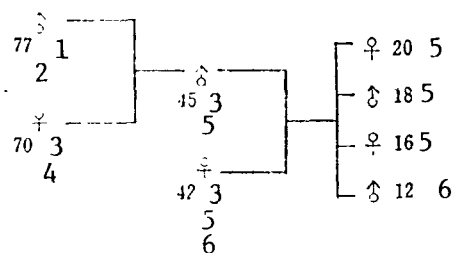
Figure 6. Family construction for the investigation objectives.

1- (Lived separately);   2- dealer of fish products;   3- (Lodger);
4- (Susceptible);   5- fisherman;   6- (Recognized)
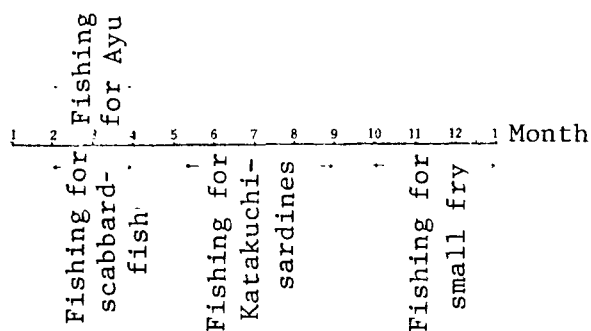
Figure 7. Fishing season along the Shiranui inshore (net fishing).

and second daughter was investigated, of which the net quantity of their diets

including snacks was measured respectively.   In the meantime, in regard to

the fish labor and its incidental work, their energy metabolism was measured

using both the Douglas bag method and the gas analyzer (Institute for

Science of Labor), distinguishing their respective works.   Furthermore, the

weather conditions , such as temperature, humidity, velocity, cata-cooling

power and radiant heat, were measured continually every hour from   4:00 A.M.

to 10:00 P.M. by the sanitation examination method (Japan Pharmaceutical

Association), at prescribed points on the beach close to the fisherman's

house. Especially the noise of the diesel engine of the fishing boat, etc.

was measured by a noise indicator, simple noise scale and octave analyzer

(Lion, SA-55).

71

II. RESULTS OF OUR INVESTIGATION

(1) Distribution of their life hours

The distribution of their life hours per day are indicated in Figures 8 and 9. Namely, in the fishing season of the Katakuchi-sardines, they go out fishing twice a day — at 4:00 A.M. and 4:00 P.M. and afterwards work on the processing of making dried sardines. In this case, the distribution is generally 7 hours for fishing, 5 hours for processing, 5 hours for sleep and 7 hours for leisure. During the fishing season for young Ayu, they fish 3 times a day — at 6:00 A.M., 8:00 A.M. and 2:00 P.M. and here the distribution is 8 hours for fishing, 8 hours for sleep and 7 hours for leisure. However, according to the weather condition or current condition, the evening fishing for Katakuchi-sardines and afternoon fishing for young Ayu continues for 10 to 15 days a month, and Ayu fishing during the winter season is often prevented by bad weather.

Fishing for Katakuchi-sardines is done from a main boat of approximately 2 tons equipped with a diesel engine of 15 h.p. and a fishing radar, two 3.77 ton netting boats with a diesel engine of 20 h.p. and a manual jolly boat. For young Ayu fishing, the above main boat and jolly boat are used. In addition, a tow net for Katakuchi-sardine fishing and a small roll net for young Ayu fishing are used.

Fishing for either Katakuchi-sardines or young Ayu consists of a series of continual work such as, searching a shoal, moving the boat toward a shoal, preparing the net setting, net withdrawal (by both machine operated and manual) and taking up a haul. The hours required for the operation are 60-80 minutes from the net setting to the haul in for Katakuchi-sardines, and the net setting is done 1-2 times per fishing. On the other hand, only 5-10 minutes from the net setting to the haul is required for Ayu fishing. This operation is repeated often and the net setting reaches 13-15 times a day. The distribution of hours of respective work during this period is indicated in Tables 15, 16 and 17. From these tables, you can see that the main part of

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 Time

Rising
fishing

Return
Processing work
Breakfast
Rest

Lunch
Rest
Shipping work

Return
Process work
Supper,
Bedtime

Depart, for fishing

Depart. for Fishing

Preparation for departure | Assorting

Searching for a shoal | Fish drying work

Preparation for netting | Predrying

Netting | Drying in the dryer

Net withdrawal | Selection

Taking up the haul | Encasing

Return | Shipping

Transport

Figure 8. Distribution of daily life style hours (Katakuchi-sardine fishing).

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Rising
fishing
Return
fishing

Return
Lunch
Fishing

Return
Shipping work
Supper
Rest

Bedtime

Depart. for fishing

Breakfast - Return
Depart. for

Depart. for Fishing

Preparation for departure

Searching for a shoal

Netting

Net withdrawal

Fish scooping

Return

Transport
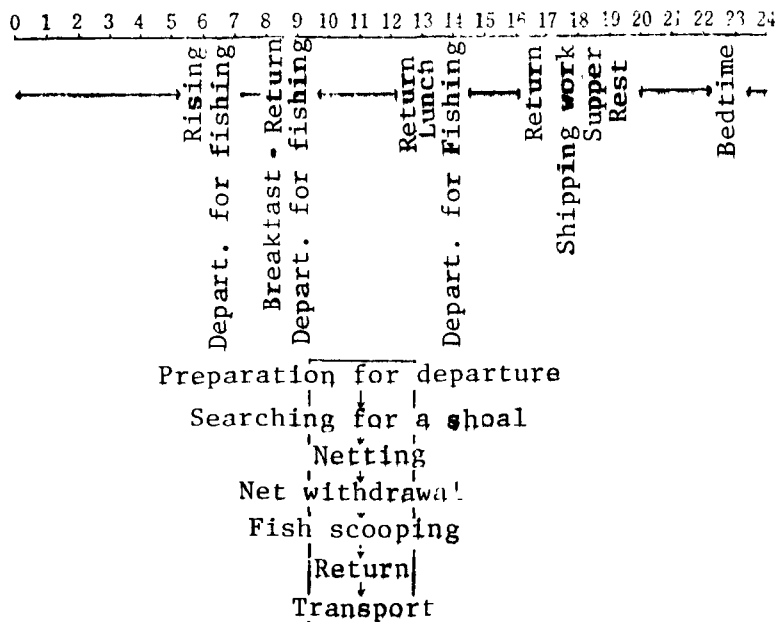
Figure 9. Distribution of daily life style hours (Young Ayu fishing).

73

fish labor at sea is the withdrawal of the net. In fishing for Katakuchi-
sardines, a considerable amount of energy is consumed in processing the dried
sardines. This work consists of assortment, boiling, drying (drying storage),
selection, encasing and shipping. The relative metabolic rate (hereafter
referred to as R.M.R.) of the respective work is shown in Tables 15, 16 and 17.
In the fish labor at sea, from 0.4, searching for a shoal and waiting while
moving (sitting position) to 2.7 for a net withdrawl, is considered as light
labor according to the distribution of hours. For this the calories consumed
due to the fishing operation are 669 for males and 323 for females per operation
(one-time net setting) for the Katakuchi-sardines and 822 calories for males and
665 calories for females for three operations (17 time net setting) for Ayu
fishing. On the other hand, for processing Katakuchi-sardines, from 3.8 for a
fish case transportation (weight: 30 kg) to 7.7 for taking them in/out of
drying storage, is considered as heavy labor. The calories consumed in the
processing work is 1020 for males and 1,268 for females when handling the raw
weight of 1500 kg.

In addition, for the manual work of withdrawing the net, the R.M.R.
is calculated as the sum total value for a series of work, and since the work
on a one-to-one basis is operated under very tense conditions, it is
difficult to set up a model labor, therefore, the values have somewhat inclined
to be lower than the actual ones.

The total calorie consumed per day by the Katakuchi-sardine fishing is
3069 Cal. for males and 2718 Cal. for females per operation, and for two oper-
ations (two net settings), it takes 3254 Cal. for males and 2857 Cal. for females
which is regarded as slightly heavy work. Furthermore, for three operations of
young Aku fishing, it takes 2544 Cal. for males and 2287 for females, which
is regarded as normal work. In addition, an example of the use of the female's
time is shown in Table 18 in which the fishing operation has been interrupted by
bad weather. That is, the total consumed calories per day are 2176 which is
about 100 Cals. less than for a usual fishing day.

(2) Nutritional conditions

The caloric intake classified according to 5 individual categories as
obtained from investigation and according to the food groups is indicated in

TABLE 15. DISTRIBUTION OF WORKING HOURS AND ENERGY CONSUMPTION (KATAKUCHI-
SARDINE FISHING, FOR ONE OPERATION)

Wife, 42-years old

Description	Hrs.	R.M.R.	
Preparation for departure	20 mins.	0.4	
Waiting on the boat(searching for a shoal)	120	0.5	
Preparation for netting	8	1.8	
Netting	15	1.0	
Net withdrawal (machine-operated)	30	2.1	* Cumulative
Net withdrawal (manual)	31	2.7	1593 Cal.
Transport of fish cases (30 kg)	21	3.8	(448 min.)
Assorting	89	4.4	
Taking in/out of drying storage	114	7.7	
Meal	55	0.4	
Working	42	1.8	
Bathing	20	2.0	
Personal things, etc.	189	0.4	
Rest	299	0.2	
Sleep	387	-10%B	
Total per day	2,730 Cal.		

Eldest son, 18 years old

Description	Hrs.	R.M.R.	
Preparation for departure (engine adjusting)	24 min.	0.5	
Steering the boat	202	0.6	
Preparation for netting	33	1.8	
Netting	47	1.0	
Net withdrawal (manual)	16	2.7	
Taking up the haul (fish scooping)	37	2.2	
Transport of fish cases	22	3.8	* Cumulative
Fish drying work	64	6.8	1689 Cal.
Taking in/out of drying storage	76	7.7	(589 min.)
Inspection inside the boat.	31	2.1	
Freezing work to freeze the fish	37	2.1	
Meal	77	0.4	
Working	7	1.8	
Bathing	15	2.0	
Personal things, etc.	45	0.4	
Rest	363	0.2	
Sleep	344	-10%B	
Total per day	3,150 Cal.		

75

TABLE 16. DISTRIBUTION OF WORKING HOURS AND ENERGY CONSUMPTION (KATAKUCHI-SARDINE FISHING, FOR 2 OPERATIONS)

Wife, 42 years old

Description	Hrs.	R.M.R.	
Preparation for departure	27 min.	0.4	
Waiting on the boat (searching for a shoal)	128	0.5	
Preparation for netting	13	1.8	
Netting	13	1.0	* Cumulative
Net rolling (machine operated)	42	2.1	
Net rolling (manual)	43	2.7	1602 Cal
Transport of fish cases (30 kg)	12	3.8	(485 min.)
Assorting	107	4.4	
Taking in/out of drying storage	100	7.7	
Meal	74	0.4	
Working	64	1.8	
Bathing	26	2.0	
Personal things, etc.	205	0.4	
Rest	271	0.2	
Sleep	315	-10% B	
Total per day		2857 Cal.	

Eldest son, 18-years old

Description	Hrs.	R.M.R.	
Preparation for departure (engine adjusting)	43 min.	0.5	
Steering the boat	160	0.6	
Preparation for netting	26	1.8	
Netting	30	1.0	
Net rolling (manual)	36	2.7	
Taking up the haul (fish scooping)	40	2.2	
Transport of fish cases	22	3.8	* Cumulative
Fish drying work	58	6.8	1607 Cal.
Taking in/out of drying storage	67	7.7	(593 min.)
Inspection inside the boat	60	2.1	
Freezing work to freeze the fish	51	2.1	
Meal	81	0.4	
Working	16	1.8	
Bathing	26	2.0	
Personal things, etc.	51	0.4	
Rest	35	0.2	
Sleep	328	-10%B	
Total per day		3,254 Cal.	

76

TABLE 17.   DISTRIBUTION OF WORKING HOURS AND CONSUMED ENERGY (YOUNG ASYU
FISHING, FOR 3 OPERATIONS)

Eldest daughter, 21-years old

Description	Hrs.	R.M.R.	
Preparation for departure	13 min.	0.4	
Waiting on the boat	236	0.5	
Preparation for netting	23	1.8	
Netting	22	1.0	
Net pulling (manual)	81	2.7	
Taking-up the haul (fish scoop assis.)	20	2.2	* Cumulative
Taking up the haul (fish scooping)			665 Cal
Stick work	10	2.1	(479 mins.)
Net withdrawal	20	2.1	
Rowing	19	2.7	
Landing work (and putting in order)	16	1.8	
Transport of fish (bucket)	19	3.8	
On the truck	5	1.2	
Light work on the land (cleaning the truck,etc.)	73	2.0	
Meal	66	0.4	
Working	145	1.8	
Bathing	15	2.0	
Personal things, etc.	25	0.4	
Rest	300	0.2	
Sleep	357	-10%B	
Total per day		2,287 Cal	

Eldest son, 19-years old

Description	Hrs.	R.M.R.	
Preparation for departure	40 min.	0.5	
Steering the boat	263	0.6	
Preparation for netting	4	1.8	
Netting	46	1.0	
Net pulling (manual)	84	2.7	
Taking up the haul (fish scooop assistance)	47	2.2	*Cumulative
Net withdrawal	20	2.1	822 Cal.
Landing work (and putting in order)	21	1.8	(568 min.)
Work in the boat (inspecting inside of boat, etc.)	43	2.1	
Driving the truck	26	1.2	
Light work on the land (cleaning the truck, etc.)	68	2.0	
Meal	64	0.4	
Working	18	1.8	

77

TABLE 17 (CONTINUED):   (Eldest son, 19-years old)

Description	Hrs.	R.M.R.
Bathing	15 min.	2.0
Personal things, etc.	26	0.4
Rest	245	0.2
Sleep	410	-10%B
Total per day	2,544 Cal.	

TABLE 18. DISTRIBUTION OF LIFE HOURS AND CONSUMED ENERGY (IN CASE THE FISHING
IS INTERRUPTED BY BAD WEATHER OR WINTER CONDITIONS)

Eldest daughter, 21 years old		
Description	Hrs.	R.M.R.
Meal	51 min.	0.4
Working	387	1.8
Personal things, etc.	35	0.4
Rest	180	0.2
Driving the truck	90	1.2
Shopping	95	1.0
Light work	40	2.0
On the truck	45	0.4
Attending the meeting	145	0.4
Sleep	372	-10%B
Total per day	2,176 Cal.	

Tables 19 and 20. The most important observation is that fish consumption
for males is very high. The mean value of fish consumption for three days
in summer is 410 g for the head of the household, and 333 g for the eldest
son. Even in February, which is considered the lowest diet consumption month
of the year, the mean value for three days is 286 g for the head of the house-
hold and 361 g for the eldest son, and on the 25th of February, 513 g for the
head of the household and 489 g for the eldest son were recorded as they had a
good haul of fish. These values are well over the 80 g of the target value
for 1975 which is set up by the National Institute of Nutrition. In this
connection, the fish they consume is derived from their fish haul, such
as scabbard-fish, garakabu, mebaru and snipefish that reside in the inland
sea or inland bay. In addition, the vegetable consumption and especially milk
and dairy products is very small, and males eat double the amount of rice and

females eat double the amount of fruits, such as watermelon and citrus fruit as the respective target values. Furthermore, a lack of caloric consumption in general is seen in a 42-year-old who has the Minamata disease and a 12-year-old. A comparison of their caloric consumption with the respective required consumption values as classified by the nutritive elements are shown in Figures 10, 11, 12 and 13. Namely, a similar pattern of caloric consumption exists between the head of the household and the eldest son, and the wife and the eldest daughter, and an inclination of high caloric or high protein due to the large fish and rice consumption notable in males. Females, on the other hand, have a notable lack of Ca, Fe, V-A, V-B_1 and V-B_2. Particularly, the second son who has the congenital disease, shows a caloric consumption of 1,389 Cals, 25.0 g of protein and 36.8 I.V. of V-A in August, and 1,487 Cals, 43.2 g of protein and 19.1 g of fat which indicates an extreme lack of a proper diet.

The caloric ratio is as shown in Tables 21 and 22. Especially, the protein calorie ratio and the animal protein ratio are high for the head of the household and the eldest son, which reflects the large fish consumption, while the second son shows extremely low values as a whole, as mentioned above.

The calories consumed daily were 2800 calories for the eldest son and 1782 calories for the wife in August, and 3041 calories for the eldest son and 1574 calories for the wife and 1487 calories for the eldest daughter in February. When these are compared with the total consumed daily calories aforementioned, there are 200 calories less for the eldest son and 900 calories less for the wife in August, and 500 calories more for the eldest son, 600 calories less for the wife and 800 calories less for the eldest daughter in February.

(3) Environmental conditions

1) Weather conditions

Daily changes in velocity (current), degrees of excess wetness, degrees of excess dryness, blackbulb temperature, temperature and humidity in August and February are shown in Figure 14. These were measured during the day for three consecutive days for the labor investigation period. The weather of the measured days were fair in August and cloudy in February; especially the weather in February was so bad as to interrupt the fishing. In summer, little labor is performed in the sun as the fishing operations occur

TABLE 19. CALORIC CONSUMPTION AS CLASSIFIED BY THE INDIVIDUALS AND BY THE FOOD GROUPS (AUGUST)　　　(Unit: g)

Objectives	45 yrs.				12 yrs.				20 yrs.				18 yrs.				12 yrs.		
August	2	3	4	Avg	2	3	4	Avg	2	3	4	Avg	2	3	4	Avg	2	3	4	Avg
① Rice	413	175	502	465	213	227	333	258	181	274	335	263	294	316	434	346	118	153	121	144
② Bread				120			40	90				30			40	14	100	29		10
③ Noodles	9		3	19			6	37			12	14			5	15				5
④ Potatoes	64		32	32	124		23	49	95		30	125	92		57	49	98			33
⑤ Sugar		13	5	6	31	5		12	7		4	4	4	2		2	24	3		9
⑥ Confectionary			70	24			80	27		160		54			60	20			150	60
⑦ Alch.beverages+soft drink	100	11		138	190		261	150	190		186	125	200	211	518	987	100		116	272
⑧ Oils and fats	18	20	30	23	35	20	24	26	27	10	30	22	10	59	95	32	26	5	3	12
⑨ Soybeans + soybean prods.					27			9	51			18	54			18	18			6
⑩ Soybean paste	10	35	21	22	18	35	15	23	36	68	31	15	36	21	24	27	12	9	17	13
⑪ Other beans														20		7	25			8
⑫ Fruits		735		245	270	565	240	358	210	720	400	443			360	120	440	545	420	468
⑬ Green vegetables		40		14		42		11		132		44	26			9				
⑭ Lightcolored vegetables	11	256	124	137	56	247	56	119	86	227	74	129	161	88	124	124	43	173	12	76
⑮ Seaweeds																				
⑯ Seafood	475	385	371	410	96	96	15	69	110		20	43	309	389	310	333				
⑰ Meat + chicken incl. whale meat	8	20	75	34	20		68	29	13	35	79	42	12	70	252	111	13		44	19
⑱ Egg	11	150		54	38	150		63	50	70		40	54	210	50	314	30	25		18
⑲ Milk																				
⑳ Dairy products					65			21									65	65		43
Totals	1419	2191	1230	1613	1322	1387	1115	1273	1186	1700	1181	1555	1266	4004	2354	2518	1459	1616	1213	1226

TABLE 20. CALORIC CONSUMPTION AS CLASSIFIED BY THE INDIVIDUALS AND BY THE FOOD GROUPS (FEBRUARY)　　　(Unit: g)

Objectives	46 yrs.				43 yrs.				21 yrs.				19 yrs.				13 yrs.		
February	23	24	25	Avg	23	24	25	Avg	23	24	25	Avg	23	24	25	Avg	23	24	25	Avg
① Rice	481	378	473	444	176	300	321	266	279	163	321	254	451	104	540	454	270	207	315	264
② Bread																				
③ Noodles	105		35	99			33	120			40	147			49	119				39
④ Potatoes	49	52		34	44	25		23	53	37	7	32	47		41	29	41	16	22	26
⑤ Sugar	2		2	1	2		28	10	2		31	11	2		1	1	2	30	3	12
⑥ Confectionary		10		4	150			50		200						720				
⑦ Alch.beverage+ soft drink												79	2160							
⑧ Oils and fats	25	31	22	26	17			6	9		1	3	30	13	22	22	16		3	6
⑨ Soybeans + soybean prods.	2		13	5			5	2	2		6	3	3		17	7	2		7	3
⑩ Soybean paste	11	15	46	25		32	35	22	13	19	33	22	23	56	55	45	15	23	15	18
⑪ Other beans							28	9			30	10						30		19
⑫ Fruits	540	250	310	367	315	220	255	263	340	150	125	205		150		50	330	240	420	330
⑬ Green vegetables	8	85		38	7		2	9			3	12			4	10				3
⑭ Light-colored vegetables	161	45	14	73	87	78	28	64	187	82	45	105	138	8		49	113	33	19	55
⑮ Seaweed	1		3	1		1	2	1	1	1	2	1	1	1	4	2			2	1
⑯ Seafood	335	9	513	296	18	82	209	103	82	20	181	84	416	178	489	361	87	13	56	52
⑰ Meat and chicken incl. whale meat	8	28		12	7	13		6	9	20	4	11	12		11	7	10	9	12	10
⑱ Egg	22		137	53	13	50		21	22	50	44	39	29	50	88	56	29	100	50	57
⑲ Milk														200		66				
⑳ Dairy products																				
Totals	1754	903	1533	1401	935	801	911	881	1128	742	830	893	3474	1060	1268	1922	1025	701	122	886

80

45-year, case (head of
household)

42-year, case (infected patient
of the Minamata disease)
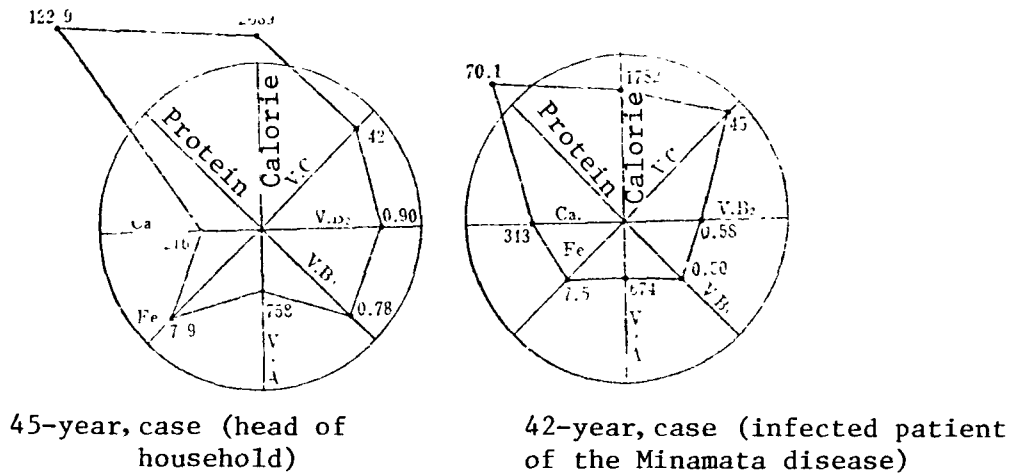
Figure 10. Caloric consumption as classified by the individuals (average for 3 days).

20 year, case

18-year, case

12 year, case (congenital patient of the Minamata disease)
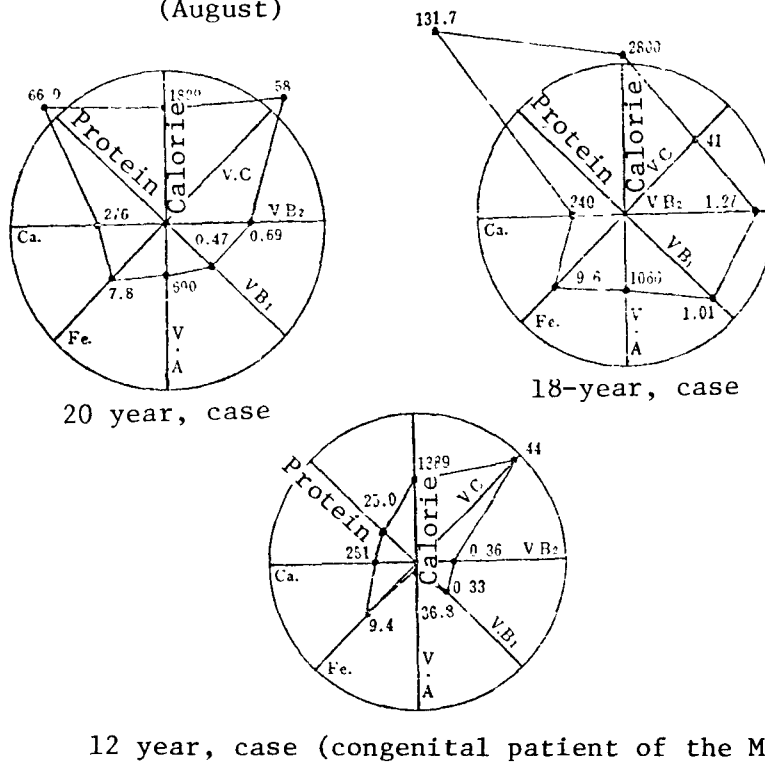
Figure 11. Caloric consumption as classified by the individuals (average for 3 days).

(February)

46-year, case
(head of household)

43-year, case (infected
patient of the Minamata disease)
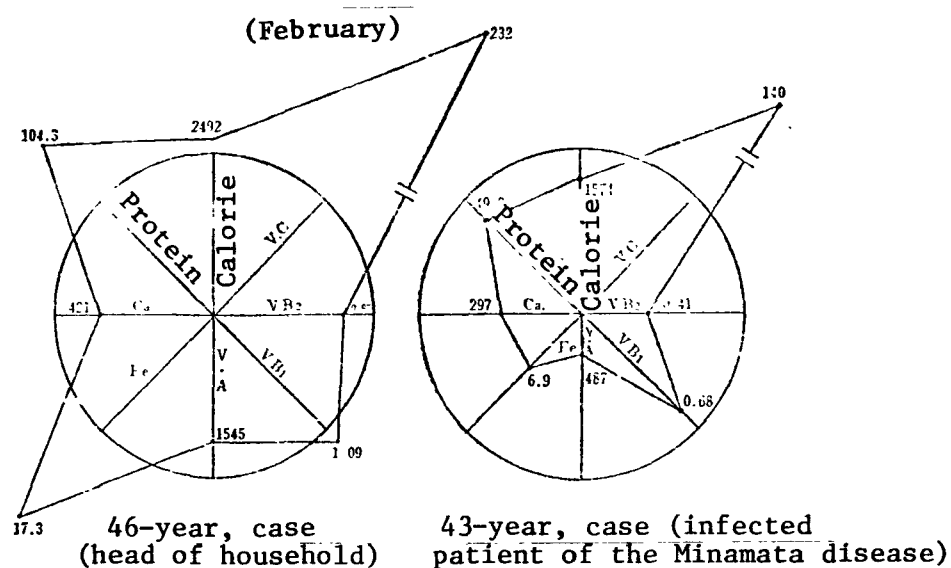
Figure 12. Caloric consumption as classified by the individuals (average for 3 days).

(February)

21-year, case

19-year, case

13-year, case (congenital
patient of the Minamata disease)
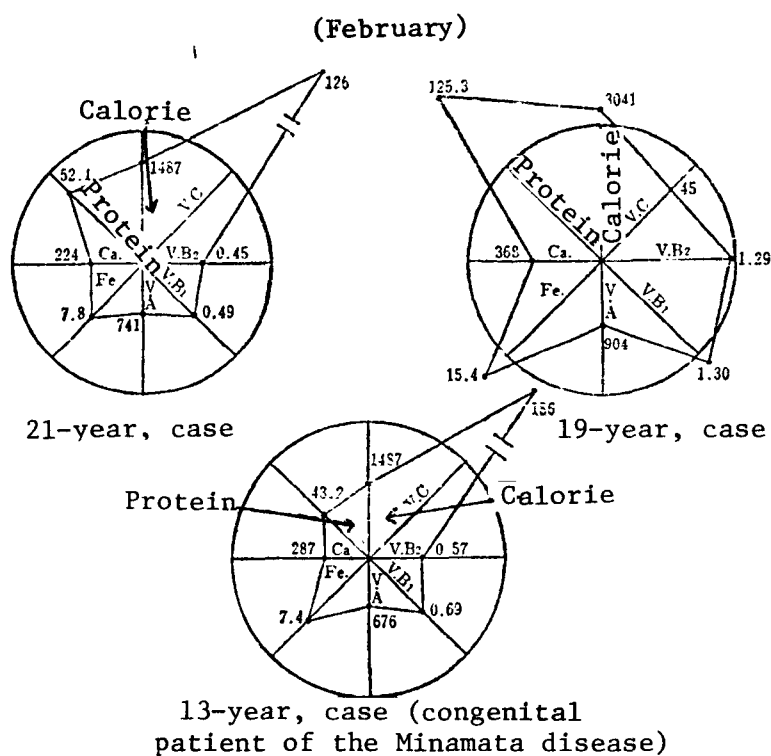
Figure 13. Caloric consumption as classified by the individuals (average for 3 days).

82

TABLE 21. CALORIC RATIO   (August)

Objectives	Cereals cal. ratio	Protein, cal. ratio	Animal protein ratio	Animal fat ratio	Fats, cal. ratio
45 year-male	63	18.3	74	60	14.4
42 year-female	37	15.7	65	28	21.1
20 year-female	58	14.4	31	39	14.3
18 year-male	45	18.1	65	50	20.9
12 year-male	36	7.2	15	6	3.9
Target value for 1975	50 ~ 60%	About 15%	40 ~50%		20 ~ 25%

TABLE 22. CALORIC RATIO   (February)

Objectives	Cereals cal. ratio	Protein cal. ratio	Animal protein ratio	Animal fat ratio	Fats cal. ratio
46-year-male	62	16.7	67	42	19.6
43-year-female	60	12.7	45	41	10.7
21-year-female	61	14.0	51	55	11.4
19-year-male	52	16.5	73	29	26.5
13-year-male	63	11.6	43	49	11.6
Target value for 1975	50 ~ 60 %	About 15%	40 ~50%		20 ~25%

early in the morning or in the evening. In winter, on the contrary, the net withdrawal work takes place in the sea at temperatures from 0 - 10° C and a humidity of 50-60%, which should be considered and evaluated as abnormally low temperature conditions.

2) Noise due to the diesel engine.

As aforementioned, fishing for Katakuchi-sardines is operated by a main boat, two netting boats and a jolly boat, and among which, a diesel engine is

83

equipped in the main boat and the netting boat. The operation of the engine is done in the following 4-steps according to the fishing conditions. Namely, the idling condition (net withdrawal while the boat is stopped, taking up a haul), low speed condition (net setting, net pulling), medium speed condition (moving by searching a shoal, within a relatively short distance) and high speed condition (moving toward a shoal, transporting a haul, for a relatively long distance). Under these 4-steps for operating the engine, the noise is continuous and very stable for it remains at a certain level without intervals while the work takes place. Table 23 shows the extent of the noise in relation to the worker's ears on the main boat and netting boat, the hours exposed to the noise per fishing operation and their distributions. It is distributed between 79 - 83 phons while idling and 114-116 phons while in high speed, and as a whole, the extend of the noise is high. The result of a frequency analysis while idling and in high speed is as indicated in Figure 15, according to the octave analysis. You can see the peak at about 100 Hz, and it retains a high level up to a considerably high frequency range. These noises as an absolute value exceed the permissible sound value (480 min. value, 240 min. value) recommended by the Japan Industrial Hygiene Society. Also, the noise on the fishing boat when the engine is not operated is still as high as 68 - 72 phons which is more than expected, due to the wave sounds or the noise from the other fishing boats. In addition, the noise distribution on the fishing boat is as follows: the muffler part of the engine is naturally the highest which is 116 - 118 phons, 101 - 107 phons in the stem part, 101 - 109 phons in the stern part and 107 - 113 phons in the steering room.

Furthermore, while fishing for young Ayu which is done by one main boat and one jolly boat, there are 78 - 82 phons while idling, 82 - 84 phons at low speed, 85 - 86 phons at medium speed and 88 - 93 phons at high speed.

III. CONSIDERATIONS

The difference between the Minamata disease and methyl mercury poisoning, which is poisoning present in certain occupations in factories, is that the Minamata disease is transmitted to humans through certain foods at the inception
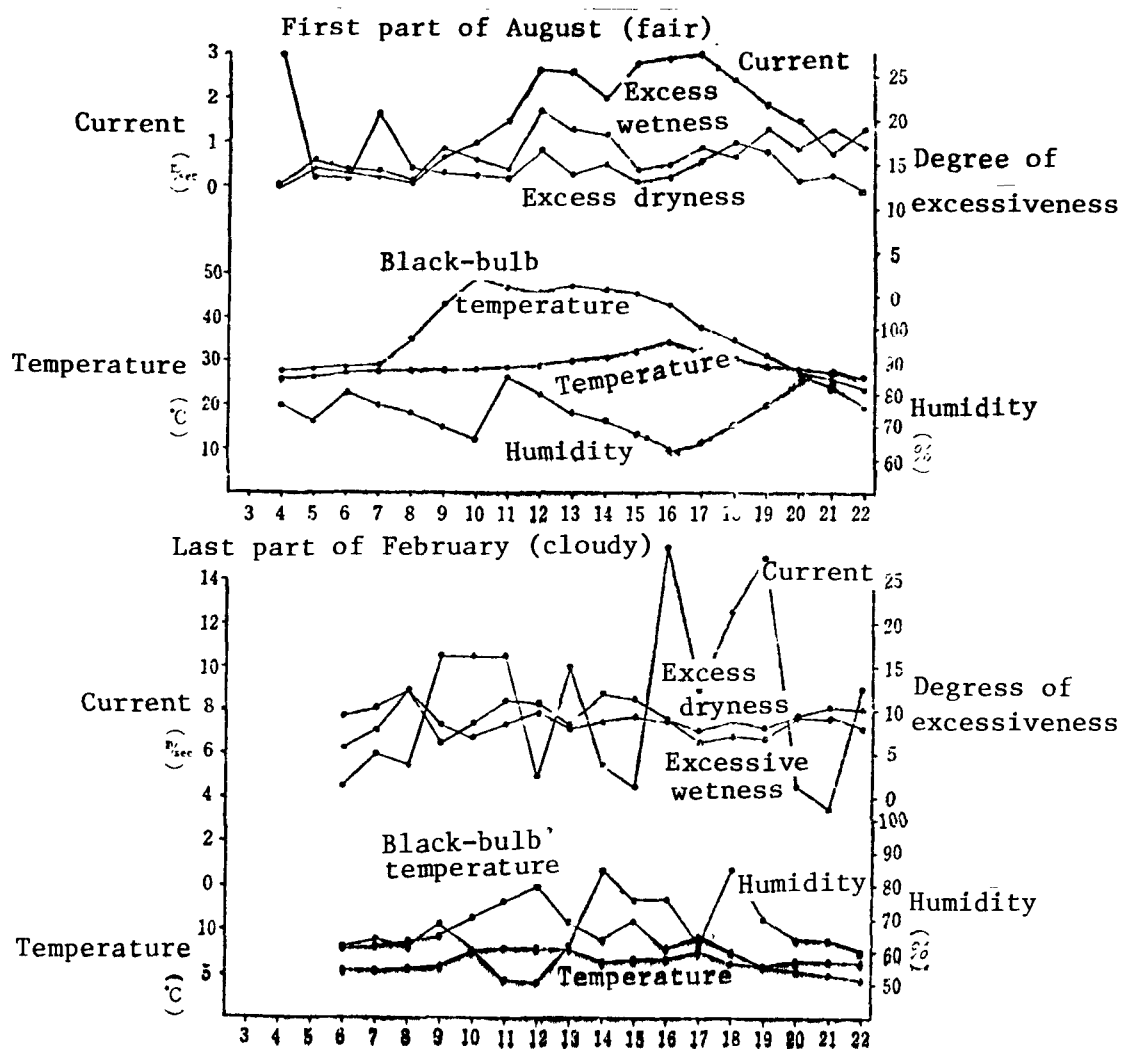
Figure 14. Daily changes in the weather conditions.

(for each fishing operation)

Operation of engine	Netting boat		Main boat	
	Noise level	Exposed hours	Noise level	Exposed hours
Idling	79~83phon	79'51" (36.5%)	79~85	13'55" (15.8%)
Low speed	93~95	46'28" (21.5%)	92~94	37'20" (42.5%)
Medium speed	104~107	90'12" (42.0%)	104~105	36'50" (41.7%)
High speed	114~116		—	0

Remarks   When fishing for 216 mins.   When fishing for 88 mins.
31 secs.   5 secs.
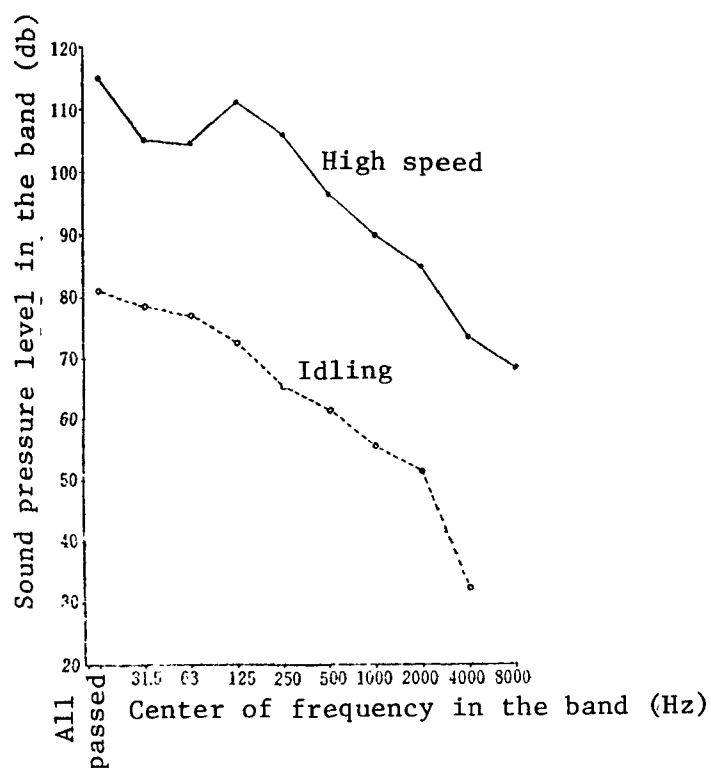
Figure 15. Frequency analysis of noise due to the diesel engine on the
fishing boat.

of the disease [13]. In regards to industrial poisoning in the factory, the content of the poisonous substance in the working environment, the physical or chemical characteristics, the retaining hours and the time lapse are the important problems. In regards to the Minamata disease, the problem is not only the content of mercury in the fish, but also the circumstances surrounding the fish consumption as a condition of exposure [14]. At the same time, an understanding of the actual condition and diets of the fishermen's lives and the Minamata diseased patients is the essential matter for investigating the outbreak of the Minamata disease and its progress afterwards. Furthermore, in order to control the health conditions in the future of the local inhabitants and the Minamata diseased patients, a basic understanding of the fishermen's lives and diets is needed.

The investigation dealing with the fisheries conducted by Kitamura, et al., at the early stage (the interview investigation of a fisherman's family and the analysis of fishery statistics)[15] is considered a worthy study from the above viewpoint. Regarding the above study, we tried to positively grasp by a case study the actual conditions of the fishermen and their families' lives and eating habits. .

Despite the dependence of Japan on seafood for the greater part of protein resources, references concerning inshore fishery studies are very short except for a study by Kitoshi, et al., [16].

The medical labor characteristics for Katakuchi sardine fishing and young Ayu at the Shiranui inshore are described below. That is, R.M.R. does not exceed 3 in any kind of fishing work at the sea. In other words, the work is relatively light as a labor strength. On the contrary, the processing work to make dry fish of the Katakuchi sardine fish is very trying which can be considered heavy work as a labor strength. The distribution of daily hours completely depends on the weather conditions or the ocean conditions such as sea currents, and the individual fishing work requires complete attention. The fishing condition, that is, the estimated haul of fish on any day is unpredictable. Therefore, it is difficult to balance the hours of work with the

hours of rest, which has caused a lot of unevenness in the density of labor, and thus, it is not necessarily easy to construct a definite proportion. However, it seems that a working pattern, which is completely united with the natural phenomena and is not restrained by artificial power, cannot be evaluated by the past labor medical concept.

In this regard, the calorie consumption for the Takakuchi sardine fishing for two operations is equal to bricklaying or moulding work and for young Ayu fishing, it is equal to keypunch work [17].

In addition, there is a report [17] in regard to the labor strength inshore fishing, in which the total calorie consumption per day is reported to be 2,580 cal. for a fisherman who operates the net fishing in the Yamaguchi Prefecture. However, this value is less than Katakuchi sardine fishing and approximately equals the total calorie consumption per day in young Ayu fishing which is investigated by us.

From the results of our investigations this time, we can cite significant problems below due to environmental conditions such as the noise of the diesel engine and the work at sea under low temperature conditions in winter. The noise of a diesel engine for a fishing boat shows a slightly higher level than the noise of a diesel engine for a bus or truck, which was investigated by Itoh, et al., [18]. However, they are similar when viewing the contribution of spectrum. As indicated in the report of 1971 by this study group, noise and the exposure are enough to cause deafness or cause a hearing disturbance which is suspected of the noise deafness. In regard to working at sea under low temperature conditions in winter, there is a report [19] which mentioned that the circulating of the finger periphery of fishermen, who operate the net fishing on the icy water of the northern sea, is superior to that of the controlled people, and the partial cold-resistance is high therefore. We intend to further investigate by observing the function as well as the relation of the peripheral nerve function.

Next, we describe the fishermen's diets, especially as regards fish consumption. Of late, in the controversy on the interim restrictive value

relative to the safety standard of the P.C.B., it has been discussed how
to estimate the amount of exposure of fishermen considered as consuming lots
of fish. Then, in order to set the interim restrictive value, we used the
national mean value classified by the classes based on the People's Nutrition
Investigation of 1968 as a basic reference. According to that mean value, the
seafood intake is 75.9 g min/day, 139 g max/day and 86.3 g av./day and the
intake ratio of inshore and pelagic fish is respectively 57.0 g min/day and
189 g min/day, 98.2 g max/day and 32.7 g max/day, 64.7 g/av/day and 21.6 g av/day
[20], about which the comment is added that the intake value may not exceed
200 g, however, all of them are fishermen. The problem here is at what level
the maximum consumption is judged. In the examples of fishermen of the Shiranui
inshore who consume the inshore fish, that is, the seafood of their own produce,
the average net consumption for three days is from 286g day in winter to 410 g
day in summer, which is far above the estimated value by the Investigation
Committee of Food Sanitation. According to the preinvestigation conducted in
February of 1972 prior to the investigation this time, in which 4 fishermen and
non-fishermen families of the Minamata district seacoast were examined by the
self entry method, it was observed that the fish consumption is about 200 g even
in the non-fishermen's families. From these results, a reinvestigation regard-
ing the fish consumption condition is required in accordance with the distri-
bution channel of seafood by focusing on the fishermen's families. Upon which,
the reestimation of the present safety standard and the present contamination
condition due to the fish products in the Minamata Bay area is required,
besides it is considered that the dose-response relating to the effect of
methyl mercury on the living body should be reinvestigated.

On the other hand, the wife, especially, and the second son who have
suffered from the Minamata disease, generally show an imbalance in the caloric
consumption plus a shortage of absolute caloric amount is observed. In this
regard, the second son who is affected by the congenital Minamata disease has
a caloric intake of 1389 in summer and 1487 in winter which slightly exceeds
the base metabolic amount of 1287 cal. per day for that age. Therefore, strict
health control measures of their whole daily life should be investigated promptly,
not only for those stricken by the congenital Minamata disease who are in the

physical and mental growth period, but also for all those stricken by the
Minamata disease.

Based on our present investigation, we shall continue to collect the
basic references relating to the fishermen's families along the Shiranui
seacoast by investigating the actual conditions of the fishermen's labor for
net fishing and their life environment.

CONCLUSION

First, we tried to find out the effect of the environmental contamination
due to the factories' waste waters containing organic mercury on the health
standard and the causes of death in the said districts. Namely, we investigated
the health indicators as well as the classification of the causes of death
based on the information in the death certificates and the medical death
certificates of those who had lived in the said districts during the 24 years
from 1948 to 1971. The results are as follows.

1. In the Minamata district, the stillbirth rate is slightly higher and
the frequency of mortality by age group is less in the older age group as com-
pared with the Ariake district, which is considered nearly free of contamina-
tion.

2. The PMI and the death rate of the old age group over 65-years are
respectively 62.4% and 41.9% for males, 64.5% and 46.1% for females, and
those for females are significantly (statistically) less than in the
Ariake district which are respectively 70.3% and 51.9% for males, 76.0% and
62.3% for females, and even the corrected PMI changed by correcting the ages,
the females show younger values than in the Ariake district.

3. The average death age is 50.36 ± 28.91 years for males and
52.50 ± 29.49 years for females in the Minamata district which are younger
than 56.56 ± 27.74 years for males and 61.22 ± 28.06 years for females in
the Ariake district, and even of the corrected average death ages, the females
show slightly younger values than in the Ariake district.

4. When compared, the construction of cause of deaths classified by the group of cause of deaths, Minamata district has less groups of deaths by adult disease both in male and female than Ariake district, and contrary, more groups of deaths by non-disease in male and by bacterial infection in female.

5. A significant difference was observed between the districts when the main causes of deaths were compared. That is, in the Minamata district, the deaths are mostly caused by inflammation of the intestines, other laxative diseases, car accidents and other unexpected accidents (mostly by drowning) for males, and tuberculosis, meningitis and other unexpected accidents (mostly by drowning) for females, while less deaths by senility (not accompanied by mental disease), non-inflammative diseases of the nerve center system for males, and cardiac diseases and senility (not accompanied by mental disease) for females were reported.

Secondly, we investigated the results of the inhabitants' examinations in the Minamata, Goshoura and Ariake districts in order to study the way of inhabitants examination of Minamata disease, and the results are as follows:

1. We pointed out the validity and limitation in the screening examination method of the Minamata disease based upon complaints of patients. The validity of screening is approximately 80% in sensitivity and approximately 60% in specificity when using combinations of the 4-item complaints, which are disorder of perception, stricture of visual field, disorder of movement and hearing disturbance which are considered important for diagnosing the Minamata disease.

2. As a result of investigating the validity of the screening process of the Minamata disease in regards to the examination of some organs, several problems are pointed out for selecting examination items and the application of the screening level, and a reinvestigation of the above points and their combinations are required.

91

Thirdly, for the purpose of grasping the actual conditions of the fisher-
men's labor and their diet along the Shiranui seacoast, we conducted investiga-
tions of the actual conditions of a fisherman's family (Minamata disease
patients) in the Tsunaki-machi, Ashikita-gun, Kumamoto Prefectures during the
fishing seasons of the Katakuchi-sardine and the young Ayu.

The results are:

1. The labor strength for the labor at sea is relatively light;
however, the daily caloric consumption including the processing work is
slightly heavy or corresponds to normal work, and the distribution of life
hours is irregular.

2. The working environment, particularly the noise due to the
operation of the diesel engine, and the sea labor under low temperature con-
ditions in winter need to be noted.

3. Fish consumption of fishermen's families is far above the estimated
values by the Investigation Committee of Food Sanitation, etc.

4. The daily caloric consumption of those stricken by the Minamata
disease is apparently low as a whole, and from such a viewpoint too, it is
pointed out that the daily health control measures of the patients should be
promptly investigated.

REFERENCES

1.   Shigeru Nomura, et al. Progress of Minamata Disease and its Epidemic
          Study. The Results of Health Examinations of Inhabitants in
          Minamata and Goshoura districts (Part 1). The report written by the
          investigation group on the "Minamata Disease Ten Years Hence", Medical
          Department, Kumamoto University, 1972, pp. 6 - 36.

2.   Swaroop, S. and K. Uemura. Proportional Mortality of 50 Years and Above.
          Bull. Wld Hlth. Org., Vol. 17, 1957, pp. 439-481.

3. WHO Study Group. Report on the Measurement of Levels of Health.
    Wld Hlth Org. techn. Rep. Ser. No. 137, 1957.

4. Toshiaki Miyoshi, Manzaburo Okamoto: The Significance of the
    Average Death Age as a Death Index Classified by Causes of Deaths,
    National Hygiene, Vol. 23, 1954, pp. 73 - 82.

5. Ministry of Health and Welfare, Statistics and Survey Division:
    The Comparison of Causes of Deaths between Pre-War and Post-War
    Ages According to the Life Table, Index of Welfare. Vol. 15, 1968,
    pp. 37 - 43.

6. Kazuo Shirosaki, et al.: The Corrected Average Death Age as a
    Health Index. People's Hygiene, Vol. 36, 1970, pp. 123-129.

7. Haruo Katsunuma, Akira Koizumi. The Conditions of Applicability of
    Proportional Mortality. Indicator (Kamimura-Swaroop) as a Health
    Index for a group of Inhabitants, People's Hygiene, Vol. 28, 1962,
    pp. 101-105.

8. Tadao Takeuchi, et al.: a) Minamata Disease Ten Years Hence and its
    Change. Japan Medical News Report, No. 2402, 1970, pp. 22-27:
    b) Subclinical Minamata Disease — from the Records of Dissection
    by Dr. Hisashi Matsumoto — Short Commentary Report of Dissection,
    Saikai Medical Report, Vol. 252, 1969, pp. 9-12.

9. Wilson, J. M. G. and G. Jungner. Principles and Practice of Screening
    for Disease. Public Health Papers (WHO) No. 34, (Japanese transla-
    tion), 1968.

10. Haruhiko Tokuomi. Clinics of Minamata Disease — Minamata Disease in
    Adults. Minamata Disease (Compiled by the Investigation Group of
    Medical Department, Kumamoto University, 1966, pp. 48-81.

11. Tadao Tsubaki, et al. A Clinical Diagnosis of Organic Mercury
    Poisoning along the Agano River Coast, Progress of Neurology,
    Vol. 13, 1969, pp. 85-88.

12. Masayori Tatetsu, et al. A Neuro-Psychiatric Study of the
    Minamata Disease — A Clinically Epidemiological and Symptomatological
    Study of Minamata Disease. The Report written by the Investigation
    Group of the "Minamata Disease Ten Years Hence" of the Medical
    Department, Kumamoto University, 1972, pp. 41-65.

13. Shoji Kitamura. Epidemiology of the Minamata Disease — Epidemiological
    Approach of Organic Mercury Poisoning, The Latest Medical Science,
    Vol. 26, No. 10, 1971, pp. 1966-1972.

14. Shigeru Nomura. Occupation Disease and Pollution Disease as an
    Occupational Illness, Science, Vol. 41, No. 3, 1971, pp. 138-145.

15. Shoji Kitamura, et al.   The Results of an Epidemiological Investi-
gation Relating to the Ill-defined Disease of the Nerve Center
System which Occurred in the Minamata District.   Journal of
Kumamoto Medical Society.   Vol. 31, (Supplement 1), 1957, pp. 1-9.

16. Yoshihito Kitoshi.   The Investigation Report of Fishery Labor of
Net Fishing for Sardines at Nobomura, Nishikanokine-gun, Nagasaki
Prefecture, Fisheries Agency, 1952.

17. Kokichi Numajiri.   Energy Consumption of the Working People, Institute
for Science of Labor, 1972, pp. 23-24.

18. Takeshi Ito.   Noise and Its Control, Corona Co., 1959, pp. 44-51.

19. Nelms, J.D., et al.   Cold Vasodilatation and Cold Acclimatization in
the Hands of British Fish Filleters.   J. Appl. Physiol., Vol. 17, 1962,
pp. 444-448.

20. Investigation Committee of Food Sanitation: The PCB Regulation for the
PCB Remaining in Food (Report), 1972.

21. Shoji Kitamura.   Accumulation of Agricultural Medicines and Heavy Metals
in the Human Body and its Significance.   Mercury, Journal of Japan
Public Hygiene, Vol. 18, No. 10, 1971, pp. 126-127.

22. Ferrer, H.P.   Screening for Health.   Theory and Practice.   Butterworths,
London, 1968.

4. A STUDY CONCERNING EFFECTS OF CONSUMING FISH CONTAMINATED BY
   ORGANIC MERCURY ON RESIDENTS ALONG THE SHIRANUI SEA

Neuro Psychiatry Department, Kumamoto University Medical School

Group member: Masatoshi Tachitsu

Research Assistants: Kazuo Seida, Masasumi Harada, Taichei
Miyakawa, Hisao Tomonari, Satoshi Inoue, Toru Teraoka,
Shiro Sumiyoshi, Eiichi Murakami, Fumie Yasuoka, Kiyoshi
Nakamura, Motonori Deshimaru, Takeshi Tsukiyama, Noriuki
Hotta, Shozo Egami, Teruo Hirahara, Ryuichi Minami, Noritake
Tanaka, Hideo Hattori, Shotaro Hanawa, Kosuke Kono, Hideaki Goto,
Tadashi Fujino, Hitomi Goto, Toshio Fujimoto, Koichi Fujiwara,
Norihito Koga, Shunsuke Kuwabara, Toshio Fujioka, Noriko Hotta,
Kanji Matsumoto, Keikichi Kashima, Tomosuke Sakai, Kou Shikai,
Masahiro Nishi, Tadashi Kuramoto, Yoriko Fujimoto, Minhei Miyakawa

The aim of this study is to clarify the number of patients and their
health conditions resulting from organic mercury poisoning among people along
the Shiranui Sea, and to provide information on planning living situations and
medical relief for the patients and on preventing the outbreak of the disease.
At the same time, this study aims to clarify problems concerning the stages of
organic mercury attacking the human body from the clinical standpoint.

In order to achieve the above objectives, the following were done —
simultaneous examination of residents and attempts at treating organic mercury
poisoning patients detected from the examination; investigation of the
poisoning conditions of people who were moved out to some other prefectures
from the areas contaminated by organic mercury; analysis of the relation-
ship between the occurrences of poisoning and the amount of mercury in
residents' hair and fish in the contaminated areas.

PART 1

SIMULTANEOUS EXAMINATION

Chapter 1. Subjects and Methods of Investigation

Residents in Tsukinoura, Detsuki and Yudo of Minamata City and Arashiguchi, Koshiji, and Sotohira of Goshoura town, Tengusa County were selected as subjects for the investigation. The Minamata area mentioned above is considered to be the area which was most contaminated by organic mercury in the past. Actually, prior to the investigation, i.e., between December, 1956 and July, 1971, 67 persons out of 85 (78.3%) who were determined by Kumamoto prefecture to be suffering from organic mercury poisoning, i.e., Minamata disease, were from the Minamata area. Twenty-six persons out of these 67 (38.8%) were already dead. Health conditions of residents in Goshoura, which is only 16.5 km away across the sea from Minamata City, were also considered to be affected by organic mercury. In fact, in January, 1972, two persons among residents of Oura, Goshoura town, were determined to have the Minamata disease. For the purpose of comparing with these two areas, residents in Akasaki, Sugo, and Oura of Ariake town, Tengusa county, of the southern shore of the Ariake Sea, were selected. People in these areas were considered at first not to be contaminated by organic mercury.

Table 1 illustrates the number of people registered in these areas and their ages. According to Table 1, the ratio of people over 65 in these areas is high. For the Goshoura and Ariake areas, the ratio of children under 14 is high and that of working age people between 15 - 64 years old is low, and the ratio of people between 20-29 is remarkably low, compared with that of the entire nation. For the Minamata area, the ratio of the labor force is almost the same as that for the entire country.

The investigation of residents was done in about a year and a half. First, it began by asking people to list their health conditions on questionnaires sent from the Public Hygiene Department of the Medical School of the Kumamoto

96

TABLE 1. AGE STRUCTURE OF PEOPLE IN INVESTIGATED AREAS

| Age | Total residents | | | | | | Examined residents | | | | | | Entire country |
| | Minamata | | Goshoura | | Aviake | | Minamata | | Goshoura | | Ariake | | |
	Actual No.	%	Actual No.	%	Actual No.	%	Actual No.	%	Actual no.	%	Actual No.	%	%
0 – 4	81	7.2	140	7.6	67	5.8	39	4.2	131	7.6	49	5.4	8.5
5 – 9	79	7.1	149	8.1	121	10.4	62	6.7	146	8.5	111	12.3	7.9
10 – 14	103	9.2	263	14.3	147	12.6	95	10.3	255	11.8	130	11.4	7.6
15 – 19	100	8.9	108	5.9	56	4.8	69	7.5	96	5.6	22	2.4	8.7
20 – 24	53	4.7	65	3.7	40	3.4	33	3.6	58	3.4	20	2.2	10.4
25 – 29	65	5.8	51	1.6	36	3.1	17	5.1	67	3.9	24	2.7	8.7
30 – 34	67	6.0	100	5.4	63	5.4	57	6.2	90	5.2	44	4.9	8.1
35 – 39	73	6.5	108	5.9	70	6.0	68	7.4	99	5.7	53	5.9	7.8
40 – 44	99	8.8	112	6.1	83	7.1	84	9.1	104	6.0	65	7.2	7.1
45 – 49	68	6.1	120	6.5	72	6.2	64	6.9	112	6.5	54	6.0	5.7
50 – 54	77	6.9	120	6.5	67	5.8	70	7.6	110	6.4	51	5.6	4.6
55 – 59	72	6.4	125	6.8	82	7.0	70	7.6	117	6.8	64	7.1	4.3
60 – 64	57	5.1	100	5.4	73	6.3	52	5.6	96	5.6	64	7.1	3.8
65 – 69	46	4.1	76	4.1	63	5.4	40	4.3	73	4.2	56	6.2	2.9
70 – 74	41	3.7	72	3.9	61	5.2	39	4.2	71	4.1	49	5.4	2.1
75 – 79	22	2.0	49	2.7	35	3.0	21	2.3	49	2.8	27	3.0	1.2
80 – 84	11	1.0	35	2.0	21	1.8	11	1.2	33	1.9	15	1.7	0.6
85 – 89	3	0.3	11	0.6	5	0.4	3	0.3	11	0.6	3	0.3	0.2
90 – 94	0	—	1	0.1	2	0.2	0	0	1	0.1	2	0.2	0.1
95 – 99	2	0.2	4	0.2	1	0.1	0	0	4	0.2	1	0.1	0.01
Unknown	1	0.1	—	—	—	—	—	—	—	—	—	—	—
0 – 14	263	23.5	552	29.9	335	28.8	196	21.4	532	30.9	290	32.1	23.3
15 – 64	731	65.3	1045	56.6	642	55.1	614	66.5	949	55.1	461	50.0	69.1
Over 65	126	11.3	248	13.4	188	16.1	114	12.3	242	14.0	153	16.9	7.1
0 – 15	283	25.3	584	31.7	356	30.6	212	23.0	561	32.6	301	33.3	25.7
16 – 40	356	31.8	458	24.8	269	23.1	274	29.7	461	33.3	166	18.6	43.6
Over 41	481	43.0	803	43.5	510	46.3	438	47.3	761	44.2	433	48.1	30.7
21 – 50	432	38.6	600	32.5	372	31.9	360	38.9	537	33.3	269	32.7	46.6
Over 51	316	28.2	555	30.1	393	33.7	293	31.7	551	32.0	319	35.3	18.5
0 – 10	171	15.3	327	17.7	215	18.5	111	12.0	315	18.3	154	20.4	17.9
Over 11	949	84.7	1518	82.3	950	81.5	813	88.0	1408	81.7	720	79.6	82.1
5 – 30	418	37.3	686	37.2	412	35.4	321	34.7	636	36.9	315	34.6	44.9
Over 31	621	55.4	1019	55.2	686	58.9	564	61.0	956	55.5	540	59.7	55.0
0 – 99	1120	—	1845	—	1165	—	924	—	1723	—	904	—	—

Note: Age structure of entire population in the country is from a census in 1970. The total number is 104,665,171.

University (initial examination). Then as the first simultaneous
examination, the direct examinations of all the residents were carried out
by members of this Neuro-Psychiatric Department and the Pediatrics Department,
by referring to the items entered in the above questionnaire (Table 2). Neuro-
psychiatric examinations were done from August 21-28 in the Goshoura area, from
September 7-12 in the Minamata area, and from October 22-26 in the Ariake area.
However, the examinations continued until January, 1972 in order to supplement
them. The results of this first examination were reported in "Epidemical,
Clinical and Pathological Studies of the Minamata Disease after 10 Years",
issued in March, 1972.

Since April, 1972, the examination has continued in the Minamata area,
especially focused on people who were not examined during the first medical
examination. However, in the examination mentioned above, many cases were
observed to be difficult to diagnose. Therefore, in order to accurately
diagnose, as well as to establish the conditions of the disease in detail,
with more precise examinations, the second simultaneous medical examination
(the third examination) was held. Subjects for the examination were pri-
marily people who were diagnosed or suspected of organic mercury poisoning, or
people in whom the effects of organic mercury were detected during the first
examination (Table 2). The examination at each area was carried out as
follows: Ariake area: subjects were examined during July 27, 1972
and March 23, 1973 by either asking them to come to the area elementary
school or visiting them in their homes; Goshoura area: subjects were
examined during September 2, 1972 and February 28, 1973 by either asking them
to come to the area elementary school or day care center or by visiting them
in their homes in case they could not come; Minamata area: subjects were
examined during October 25, 1972 and March 14, 1973 by either asking them to
come to the Minamata City Hospital, or visiting them in their homes when they
could not come to the hospital. Table 2 illustrates the number of people
who were examined. Also, x-ray pictures of their spines were taken at
the Radiology Department of the hospital attached to the Kumamoto University
Medical School for people who were suspected of spinal diseases. The date
and the number of subjects for this case are as follows: Ariake area patients:

TABLE 2.   NUMBER OF RESIDENTS AND NUMBER OF EXAMINED PERSONS

Area	Total No. of residents			Persons examined 1st time			Persons examined 2nd time		
	Male	Fem.	Total	Male	Fem.	Total	Male	Fem.	Total
Minamata	524	596	1120	425	540	965	144	169	313
Goshoura	895	950	1845	819	904	1723	62	71	133
Ariake	564	601	1165	410	494	904	12	15	27

10 persons between July 27, 1972 and August 2, 1972; Minamata area patients:
39 persons between February 16, 1973 and February 28, 1973; Goshoura area
patients: 35 persons between March 2, 1973 and March 7, 1973. Also, since
it was felt that in the Minamata area many people suffered from recurrent liver
diseases, several persons among those who were examined during the second
simultaneous medical examination were examined for alkaline phosphatase in
their blood, GOT, GPT, and cholesterol counts. Also, some of the patients
examined by the Neuro-Psychiatric Department were examined by specialists from
the Ophthalmology and the Otorhinolaryngology Departments of Kumamoto Univer-
sity. The contents of this report are the result of the investigation men-
tioned above.

The Minamata area is further divided into three: Tsukinoura and Yudo are
directly facing the sea, whereas Detsuki does not have a shore line. Village A
of Tsukinoura surrounds the inlet and is located approximately 1,700 m at a
direct distance from the outlet of the drainage of the nitrogen plant. This
inlet is considered to be one of the worst areas contaminated by organic
mercury. Among people in Village A, 4-5 persons are fishermen and the rest
are engaged in work, such as ship carpentry, stone masonry, and temporary labor.
They are poor, and few are farmers. The "Minamata disease" was first noticed
here and patients were isolated in the contagion hospital. Their parents and

siblings were also isolated from their neighbors. Other parts of Tsukinoura contain farm land and from the center of the land to the shore of Village A is about 200 m in a direct line. Many people in Detsuki engage in farming or are employed by the nitrogen plant. The distance from the center of Detsuki to Village A is 500 m in a direct line. Yudo also faces the inlet surrounding the Fakuro Bay and the distance between this inlet and the plant drainage is approximately 2,250 m. One-half of the people in this area are engaged in farming, and the rest in fishing. The distance between the center of this area and the shore is about 100 m. It is believed that currently the people engaged in fishing are about 1/3 of the total population.

Chapter 2. Results of Investigation

Section 1. Rate of Persons Having the Disease

The examinations of residents were continued from the beginning of the first simultaneous examination until the end of the second simultaneous examination, i e., from August 21, 1971 until March 14, 1973. Table 3 illustrates the frequency of the actual number of persons having diseases against the total number of people who were examined; i.e., disease rate, in the areas examined during the period, grouped by types of neuro-psychiatric diseases.

The frequencies of total neuro-psychiatric patients are 59.48 ± 1.58% in the Minamata area, 27.63 ± 1.08% in the Goshoura area, 31.30 ± 1.54% in the Ariake area, and 10.30 ± 0.62% in Heianza Island, Okinawa [3]. The difference between the number at each area of this investigation and that at Heianza Island is significant $(\chi^2 > 528.23,\ P < 0.001)$. Similarly, the differences between the number for the Minamata area and that for the Goshoura area, and between the Minamata area and the Ariake area are significant $(\chi^2 > 283.71,\ P < 0.001)$. Residents of Heianza Island are either farmers or fishermen. 2,379 of the residents centered around Herian were all examined together in 1965 neurologically and psychiatrically by a team of the Neuro-Psychiatry Department of

Kumamoto University.

These three areas including the Ariake area contains patients who exhibit the symptoms of the Minamata disease. The frequencies of subjects who were diagnosed as having the Minamata disease in these three areas are 28.50 ± 1.45%, 1.97 ± 0.33% and 0.89 ± 0.31%, respectively. The differences between these numbers are significant for the Minamata and the Goshoura areas ($\chi^2 \gtrsim 121.61$, P<0.001) and for the Minamata and the Ariake areas ($\chi^2 \geq 273.92$, P<0.001) but the difference is insignificant for the Goshoura and the Ariake areas ($\chi^2 \approx 3.76$, P<0.05).

If the Minamata area is further subdivided into smaller areas based on the distance from the shore, depending on the fish consumption, etc., the frequency of the Minamata disease is 74.58 ± 5.67% at Village A, 26.17 ± 3.01% at Tsukinoura excluding Village A, 32.34 ± 2.33% at Yudo, and 15.52 ± 2.13% at Detsuki (Table 4). The differences among these numbers are significant among Village A and the other areas $\chi^2 \approx 2.87$, P<0.001). Also, the differences between the numbers for Detsuki and Tsukinoura (excluding Village A), and between Detsuki and Yudo are significant ($\chi^2 \approx 25.39$, P<0.001). However, any significant difference between the numbers for Tsukinoura (excluding Village A) and Yudo cannot be proven ($\chi^2 \gtrsim 2.33$).

The frequency of people with the Minamata disease as well as with a suspicion of the Minamata disease, is 83.05 ± 4.88% at Village A, 32.24 ± 3.23% at Tsukinoura excluding Village A, 35.07 ± 2.38% at Yudo and 18.62 ± 2.29% at Detsuki (Table 4). The difference between the number for Tsukinoura (excluding Village A), Yudo, and Detsuki is significant ($\chi^2 \gtrsim 71.10$, P<0.001). The difference between the numbers for Tsukinoura (excluding Village A) and Yudo and that of Detsuki is also significant ($\chi^2 \geq 25.28$, P< 0.001).

TABLE 3. DISEASE RATE

		Minamata area		Goshoura area		Ariake area	
		Actual No.	%	Actual No.	%	Actual No.	%
Total residents		1120	–	1845	–	1165	–
Examined persons		965	86.1	1723	93.4	901	77.3
Persons with nervous and mental disorder	Total disorder	573	59.4	476	27.6	282	31.3
	Minamata disease	275	28.5	34	2.0	8	0.9
	Suspected Minamata disease	38	3.9	31	1.8	2	0.2
	Deferred diagnosis	15	1.6	29	1.7	6	0.7
	Other diseases	221	23.9	382	22.2	266	29.5

TABLE 4. EXISTENCE RATE OF ORGANIC MERCURY POISONING (MINAMATA DISEASE) AND ITS SUSPECTED PATIENTS AMONG PEOPLE IN INVESTIGATED AREA

Investigated area		Examined residents	Minamata disease		Minamata disease + its suspect	
			Actual No.	%	Actual No.	%
Minamata	Village A	59	44	74.6	49	83.1
	Tsukinoura excluding Village A	214	56	26.2	69	32.2
	Yudo	402	130	32.3	141	35.1
	Detsuki	290	45	15.5	54	18.6
	Total examined areas	965	275	28.5	313	32.4
Goshoura		1723	34	2.0	65	3.8
Ariake		901	8	0.9	10	1.1

102

The frequencies of patients of other diseases, i.e., people who do not show the symptoms of organic mercury poisoning, are 22.90 ± 1.37%, 22.17 ± 1.00%, and 29.52 ± 1.52%, respectively, for these three areas (Table 3). The differences between these numbers are significant for the Minamata and the Ariake areas ($\chi^2 = 10.59$, $P < 0.01$) and for the Goshoura and the Ariake areas ($\chi^2 = 17.19$, $P < 0.001$). However, no significant difference between numbers for the Minamata and the Goshoura areas is proven.

Section 2.   Classification of Acquired and Hereditary
              (Congenital) Minamata Disease and Combination
              with other Mental Diseases

Minamata disease patients or suspected Minamata disease patients mentioned above are further divided as follows:

In the Minamata area:

Acquired Minamata disease: 268 persons

Hereditary or congenital Minamata disease: 6 persons

Hereditary + acquired Minamata disease?, or

weak mind + acquired Minamata disease? :   1 person

Among hereditary Minamata disease patients, one weak mind + acquired Minamata disease patient (female born in November, 1941), one mental breakdown + acquired Minamata disease (30 years old) are included. The former was born in Goshoura, moved to Yudo at the age of 8; mental retardation was observed right after her birth; at the examinations in September and December, 1972, the following were proven: mental retardation, noticeable loss and dullness of the senses around the mouth and the extremities of four limbs, loss of hearing, loss of balance, speech problem, etc.; her mother (55 years old) is also a Minamata disease patient. The latter is a female born in March, 1942 in Yudo; when she was a sophomore at a senior high, she fell ill with the suspicion of the disease, later entered mental hospitals several times, such as the Psychiatry Department of Kumamoto University, diagnosed as nervous

breakdown; on the examination in February, 1972, the following were proven:
cold and superficial feeling, decline in mental alertness, jerky motion and
motionless style, phony laugh, stiffening of feeling, problem in personal
relations, dullness in the extremities of four limbs, minor loss of balance;
both father (57 years old) and mother (49 years old) were diagnosed as having
the Minamata disease. One last example of difficulties in determining
whether the mental retardation which was estimated to start at the beginning
of the infancy was due to organic mercury poisoning or not, is as follows:
born in April, 1957, in Yudo; slow in starting walking and the grades at the
elementary school were low; had epileptic fits since the sixth grade; the
conditions in December, 1972 were: mental retardation, narrow centripetal
vision, high degree of dullness in senses at the extremeties of four limbs,
loss of balance, outbreak of the θ wave in brain waves, jerky motion, body
stiffening, cold feeling, inability to be friendly, etc.

In the Goshoura area:

    Acquired Minamata disease: 34 persons
    Hereditary + acquired Minamata disease?   or
        weak mind + acquired Minamata disease?   1 person
    Suspicion of hereditary Minamata disease: 1 person

An example of difficulties in determining whether the disorder in
mental development which was noticed at the early infancy is due to organic
mercury poisoning or not is a female born in November, 1957; started walking
at the age of 3, did not speak at all; at the examination in September,
1972, the following were proven: mental retardation, deaf-mute, excessive
centripetal vision narrowness, high degree of dullness in the senses of
aching and touching around mouth and the terminals of four limbs, loss of
balance in moving. This patient's mother (38 years old) has a high degree of
dullness in the senses of aching and touching at the extremities of upper and
lower limbs and minor loss of balance, but does not have subjective symptoms;
thus unclear when the disease occurred. The 14-year-old patient mentioned above
was included among the patients with acquired Minamata disease. An example
of suspicious hereditary Minamata disease is a boy born in August, 1960. The

following were observed: started walking and talking at the age of a year and a half and 3 years, respectively; at the March, 1972 and March, 1973 examinations, the following symptoms were observed: mental retardation, sensory disorder of warmth and vibration at both hands and feet, loss of balance, excessive centripetal vision narrowness, minor fits, epileptic abnormal brain waves (irregular exceptional wave, frequent occurrences of δ waves, etc.); his mother (born in September, 1937)also has mild mental disorder and epileptic abnormal brain waves (sudden occurrences of irregular exceptional waves, sudden outbreak of sharp waves by flashing light stimula).

In the Ariake area, no patients with hereditary Minamata disease were found.

Section 3. Examples of Patients Diagnosed as having the Minamata Disease

I. PROGRESS OF EXAMINATION

As shown in Table 5, the number of cases diagnosed as having the Minamata disease increased in the Minamata and Ariake areas and decreased in the Goshoura area from a comparison of the results from the first and second examinations. The number of cases suspected as having the Minamata disease was remarkably decreased in the second examination in every area.

II. EPIDEMICAL AND SOCIOMEDICAL ITEMS

Occupation

Profession or business of Minamata disease patients who were identified at the simultaneous examination in the Minamata area is as follows: fishermen: 69, half-fishermen - half farmers: 6, farmers: 32, nitrogen plant workers: 22, other companies employees: 20, carpenters: 14, temporary laborers: 9, self-employed: 8, public officers: 7, sailors: 4, drivers: 3, lumber workers, doctors, nurses, self-defense force soldiers, etc: 2 -1. As depicted above, persons who

TABLE 5. HISTORY OF DIAGNOSES

Area	Course of diagnosis	Minamata disease	Suspected case
Minamata	First examination	185	105
	Second examination	275	38
Goshoura	First examination	39	39
	Second examination	34	31
Ariake	First examination	5	24
	Second examination	8	2

engaged in fishing were only 69 out of 215 persons, i.e., 32%, or 35% even if the persons whose work is half farming and half fishing were included.

The occupations of Minamata disease patients in the Goshoura area are as follows: 15, half fishermen- half farmers: 2 farmers: 5, public officers and self-employed: 2 each, carpenter, pearl cultivater, inn owner, monk, etc.: 1 each, unknown: 3, welfare supported: 1, etc.

In the case of the Ariake area, they are: fishermen: 6, half farmers - half fishermen: 2.

Economic conditions

We, the staff at the Neuro-Psychiatry Department, examined Minamata disease patients by visiting their homes from 1961. At that time, we noticed that these patients' families, or in general, villages to whom they belonged, were extremely poor. The eaves and walls of many of their houses did not stand straight. Many houses were dark and small. For some patients' houses, roofs were broken so that the sky could be seen from the inside and walls were fallen so that the wind blew through the room. Some of the patients could not afford to provide their daughters with mandatory education, and some of them had to force two or three of their daughters to become housemaids. Presently,

106

the conditions are considerably improved. Under the economic conditions
mentioned above, they did not have a choice except eating fish caught at the
nearby sea for their meals. Many of the families whose entire or majority of
members suffered from the Minamata disease seriously or fatally were exceptionally
poor.

Some Minamata disease patients who exhibited clear symptoms and suffered
seriously did not take any treatments for a long time, since they could not
afford the transportation cost to the medical facility or they could not afford
the cost of medical care.

Consumption of fish, and amount of mercury contained in shellfish.

All of the patients consumed a great deal of fish. The methods of obtain-
ing fish are as follows: when they were fishermen and caught fish by themselves;
when they were not fishermen but caught fish by themselves; when they obtained
fish from relatives or neighbors who were frequently fishermen; when they were
given as a remuneration for their ship carpentry work or accupuncture work;
when they bought fish.

Especially in the Minamata area, poor families, families without farm land,
families who lived near the shore where fish could be easily caught and
fishermen's families naturally ate a great deal of fish every day. In some
cases, no other way besides eating fish existed in order to survive.

In the Ariake town, seven cases were fishermen: five cases used the
Ariake Sea as their fishing ground, and two other cases (couple) used mainly
the Ariake Sea and went fishing to the Shiranui Sea for about five months
(actual fishing period was two months) in 1963. The remaining case was a
farmer who purchased fish and ate fish occasionally.

The results of measuring the amount of total mercury in bina, caught
from the shore of the Ariake area in April, 1972 are as follows: bina caught
at Ariake: 0.000 PPM (caught on April 8), 0.054 PPM (April 17); bina caught at
Suko: 0.061 PPM (April 19); bina caught at OShimako: 0.030 PPM (April 20).

Date of illness

Years when the Minamata disease patients in the Minamata area started suffering from the disease range from 1942 to 1972 (Table 6).

An 18-year-old boy who fell ill in 1972 (household number: 551) said that he started having speech problems this year. The examination held in February 13, 1973 proved that he had speech problems, sensory disorder in touching, aching, warmth, coldness and motion (worse at the extremities of four limbs, and some around his mouth), mental retardation, high blood pressure (158/82). His grandfather (69 years old) living with him also had the Minamata disease. The second example is a case of a 58-year-old housewife (household number 558). She had felt burning sensation in her head since April, 1972. Her body started shaking in September, and started having a buzzing in her ears in October. The examination in March 7, 1973 showed that she had remarkable subjective symptoms (aches in her waist, easy to fall and get injured, dizziness, etc.), and minor loss of balance at the knee bend test, medium degree of muscle tension, shaking, dullness of the senses higher at the terminals of four limbs and also around her mouth, hearing difficulty, minor mental retardation, depression, high blood pressure, etc. Her daughter (35 years old) was also diagnosed as having the Minamata disease.

For the first case in the Goshoura area, a subject fell ill in 1972 (male; household number: 593). The initial symptoms were aches in lower limbs and the bottom of his feet. Although symptoms in September 3, 1972 (74 years old) were minor, he showed speech problems, loss of balance, loss of strength, vision narrowness, dullness of the senses worse at the extremities of four limbs, dullness of the senses around his mouth, hearing difficulty, medium degree of mental retardation, high blood pressure, etc. The subject for the second case (male; household number: 599) showed blurred eyes, and languid legs in 1972. His conditions on September 3, 1972 (66 years old) were minor, but showed loss of balance, muscle tension, narrow vision, dullness in the sense of warmth at the extremities of four limbs, medium degree of muscle contraction (calf, biceps, etc.), hearing difficulty, noticeable mental

108

retardation, etc.

The case in which the disease was suspected to start in 1941 was a girl born
in Yudo in November, 1941 (household number: 757). Her birth was normal and
she weighed 700 kg. She was nursed with her mother's milk until her first birthday.
However, from the beginning, her mental development was slow. She started
walking alone and speaking at the age of 7. Until then, she suffered from
spontaneous paralysis. She entered into Ryonan dormitory in Motowatari in 1969.
She has been frequently in bed due to paralysis in her right foot since the
fall of 1972. Her conditions at December 6, 1972 were as follows: small built,
walking with her left foot dragging, muscle contraction of her left hand and
foot, loss of the senses of aching and warmth (40° C) at 1/5 of both of her arms
from the edge and 1/3 of her left lower limb, dullness of the senses of touching
and coldness at the terminals of her four limbs, medium degree of hearing
difficulty, minor loss of balance, high degree of speech problem, high degree
of mental retardation, etc. Her mother (55 years old) was also a Minamata
disease patient.

The case in which it was considered that the illness started in
1942 was, as reported in the previous report, a subject both of whose parents were
Minamata disease patients (household number: 592). The patient's sister, who
lived with them until July, 1971, was also a Minamata disease patient.
This patient was born in Tsukinoura in October, 1938, grown up normally.
He suffered from paralyzing fits four times in February, 1942. He did not
have fevers but had stopped growing mentally since then. The examination in
November, 1972 showed symptoms, such as a high degree of mental retardation,
noticeable loss of balance in getting up, walking, finger movement,
and nose-pointing test, high degree of speech problem compared with his attitude
and clothes which were in good order, abnormality in eye movement which brought
the suspicion of the Minamata disease:although vision measurement was not
possible,athetosis of left hand and leg, remarkable.

TABLE 6. YEARS WHEN PATIENTS WERE DIAGNOSED AS HAVING THE MINAMATA DISEASE.
THE NUMBER IN PARENTHESES IS FOR THE HEREDITARY MINAMATA DISEASE

	1941	1942	1943	1945	1946	1948	1949	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961
Minamata area	1	1	--	—	3	1	1	3	4	4	3	$21_{(1)}$	$36_{(2)}$	$25_{(1)}$	$6_{(1)}$	12	22	6
Goshoura area	-	--	—	—	—	—	—	—	2	--	1	1	--	1	—	3	1	1
Ariake area																		

	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	Unknown
Miramata area	$30_{(1)}$	12	7	9	16	12	4	13	9	3	2	6
Goshoura area	4	—	5	2	—	3	—	1	2	—	2	4
Ariake area	—	2	—	—	—	—	1	3	—	—	—	2

From Table 6, years when many patients started having the disease were 1955 - 1962 and 1966 - 1969 in the Minamata area and 1959 - 1967 in the Goshoura area.

Illness within a single dwelling family

From Table 7, for the Minamata area, for instance, 81.8% of patients have other Minamata disease patients, and 86.2% of patients have other Minamata diseases or suspected Minamata disease patients within their families living together. In the Minamata area, there were many cases with suspected Minamata disease and for many cases, they had Minamata disease or suspected Minamata disease patients within their families living together. However, the frequency of this phenomenon decreased among patients in the Goshoura area. There were 2 in the Ariake area.

Among these families, there were many cases where an entire family had suffered from the Minamata disease. For example, there were cases where a couple and all of their six children, a couple and 7 out of 10 children who came for the examination, a couple and all of their three daughters, a couple and 6 out of 9 children, and a couple and 5 out of 6 children who came for the examination were suffering from the Minamata disease.

110

Abnormality seen in domesticated animals

For the Minamata area, among 216 persons diagnosed as having the Minamata disease, 144 persons (66.7%) claimed that their domesticated animals died mad, 16 persons claimed that neighbors' domesticated animals died mad, 26 persons showed unknown, and 30 persons (13.9%) indicated no such incidents. Also for persons who claimed their domesticated animals die mad, nine were among 19 persons who were suspected of the Minamata disease from the examination, 3 were among 16 persons who were held for further diagnoses, 3 were among 6 patients of other diseases. For domesticated animals which died mad, 131 persons who were diagnosed as having the Minamata disease indicated cats, 15 persons - pigs, 13 persons - dogs, 9 persons - chickens and 1 person each claimed a goat and a rabbit, respectively. One hundred thirty-four persons were investigated in the Goshoura area. Five persons who were diagnosed as having the Minamata disease, 5 persons who were suspected of the Minamata disease and 3 persons who were held for further examination observed that their cats (11) and chickens(2) either died (5 cats) or showed abnormality such as dizziness, spinning, and loss of strength.

For the Ariake area, cats owned by 2 persons who were diagnosed as having the Minamata disease and a cat owned by a person who was held for further examination died.

TABLE 7. OCCURRENCES OF THE DISEASE AMONG MEMBERS OF A FAMILY.
THE NUMBER IN PARENTHESES IS WHEN THE SUSPECTED PATIENTS ARE ADDED

	Minamata area		Goshoura area		Ariake area	
	Actual no.	%	Actual no.	%	Actual no.	%
Minamata disease	225(237)	81.8(86.2)	5 (13)	14.7 (38.2)	2(0)	25(0)
Suspected Minamata disease	9 (10)	23.7(26.3)	1 (8)	3.2 (25.8)	0(0)	0(0)
Deferred diagnosis	3 (8)	20.0(53.3)	0 (2)	0 (6.9)	0(0)	0(0)

III. AGE AND SEX

Table 8 shows patients' ages and sex.

For patients in the Minamata area, the rate of those over 40 years old, especially over 60 years old, is high compared with that of the total patients examined. Thirty-two out of 34 patients in Goshoura and all of nine patients in the Ariake area were over 50 years old. A similar tendency is seen among patients, surviving or dead, who were known to have the acquired Minamata disease in Kumamoto prefecture (Table 8). On the other hand, the number of Minamata disease patients under 39 years old in the Minamata area is small. Similarly, a small number of people under 39 years old are known acquired Minamata disease patients in the Goshoura and Ariake area (Table 8).

For 275 cases found at the simultaneous examination, the ratio between male and female Minamata disease patients is 897:100. The ratio for the entire persons examined in the Minanata area is 83.0 : 100 (Table 8).

IV. AGES WHEN THEY FELL ILL

From Table 21 shown later, 42.5% of patients fell ill when they were younger than 39 and 54.2% of them fell ill when they were older than 40.

V. INITIAL SYMPTOMS

Initial symptoms for 215 patients in the Minamata area were as follows: numbness in their hands and feet (75 cases), headaches (21 cases), waist aches (20 cases), shivering (18 cases), dullness (16 cases), muscle spasms (10 cases), weakening (10 cases), dizziness (8 cases), early fatigue (7 cases), easiness in falling (7 cases), etc.

TABLE 8. CURRENT AGES OF MINAMATA DISEASE PATIENTS

Age	Minamata area Total examined persons %	Tot. Min. disease patients M Act.no.	Tot. Min. disease patients F Act.no.	Tot. Min. disease patients T %	Heredit. Min.disease pts. M Act.no.	Heredit. Min.disease pts. F Act.no.	Goshoura Ariake area Min.dis. patient M Act.no.	Min.dis. patient F Act.no.	Min.dis. patient T %	Min.dis. patient M Act.no.	Min.dis. patient F Act.no.	Acquired Min. dis. patients Alive Act.no.	Acquired Min. dis. patients %	Total exam. persons Dead Act.no.	Total exam. persons Alive Act.no.	Hereditary Min. dis. patients Dead Act.no.
0 – 4	4.3	–	–	–	–	–	–	–	–	–	–	–	–	5	–	1
5 – 9	6.7	–	–	–	–	–	–	–	–	–	–	–	–	5	–	1
10–14	10.3	3	2	1.8	2	–	–	1	3	–	–	1	0.3	–	6	1
15–19	7.5	6	7	4.7	–	4	–	–	–	–	–	5	1.7	–	13	–
20–29	8.7	8	11	6.9	–	–	–	–	–	–	–	19	6.4	–	–	–
30–39	13.2	11	13	8.7	–	–	–	1	3	–	–	19	6.4	3	–	–
40–49	16.1	25	28	19.3	–	–	–	2	6	–	–	47	15.8	4	–	–
50–59	15.2	25	30	20.0	–	–	3	5	24	2	–	55	18.5	5	–	–
60–69	10.0	21	32	19.3	–	–	7	2	26	1	1	84	28.2	14	–	–
70–79	6.5	26	19	16.4	–	–	6	6	35	2	2	59	19.8	9	–	–
80–	1.5	5	3	2.9	–	–	1	–	3	–	–	9	3.0	10	–	–
Over 0	100.0	130	145	100.0	2	4	17	17	100	5	3	298	100.0	55	19	3

113

TABLE 9. CHANGES AFTER DIAGNOSIS OF THE MINAMATA DISEASE.

THE NUMBER IN PARENTHESES IS FOR THE HEREDITARY MINAMATA DISEASE

Area	Worsen	Fluctuation	Steady	Better	Unknown	Examined persons
Minamata	152	32	45(3)	27(3)	19	275
Goshoura	14	1	10	2	7	34
Ariake	4	2	2	–	–	8

VI. PASSAGE FROM THE BEGINNING OF ILLNESS TO MEDICAL EXAMINATION

Subjective symptoms observed yearly by 275 patients in the Minamata area from the beginning of illness to the examination were as follows: worsening: 152; repetition of sudden worsening and sudden recovery: 32, steady: 45, improvement: 27, unknown: 19 (Table 9). Many patients observed that their symptoms worsened year by year, according to the above figure. For the Goshoura and Ariake areas, many patients got worse each year.

Many patients took examinations continuously at nearby hospitals after they fell ill. Some of the patients received examinations by changing their medical facilities frequently. However, for many cases, no noticeable improvement in their subjective symptoms were observed. That is, even after many visits to medical facilities or entering hospitals 1/2 - 2 years, their diagnoses were not clear in most cases. It was frequently seen that many of them showed symptoms for the Minamata disease and their noticeable progression. Many patients resorted to moxa.

114

TABLE 10.   OUTLINE OF SYMPTOMS (Exclude the hereditary (congenital) Minamata disease)

Type of disease	Area	Subjective symptoms	Speech problem	Loss of balance	Loss of strength	Muscle tension	Loss of muscle tension	Abnormal inherent reflex	Poor reflex	Muscle contract	Weakening of muscle	Excess movement	Jerky movement	Parkinsonism	Narrow vision	Sensory disorder	Hearing loss	Loss of the senses of smell and taste	Loss of vegetable function	Epilepsy	Mental retardation	Emotional disorder	Other mental disorders	High blood pressure	Total number of persons examined
Minamata disease	Minamata(an)*	255	168	252	181	170	39	221	49	34	24	256	66	22	160	261	226	91	165	24	241	230	43	134	269
	% (%)	94.8	62.5	93.7	67.3	63.2	14.5	82.2	18.2	20.1	8.9	95.2	24.5	8.2	59.5	97.0	84.0	33.8	61.3	8.9	89.6	85.5	16.0	49.8	—
	Goshoura(an)	32	15	27	26	16	5	31	3	9	1	16	4	2	26	34	14	6	7	1	24	24	10	19	34
	Ariake (an)	8	7	8	7	7	2	8	3	2	1	—	—	—	7	8	5	—	2	—	8	7	—	6	8
Deferred diagnosis	Minamata(an)	19	3	12	10	9	1	17	2	—	2	5	1	1	4	19	11	3	12	2	12	11	1	7	19
	Goshoura(an)	29	14	31	11	10	1	20	3	1	2	14	3	3	14	30	14	5	3	2	28	23	1	12	31
Susp. Minamata disease	Minamata(an)	14	3	5	5	7	3	13	5	1	1	4	2	—	2	14	14	1	6	—	6	7	—	5	16
	Goshoura(an)	26	9	14	7	12	2	21	2	3	1	6	3	2	7	25	11	2	5	3	17	18	—	10	29
	Ariake (an)	5	4	5	2	3	1	4	—	—	—	—	—	—	4	5	4	—	3	—	6	5	—	3	6

* (an) = (actual number)

Note: Suspected Minamata disease patients in the Ariake area were not included in this table.

115

VII. CONDITIONS AT THE EXAMINATION

A. Acquired Minamata disease

1. Summary

Table 10 illustrates major symptoms of patients diagnosed as having the Minamata disease. Major symptoms of 269 patients in the Minamata area are as follows in the order of frequencies: sensory disorder 97.0%, subjective symptoms 94.8%, loss of balance 93.7%, mental retardation 89.6%, memory difficulty 85.5%, hearing difficulty 84.0%. They are followed by loss of strength 67.3%, speech problem 62.5%, centripetal vision narrowness 59.5%, etc. Also, 49.8% showed high blood pressure and 16% showed mental disorders besides retardation and memory loss.

2. Subjective symptoms

The following show major subjective symptoms of 215 patients who were examined rather in detail: numbness in hands and feet (82%), aches in hands and feet (50%), other abnormal senses (9%), aches in head, back, waist, four limbs, etc. (79%), dullness (65%), loss of sight (55%), narrow vision (31%), loss of hearing (60%), a buzz in ears (51%), loss of smell (28%), loss of taste (22%), easiness of falling (58%), difficulty in putting slippers on (36%), sandals slip off (40%), difficulty in moving fingers (37%), difficulty in moving hand (37%), easiness in dropping things (43%), difficulty in buttoning (34%), difficulty in clear talking (36%), difficulty in talking (41%), loss of strength in hands and feet (57%), body stiffness (this is mostly stiffening paralysis partially on four limb muscles and comes with uncomfortable feeling (71%), muscle spasms (56%), trembling hands and feet (43%), insomnia (43%), early fatigue (68%), intense body (61%), laziness (40%), need to stop work to sleep (16%), forgetfulness (75%), fits which cause dizziness (42%), black out (16%), etc.

These subjective symptoms sometimes become controlling symptoms in clinical cases: 22 cases in the Minamata area and 2 cases in the Goshoura area.

3. Neurological symptoms

Sensory disorders

Table 11 shows that sensory disorders are so extensive that in 95% of the patients even the tips of their extremities are affected. Many are affected also around their mouths (54%) As for the degree of disorder, 67% manifested

TABLE 11. TYPE AND DESCRIPTION OF SENSORY DISORDERS AND SENSES AFFECTED AFFECTED: CONGENITAL MINAMATA DISEASE PATIENTS ARE EXCLUDED

Type of disease	District	Tip of upper and lower extremities	Mouth area	Islet type	Whole body	Left/right body half	Upper/lower body half	Irregular	Loss	Abnormal sensation	Affected sensations Pain	Touch	Warmth	Coldness	Tremor	Position	Motion	No. of cases examined
Minamata disease	Minamata (actual number)	200	117	70	70	37	21	37	146	26	213	209	209	207	187	128	104	217
	Minamata (%)	95	54	32	32	17	10	17	67	12	92	96	96	95	86	59	45	—
	Goshonoura (actual number	34	24	12	8	11	6	11	5	6	30	25	27	25	13	2	2	34
	Ariake (actual number)	8	4	1	2	2	1	—	5	—	8	7	8	5	7	2	1	8
Probable Minamata disease	Minamata (actual number)	17	9	7	2	7	—	1	5	2	16	17	18	13	15	8	5	19
	Goshonoura (actual number)	29	14	6	1	5	4	5	6	3	25	23	28	26	16	2	2	31
Diagnosis reserved	Minimata (actual number	13	2	2	—	—	—	2	—	1	12	7	8	9	4	2	4	16
	Goshonoura (actual number	27	7	2	—	2	6	7	'2	2	19	12	19	19	3	1	1	29
	Ariake (actual number)	3	—	—	—	—	—	1	—	—	4	4	2	1	2	2	2	6

Note: Probable cases of Minamata disease in Ariake district are not shown in this table.

some loss of sensation, while 32% were affected throughout the whole body. Tests of the sense of pain, touch, warmth, and coldness also indicated an advanced degree of disorder. The disorders of the sense of tremor, especially senses of position and motion, were difficult to establish in many cases. Abnormal sensations were observed in 12%, primarily of the tip of extremities. Other complaints consisted of feelings of a worm crawling on one's back, a quivering of the lips, and a burning sensation of the tongue.

Loss of coordination

The results of tests in which the phenomenon of loss of coordination appeared under comparatively simple conditions and at a great frequency are as shown in Table 12. The tests consisted specifically of kinetoses (in this test, loss of coordination was confirmed in 90% of Minamata disease patients), straight line walk test (69%), finger-nose test (64%), right turn test (63%). Romberg's symptoms were seen in only 37% of the patients.

Involuntary movements

A description of involuntary movements is provided in Table 13. As is shown, many cases of tremors were observed in both Minamata and Goshonoura districts. During the examinations, fascicular contractions were frequently directly observed.

Symptoms on left/right side of body

Table 14 shows that of the 38 cases (of which 18 cases involve males) in the left/right half of the body, 30 cases (of which 16 are males) are severe, and the remaining 8 are of moderate degree. Thirty-nine cases (of which 18 were males) showed marked sensory disorders in half of their body; eight cases (of which 5 were males) manifested both paralysis of movement and sensory disorders, all on the same side of their body. A peculiar characteristic among the Minamata disease patients was that, despite a serious paralysis of movement in the left/right sides of their bodies, no difference was

TABLE 12. SYMPTOMS OF LOSS OF COORDINATION AS A RESULT OF VARIOUS TESTS: CONGENITAL MINAMATA DISEASE EXCLUDED

Type of disease	District	Natural walk	Walk on straight line	Right turn	Romberg	Standing on one foot – Eyes open	Standing on one foot – Eyes closed	Bending backwards stest	Squatting test	Finger-nose test – Detachment	Finger-nose test – Intentional tremor	Finger-nose test – Under measured	Finger-nose test – Over measured	Foot test – Detachment	Foot test – Intentional tremor	Foot test – Under measured	Foot test – Over measured	Kinetoses	Resilience exercise	Rising-lying down test	Penmanship	Line drawing	Instruction test	No. of cases examined
Minamata disease	M-ac	32	149	136	80	132	195	165	83	136	96	46	91	133	39	34	71	195	18	129	84	93	96	217
	M-%	42	59	63	37	61	90	76	38	64	44	21	42	64	18	16	33	90	8	59	39	43	44	—
	G-ac	6	16	4	6	12	26	26	15	17	9	1	7	15	1	2	1	24	3	16	14	14	19	34
	G-%	3	5	1	2	4	4	4	3	6	4	1	—	5	—	—	—	8	1	2	2	2	1	3
Pr.M	M-ac	—	5	3	3	1	11	11	1	7	2	2	7	6	—	—	4	6	1	4	1	1	1	19
	G-ac	6	17	4	6	13	27	27	16	17	9	1	7	15	1	2	1	25	3	16	14	14	10	31
Diagnosis reserved	M-ac	1	3	2	1	4	9	7	2	2	2	—	—	3	—	—	1	6	—	4	2	1	2	17
	G-ac	5	12	3	2	11	20	24	12	5	7	—	2	7	3	2	3	22	3	9	4	7	7	29
	A-ac	2	2	6	1	1	2	3	3	1	1	1	1	2	3	3	3	1	4	3	5	4	4	6

M-ac — Minamata (actual number); M-% — Minamata (%); G-ac — Goshonoura (actual number); G-% — Goshonoura (%); A-ac — Ariake (actual number); Pr.M — Probable case of Minamata disease

119

TABLE 13. BREAKDOWN OF INVOLUNTARY MOVEMENTS; FIGURES IN PARENTHESES
INDICATE PERCENTAGE

	Minamata district		Goshonoura district	
	Minamata disease	Probable case of Minamata disease	Minamata disease	Probable case of Minamata disease
Shaking	120 (55.8)	6 (31.6)	14 (40.0)	12 (38.7)
Athetosis	8	-	1	1
Hiolea	4	-	1	-
Involuntary movement, but seemingly voluntary	5	-	-	2
Tic	1	-	-	-
	8	-	-	-
Fascicular contraction	20	1	1	-
No. of cases examined	217	17	34	31

TABLE 14. COMBINATION OF PARALYSIS OF MOVE-
MENT AND SENSORY DISORDERS IN LEFT/RIGHT
HALF OF BODY; BRAIN HEMORRHAGE. FIGURES IN
PARENTHESES SHOW NUMBER OF PERSONS STRICKEN
WITH BRAIN HEMMORRHAGE OUT OF THE PRECEDING NUMBER

		Sensory disorders			Total
		++	+	0	
Movement disorders	++	8 (4)	—	22 (2)	30 (6)
	+	8	—	—	8
	0	23	—	—	23
Total		39 (4)	—	22 (2)	

Note: ++ — considerable difference in dis-
orders between left/right side; + — moderate
difference between left/right side; 0 — dif-
ference between left/right side not established.

found in sensory disorders between the left/right sides in 22 cases, and that despite a severe sensory disorder in half of the body, no difference in more-ment disorder between the left/right sides was noted in 23 cases. There was a total of 6 cases of brain hemorrhage resulting in a paralysis of movement, of which 4 cases involved also a sensory disorder of half the body, and 2 cases manifested no difference in sensory disorder between the left/right sides. In the cases involving paralysis of movement, 35 were affliced with symptoms of half of the body without accompaniment of brain hemorrhage, while 24 were afflicted with sensory disorders. All 6 cases with brain hemorrhage suffered from a paralysis of half of the body.

Out of 214 Minamata disease patients, 6 or 2.80 $\pm$ 1.13% were 40 or over, and had a paralysis of half of the body as a result of brain hemorrhage. This percentage was close to that of residents of Heianza Island [5] where — out of 766 individuals — 20 persons or 2.61 $\pm$ 0.57% had a similar condition. In the case of general, movement-type paralysis of half the body, the figures for Minamata disease patients were 17.75 $\pm$ 2.61%, and for Heianza Island residents, 34 persons out of 766, or 4.44 $\pm$ 0.74 — a significant difference $(\chi^2 \cong 37.00. \ P < 0.001)$. For the inhabitants of Minamata, movement-type paralysis designates those who drag one foot while walking and demonstrate a slight difference between the left/right in innate reflexes or muscular tension. As for the inhabitants of Heianza Island, the term embraces even those who simply manifest a difference between left/right in their innate reflexes. The fre-quency rate for symptoms of half of the body combined with both movement and sensory type of paralysis was 61 persons or 28.50 $\pm$ 3.09% out of 214 Minamata disease patients, as oppored to 4.47% for Heianza inhabitants — a significant difference $(\chi^2 \cong 110.66. \ P < 0.001)$.

Brain disorders, dizziness, giddiness

Among Minamata disease patients in Minamata district, 81 (37.7%) out of 215 cases examined had fainting spells, and either grasped some object for support or assumed a squatting position, while 33 cases (15.3%) felt dizzy or

giddy. Such seizures were noted in 6 cases (17.1%) among Goshonoura in-
habitants.

Epilepsy_(symptomatic_epilepsy)

Epilepsy among Minamata disease patients was 8.92 ± 1.74% in the Mina-
mata district, and 2.86 ± 2.82% in the Goshonoura district. In many cases,
the types of seizures were uncommon. For example, clonic spasms may occur
with or without disorders of the brain, or there may be sudden eruption of
electrical discharges without any mental disorders or seizures. The latter
case was seen in residents (2 cases) of A community in the Minamata district
in one of three sisters residing in Osaka, but they were not considered to
be epileptics.

4. Mental Symptoms

The mental conditions of Minamata disease patients may be classified
into reactions against the environment and an abnormal mental state.

(i) Reaction against environment

Existing in the mental state of Minamata disease patients is a reactive
element against one's illness or social influences, believed to fall within
the normal range, but considered unusual.

The patient possesses, relative to his illness, a sense of anxiety and
inferiority with respect to the following matters. For example, propriocep-
tive symptoms accompanied by pain, difficulty and lessened efficiency in
daily work due to decline of motor and mental functions, diagnosis as Minamata
disease, progress of disease and ineffectual therapeutic treatment; difficulty
and less chance for employment or marriage; transmission of disease to pos-
terity and other effects; divorse by spouse, and impact on fellow workers
and personal friends.

122

With respect to the social aspect, the Minamata disease patient is confronted with the following mental problems. For example, discrimination and lower standing as a result of sickness; envious attitude of others over compensatory payments; criticism that the intent behind application for designation as a Minamata disease patient was to receive compensation; oppressive Minamata power of administrative, company, fishery officials and urban residents; a clash of interests between enterprises constituting the financial pillar of the city and of oneself as a victim of such enterprises; distrust of the close relationship between physicians and hospital institutions, on the one hand, and the administration and enterprises, on the other; anger over the attitude of the offender, the enterprises; the irony of fishermen-patients facing livelihood and health problems because of decreased sale of fish as a result of the emergence of Minamata disease patients, and anxiety over a sick person being in the family.

For the foregoing reasons, some patients have moved from one job to another or have been divorced. In addition, because of the complex environmental factors, there were varied mental processes, standing, and interests among the patients who split into numerous groups, advocating either delegation of full authority to another party, litigation, personal negotiation, wait-and-see, or evasive attitudes.

    (ii)   Abnormal mental conditions (mental
           symptoms)

Among the Minamata disease patients, mental conditions were not confirmed in 17 (6.2%) out of 275 persons from the Minamata district, 4 (11%) out of 35 from the Goshonoura district, and 1 (11%) out of 9 from the Ariake district.

Mental conditions associated with Minamata disease are the following (Table 15).

123

TABLE 15. MENTAL DISORDERS OBSERVED IN PATIENTS WITH, OR PROBABLY WITH,
MINAMATA DISEASE

	Minamata district		Goshonoura district		Ariake district
	Minimata disease	Probable Minamata disease	Minamata disease	Probable Minamata disease	Minamata disease
Mental disorders	241	12	24	28	8
Emotional disorders	230	14	24	23	7
Delirium	-	-	-	1	-
Manic-depressive condition	16	-	8	-	-
Menopause depressive condition	1	-	-	-	-
Schizophrenic type condition	2	-	-	-	-
Neurotic condition	22	1	2		
Condition difficult to classify	1	-	-	-	-
Schizophrenia	1	-	-	-	-
Mental retardation	1	-	-	-	-

Note. Mental retardation is also included under mental disorders.

a. Those associated with organic mercury poisoning: mental disorders, emotional disorders, hallucination-delusion condition, manic-depressive condition, regressive-depressive condition, schizophrenic condition, neurotic condition, and conditions difficult to classify.

b. Combination with symptoms of organic mercury poisoning: schizophrenia (1 case), mental retardation (1 case).

Among cases dealing with the foregoing mental conditions, 1 case each —
specifically, that involving neurotic conditions from Minamata district, and
depressive plus neurotic conditions from Goshonoura district (a total of 3
cases) — were determined as not being due to either mental or emotional dis-
orders. Also, mental disorders were not established in one case each
involving schizophrenic-type conditions, schizophrenia, and neurotic condi-
tions from Minamata district (a total of 3 cases), and 1 case each involving
depressive conditions and neurotic conditions from Goshonoura district (a
total of 2).

Mental disorders

A serious degree of mental disorders was noted in 4% of Minamata district
patients, a moderate degree in 28%, and a mild degree in 65% (Table 16). In
cases of serious mental disorders, the patients required aid and supervision in
their daily livelihood because of impaired ability to think, judge, recall,
memorize, and understand. In cases of mild disorders, tests established the
degree of disturbed power of recollection, memory, and thinking, and such
disorders did not greatly affect the livelihood of the patients. The moderate
degree of mental disorder fell between the two areas above.

Hallucination-delusion condition

The case under this category involved a person born in May, 1918, and
stricken with Minamata disease around 1956 - 1957. As the disease progressed,
the individual attempted suicide in November, 1971, and was admitted to a
mental institution because of a serious depressive condition. Following ad-
mission, the patient was stricken with irregular clonic spasms, sometimes ac-
companied by poor memory, followed by the onset of hearing hallucination and
delusions. The condition of the patient as of August 17, 1972, included
hearing hallucinations, mental disorders, marked loss of coordination, poor
vision, hearing difficulty, articulation difficulty, shaking, and disorienta-
tion. Clinically, the existence of mental disorders could not be established.
The brain waves were of slow α-wave pattern, with occasional θ-waves. This

TABLE 16. MENTAL AND EMOTIONAL DISORDERS OF MINAMATA DISEASE PATIENTS

District	Mental disorders			Emotional disorders												
	Serious degree	Moderate degree	Mild degree	Expressionless	Without strength	Fatigue	No vitality	Diminished aggressive behavior	Lethargy	Inhibition of motion	Non-energetic	Disinterest	Sensitive feelings	Gloomy	Depressive mood	Euphoria
Minamata (actual number)	9	60	142	44	39	35	45	65	40	45	36	37	75	31	49	51
Minamata (%)	4	28	65	20	18	16	21	30	18	21	17	17	35	14	23	24
Goshonoura (actual number)	3	5	16	8	10	7	7	6	4	1	6	1	7	3	5	5
Ariake (actual number)	—	2	6	2	—	1	—	—	2	1	—	—	2	—	—	3

126

case was also considered to be one of the epilepsy cases associated with Minamata disease. The patient succumbed in February, 1973.

Emotional disorders

Most emotional disorders consisted of dull emotions and diminution of aggressive behavior (Table 16). The patient usually complained of various proprioceptive symptoms, demonstrated tiredness and fatigue in expression and behavior, and impolitely laid down in front of a physician while awaiting his turn for examination.

Depressive condition

As for manic-depressive disorders, there were 3 cases of manic-state (mild), 14 of depressive state, and 1 of alternate, cyclic manic-depressive state in the Minamata district. The depressive state was generally classifiable as a mild case, but four persons were observed to have a suicidal tendency. In the Goshonoura district, there were 8 cases of depressive state, of which 2 had suicidal tendencies.

These depressive states were generally in the mild category.

Menopause depressive condition

This case involved a farmer's wife born in October, 1929. When stricken in July, 1972, she felt as if her head was burning, and her condition subsequently became aggravated. Her condition as of March 7, 1973: complained strenuously and exaggerated about aches running down her neck to her shoulder, constipation, and heavy feeling in her head; repeatedly asked whether she has any strange malady and whether she was going insane; restless; constantly sighed; had her daughter massage her neck; spoke in a coquettish tone; suddenly stood up to leave, only to return and repeat foregoing statements; depressive; gloomy; sensory disorders; hearing difficulty; loss of

coordination and tremors; eldest daughter (age 35), who used to live with patient until marriage in 1963, also had Minamata disease.

Schizophrenic-type condition

Three cases involved psychoses characterized by tension. For example, one case involved a youth, age 15, who displayed inhibition of motion, immobility, muteness, stiffness, rigidity, coldness, and dark expressions, frowning, stupor, negativity, no vitality, extremely slow reaction, hard to understand, and inaccessible through communication; sensory disorders most serious at tip of limbs and also emerging in islet-shape in the right upper arm and on the back of the left hand, tremor, and mild mental disorder. The youth's father (age 42) also suffered from marked Minamata disease symptoms, such as sensory disorder, hearing difficulty, and loss of coordination, in addition to similar schizophrenic symptoms. The third case also involved a 15-year-old youth.

Neurotic conditions

Included in this category were the following: among the Minamata district patients, (a) 16 persons complained mostly of bodily and mental disorders, i.e., the recurrence of previously described proprioceptive symptoms accompanied by an outburst of emotional feeling; (b) four were characterized by depressive moods and anxiety; (c) one — by bodily and mental disorders plus depression and anxiety; (d) one — by bodily and mental disorders plus pseudo-imbecility, and (e) two — by exaggerated wavering at the time of Romberg's test.

Among the Goshonoura district patients, there was one case each of bodily and mental disorders, of bodily and mental disorders plus fear of losing his/her mind, and bodily mental disorders plus depressive-agony reactions.

Conditions difficult to classify

This case involved a woman born in April, 1903, and with 6 children, 3 of whom had Minamata disease. She was stricken around 1954 - 1955, with the onset of stiffness of hand, tremor, and excessive perspiration; she concealed her condition in fear that she may also have contracted Minamata disease after her eldest son was diagnosed as having Minamata disease; she was examined in August, 1971, November, 1972, and in March, 1973, and found to have proprioceptive symptoms, such as numbness of limbs and inability to walk properly; in stupor and immobile, with hands on her lap and leaning against a table when left unattended; appeared to be tired and fatigued; while lying down on the treatment table, crossed her arms in front of her eyes and pouted her mouth; lacked aggressiveness or "drive"; disinterested and slow in speech, yet friendly and lacked inhibition, even asking a child eating grapes nearby to give her some; short, fast steps noted in walking test; closed eyes and mouth very tight during Romberg's test; in a finger-nose test, sounded chants and thrust her head forward as if pounding rice and bent her five fingers inward in the shape of a claw and attempted to poke her nose, stopping 3 - 4 cm away from her nosetip; stroked her finger with the other hand and poked her eye while chanting; when instructed to stand on one foot with both eyes open, outstretched her fingers, extended both arms outward at her side, arched her mouth and raised one leg as if dancing the Yasukibushi (Japanese dance), asking the physician if her foot was elevated and then lowering it with an exclamatory sound; when instructed to pick up an object on the floor, would extend both her legs and gradually move the legs toward each other as if trying to catch an escaping fish; when instructed to grasp an object, would retain that position even though the object was removed from her hands; when told to stick her tongue out, would hold that pose indefinitely. The aforementioned abnormal behavior appeared unnatural, strange, and as if she were putting on an act, even humorous. As for mental functions, there was a loss of the sense of time, marked deterioration of thinking ability and of knowledge.

129

<u>Mental disorder as an unanticipated</u>

<u>accompanying symptom</u>

A case in which schizophrenia and mental retardation were respectively determined to be an unanticipated accompanying symptom of Minamata disease was previously described (Section 2, Chapter 2).

5. High Blood Pressure

The frequency of high blood pressure among the Minamata district patients with Minamata disease was 48.91 ± 3.01%. Patients aged 40 or over totalled 214, or 102 males and 112 females. Among these, 129 had high blood pressure, with 60.28 ± 3.35%. Of 766 residents (of which 298 were males) aged 40 or over from Heianza Island, those with high blood pressure numbered 238, with 31.07 ± 1.67%. The difference in the frequency of high blood pressure between Minamata district patients having Minamata disease and Heianza Island residents was significant ($\chi^2 \geq 60.93$, P<0.001).

The frequency of high blood pressure was more pronounced with increasing age. For Heianza Island, the frequency was: 40-year-old age group, 13.4%; 50-year-old age group, 20.8%; 60-year-old age group, 33.5%; 70-year-old age group, 55.2%; 80-year-old or over, 55.2%. A comparison of the age make-up between Minamata disease patients and Heianza Island residents reflected: age 40 - 49, 24.8% and 27.0%; age 50 - 59, 25.7% and 21.9%, age 60 - 69, 24.8% and 24.9%; age 70 - 79, 21.0% and 20.0%; age 80 and over, 3.7% and 6.1%. The age composition for both groups was virtually identical; however, the percentage for the 40 - 49 and 80 or over age groups for Heianza Island residents was higher. The frequency of high blood pressure for Heianza Island residents age 40 or over was 25.8% for males, and 34.4% for females, a significant difference ($\chi^2 \geq 6.23$, P<0.02). As for those over 40, the male/female ratio was 62 : 100 for Heianza Island residents, and 91 : 100 for Minamata disease patients.

6. Protein and Sugar in Urine

Of 112 Minamata disease patients examined at Minamata district, 5 (4.5%) were found to have a slight amount of protein in the urine, but no sugar was found in any. Of 8 cases of probable Minamata disease, 1 showed a slight positive reaction for urine, and none for the presence of sugar.

As for Goshonoura district, 4 and 2 persons out of 32 Minamata disease patients showed a positive reaction for protein and sugar, respectively. Out of the 20 probable cases of Minamata disease, 3 were found to have protein in the urine, and none were found with sugar in the urine.

In the foregoing diabetic cases, poor vision, sensory disorders, and loss of coordination were noted.

7. X-ray Test of the Vertebrae

Of those found to require an x-ray test of the cervical vertebrae and able to visit the Kumamoto University Hospital for such a test, Minamata disease patients numbered 44 (of which 27 were males), 17 probable cases (including 9 males), and 23 others with miscellaneous ailments (including 12 males). X-ray photographs were made of the patient's front, back, and side.

The findings based on the photographs are outline in Table 17. In the cervical vertebrae, deformative spondylosis was primarily observed, along with stricture of the disks, spinous formation in true vertebrae, and many holes in the bony structure.

A calculation based on Table 17 revealed that the elderly, aged 60 or over, constituted 81.8% of Minamata disease patients, 70.6% of probable Minamata disease patients, and 56.5% of patients with other ailments. Hitherto, it was considered that the changes of the vertebrae increased with age and were more serious. However, our figures showed 86%, 82%, and 83% for the foregoing ailments, respectively (++,+++); 30%, 24%, and 22% (no significant

TABLE 17. OBSERVATIONS FROM X-RAY PHOTOGRAPHS OF THE CERVICAL
VERTEBRAE; FIGURES WITHIN PARENTHESES INDICATE FEMALES, OTHER-
WISE — MALES

Age	Minamata disease patients				Probable case of Minamata disease				Patients with other aliments			
	++	++	+	0	+++	++	+	0	++	++	+	0
?—??	—	—	—	(2)	—	—	1	—	—	—	2 (1)	1
—40	—	—	(1)	—	—	—	1	2	—	—	—	(2)
—50	—	—	2 (2)	1	—	—	1	—	—	—	1 (3)	—
—60	1	2	3 (2)	1 (2)	—	—	1 (1)	(1)	—	—	2 (2)	1
—70	—	2	8 (3)	—	1	(1)	1 (3)	—	—	3 (2)	2 (1)	—
—79	2	3 (3)	2 (2)	—	—	1 (1)	(1)	—	—	—	—	—
12—79	3	7 (3)	15 (10)	2 (4)	1	1 (2)	5 (5)	2 (1)	—	3 (2)	7 (7)	2 (2)

Note: +++ — serious changes in cervical vertebrae; ++ — change of
moderate degree; + — change of mild degree; 0 — no change

difference; $\chi^2 \geq 0.15$) for moderate and serious changes, respectively: 92%,
92%, and 92% for total cervical vertebrae changes in those aged 60 or over
(+, ++, +++), and 63%, 60%, and 70% for total changes in those under age 60.

Next, we tested for the existence and degree of 8 symptoms, such as loss
of coordination and decrease of roughness potential, which may have some re-
lationship with changes in the cervical vertebrae, in order to determine their
effects (Table 18). We found that in cases without neurological symptoms,
there were no or little changes in the cervical vertebrae. However, the
opposite relationship was true in the case of muscular atrophy. The frequency
of occurrence of cases involving a moderate and serious degree of the same 8
symptoms appeared to have an increasing trend in moderate-serious changes of
the cervical vertebrae as far as loss of coordination, decrease of roughness
potential, and sensory disorders were concerned. However, according to the
χ^2 determination, no significant differences were found between 81.1% and
84.6% in the column on the absence of pathogenic reflexes, between 9.7% and
15.4% in the column on the frequency of occurrence of marked loss of coor-
dination, and between 6.5% and 23.1% in the column on decrease of roughness
potential.

132

TABLE 18. EXTENT OF CERVICAL VERTEBRAE CHANGES AND RELATIONSHIP
BETWEEN THE EXISTENCE AND EXTENT OF SYMPTOMS. FIGURES DENOTE THE
NUMBER OF MINAMATA DISEASE CASES; FIGURES IN PARENTHESES UNDER
ACTUAL NUMBER COLUMN DENOTE THE NUMBER OF PROBABLE MINAMATA DISEASE
CASES, AND THOSE UNDER THE PERCENTAGE COLUMN — THE NUMERICAL VALUE
FOR BOTH

	Cervical vertebrae changes: 0 - + 31 cases (13 cases)		Cervical vertebrae changes: ++ - +++ 13 cases (4 cases)	
	Actual number	%	Actual number	%
Without symptoms listed on the right (0) Loss of coordination	1 (1)	3.2 (4.5)	0 (0)	0 (0)
Decrease of potential roughness	12 (9)	38.7 (47.7)	23.1 (20.1)	3 (2)
Increase of muscular tension	12 (10)	38.7 (50.0)	23.1 (20.4)	3 (2)
Decrease of muscular tension	19 (13)	61.3 (72.7)	100.0 (100.0)	13 (4)
Abnormal innate reflex	3 (4)	9.7 (15.9)	7.7 (1.5)	1 (1)
Pathogenic reflex	27 (12)	87.1 (88.6)	84.6 (84.1)	11 (4)
Muscular atrophy	25 (12)	80.6 (84.1)	84.6 (84.1)	11 (4)
Sensory disorder	0 (2)	0 (4.5)	0 (0)	0 (0)
Marked symptoms listed (++ - +++) on the right Loss of coordination	3	9.7	15.4	2
Decrease of roughness potential	2 (1)	6.5 (4.5)	23.1	3
Increase of muscular tension	0 0	0 0	7.7 0	1 0
Decrease of muscular tension				
Abnormal innate reflex	0	0	0	0
Pathogenic reflex	0	0	0	0
Muscular atrophy	0	0	0	0
Sensory disorder	0 (2)	0 (6.5)	7.7 (11.2)	7 (1)

Examination of cases diagnosed as Minamata disease, irrespective of
changes in the cervical vertebrae, indicated sensory disorders around the
mouth, articulation difficulty, and poor vision to be prevalent in the family.

Among the Minamata disease patients, serious changes in the lumbar vertebrae were noted in a total of 10 persons, or 9 males and 1 female (6 , Minamata district; 3, Goshonoura; 1, Ariake district). Those changes consisted of serious deformity and spinous condition in $L_{3,4,5}$; narrowing and unsymmetrical cavity between L_{4-5}; ossification of the posterior, longitudinal ligament of L_{2-5}; serious deformity throughout; vertebral detachment of L_5; fibrous fusion and spinous condition of $L_5\text{-}S_1$; serious side curvature on the right convex, fusion and wedge-like deformity of L_{2-3}, and narrowing of the cavity; serious frontal curvature, narrowing of cavity in L_{2-3}, spinous condition and migratory vertebrae; gliding backward of L_5, narrowing of cavity in L_{4-5} and spinous condition; bone fracture caused by pressure on D_{12} and L_1 and spinous condition; temporal gliding of L_1, spinous condition, and fusion of spinous projections. In these cases, changes were also noted, though to a mild degree, in the cervical vertebrae. Sensory disorders, abnormal muscular tension, abnormal innate reflexes, decrease of roughness potential, and other symptoms were more serious in the lower extremities than in the upper extremities in 6 cases. There were no differences, however, in the remaining 4 cases.

8. Biochemical Test of Blood Serum

Tests were conducted on a total of 224 Minamata district residents covering 5 items listed in Table 19. Of the 224 residents, 193 were Minamata disease patients, 13 had probable Minamata disease, 14 were patients on whom diagnosis was reserved, and 4 had other ailments. A similar test was also conducted on a total of 115 residents of Goshonoura, a breakdown of which was 34, 29, 21, and 31, respectively. As for syphilitic reaction, it was noted as positive if either or both of the Ogata method or glass disk method indicated positive results. A level in excess of 13 units (K.A. Unit) for alkaliphosphatase, 40 units for GOT (Karmen Unit), 35 units for GPT (Karmen Unit) and 250 mg/dl for total cholesterol was termed as positive.

134

TABLE 19. BIOCHEMICAL TEST OF BLOOD SERUM

Persons examined	Syphilitic reaction Total no. examined	Syphilitic reaction Actual no.	Syphilitic reaction Positive %	Alkali-phos-phatase 13 or over Total no. examined	Alkali-phos-phatase Actual no.	Alkali-phos-phatase %	GOT 40 or over Total no. examined	GOT Actual no.	GOT %	GPT 35 or over Total no. examined	GPT Actual no.	GPT %	Total cholesterol 250 or over Total no. examined	Total cholesterol Actual no.	Total cholesterol %
Minimata district residents: Minamata disease	193	25	13.0	191	21	11.0	191						191	5	2.6
Probable Minamata disease	43	1		13	2	15.4	13	2		13	1		131	1	1.7
Diagnosis reserved	11	1		11	2		11			11	1		11	0	0
Other ailments	4	0	0	4	0		4	0		4	0		4	0	0
Goshonoura district residents: Minamata disease	34	5	14.7	31	1		34	1		34	0		34	0	0
Probable Minamata disease	29	3	10.3	29	3	10.3	29	2	6.9	29	2		29	1	3.4
Diagonsis reserved	21	3	14.3	21	3	14.3	21	4	19.0	21	3		21	1	4.8
Other ailments	31	1	3.2	31	6	19.5	31	1	3.1	31	0		31	0	0
Minamata disease group	269	34	12.6	267	27	10.1	267						267	10	3.7
Control group	70	5	7.1	70	11	15.7	70	4	5.7	70			70	1	1.4
Minamata district residents	224	27	12.1	222	25	11.3	222						222	10	4.5
Goshonoura district residents	115	12	10.4	115	13	11.3	115						115	2	1.7

135

The results of the tests are outline in Table 19. Figures are cited according to district and type of disease. The Minamata disease group is defined as the total of Minamata disease and probable Minamata disease cases, and the control group — as the total of diagnoses reserved and other ailment cases. Finally, the various disease types were totalled, and two major classifications for the Minamata and Goshonoura districts were established for comparison.

Items determined to reflect a significant difference in the positive ratio based on χ^2 determination are below. Specifically, the positive ratio of GOT between Minamata and Goshonoura district residents was 20.7% and 5.4% $(\chi^2=12.79, P<0.01$, and of GPT — 17.6% and 4.3% $(\chi^2=10.62, P<0.01)$, respectively. The items in which the difference was determined to be somewhat significant are below. In other words, they were the positive ratio of GPT between the Minamata disease group and the control group — 17.6% and 7.1% $\chi^2=7.70, P<0.01$. — and of GTP, 15.0% and 5.7% $(\chi^2=3.5, P<0.07)$. On the other hand, no significant difference was noted between the numerical values of the positive ratio of items below. In other words, there was no significant difference between the numerical values of 12.6% and 7.1% $(\chi^2=1.06)$ for a syphilitic reaction between the Minamata disease group and the control group; between 10.1% and 15.7% $(\chi^2=1.83)$ for alkaliphosphatase; and between 3.7% and 1.4% $(\chi^2=0.33)$ for total cholesterol. Further, no significant difference was found between the positive ratio of 12.1% and 10.4% for syphilitic reactions, 11.3% and 11.3% for alkaliphosphatase, 4.5% and 1.7% for total cholesterol between the Minamata and Goshonoura district residents, respectively.

The syphilitic reaction of blood serum was positive, in the case of males, in 13 (11.9%) of 109 persons in the Minamata district, 6 (9.5%) out of 63 in the Goshonoura district, and 0 out of 2 in the Ariake district; in the females, the count in respective districts was 16 (13.6%) out of 118, 6 (9.8%) out of 61, and 0 out of 3. The positive ratios for all three districts were: male, 10.9%; female, 12.1% and 11.5%, combined. The difference between male and female in the Minamata district was insignificant $(\chi^2=0.13)$.

Cases with a high GOT, GPT value all involved Minamata disease patients. A woman, aged 73, with a reading of 142 and 126 units, respectively, had undergone surgery for removal of a tumor from between the pancreas and the liver when she was 61 years of age; a 62-year-old male with a count of 125, 5, and 60 units claimed to have had an ulcer at age 45; a 31-year-old housewife with a count of 105 and 120 units complained of proprioceptive symptoms, such as tiredness and marked fatigue, loss of strength, and pain; a 51-year-old woman with a count of 90 and 110 units showed nothing in particular.

9. Moxa Cautery Scars

Among the Miramata disease patients, numerous moxa burns were observed on the shoulder, nape of the neck, hip, elbow joints, forearm, knee joints and on the lower thighs. They represented a record of therapy over many years against bodily pain, aches, fatigue, loss of strength, numbness, and muscular twitching.

10. Clinical Picture

Among the symptoms of patients diagnosed as having Minamata disease, some occurred frequently due to rather limited nervous system disorders. These are, for example, sensory disorders, loss of coordination, hearing difficulty, articulation disorders, and centric field of vision constriction. Table 20 is the result of study of the symptoms making up the clinical picture of cases diagnosed as Minamata disease.

Based on this table, the following points are notable: (1) there is a variety of combination (16 types) of symptoms; (2) there was a combination of 6 symptoms, and, in one case, there was only one symptom (sensory disorder); (3) among the different combinations, the greater the variety of symptoms, the greater was the frequency of appearance, and vice versa; (4) the greatest frequency was when there was a combination of 6 symptoms (A), followed by those lacking a field of vision constriction (a), those combining sensory

137

TABLE 20. COMBINATION OF MAJOR SYMPTOMS MAKING UP THE CLINICAL
PICTURE; CONGENITAL MINAMATA DISEASE IS EXCLUDED

	Minamata district			Goshonoura district			Ariake district		
	Minimata disease	Probable Minamata disease	Diagnosis reserved	Minimata disease	Probable Minamata disease	Diagnosis reserved	Minimata disease	Probable Minamata disease	Diagnosis reserved
A S+L+I+H+A+C	112	i	—	9	?	—	6	2	1
a S+L+I+H+A	43	3	1	3	8	—	—	—	1
A L+I+H+A+C	1	—	—	—	—	1	—	—	—
a L+I+H	—	—	1	—	—	—	—	—	—
B S+L+H+C	31	2	1	—	2	1	1	—	1
b S+L+H	34	3	1	—	2	1	—	—	—
C S+L+C	9	2	—	15	5	1	1	—	—
c S+L	12	4	1	7	8	1	—	—	2
D S+H+C	4	—	—	—	—	—	—	—	—
d S+H	8	12	3	—	—	—	—	—	—
E S+A+C	—	—	—	—	—	1	—	—	—
e S+A	3	—	—	—	—	1	—	—	—
f S+I	2	3	1	—	—	5	—	—	—
G S+C	—	—	1	—	—	1	—	—	—
g S	2	5	4	—	—	3	—	—	—
h L+I+A	6	1	1	—	—	—	—	—	—
I H+C	—	—	—	—	—	—	—	—	1
J C+α (C+L, C+I)	2	1	—	—	—	—	—	—	—
Total no. examined	269	37	15	34	31	16	8	2	6

Note (i) S — sensory disorder; L — loss of coordination; I —
mental disorder; H — hearing disorder; A — articulation disorder;
C — centric visual field constriction.
(ii) Where diagnosis was reserved, the figures are for patients
who received their second examination.

disorder, loss of coordination, and hearing difficulty (b), a combination of
the latter plus a field of vision constriction (B), a combination of sensory
disorder and loss of coordination (c), and the latter plus field of vision
constriction (C); (5) centric field of vision constriction occasionally ap-
peared with only sensory disorders (G), hearing disorder (I), loss of coor-
dination or sensory disorder (J).

138

11. Degree of Affliction; Degree of Affliction and
Date of Onset

From the general standpont of physical and mental disorders, the follow-
ing three levels of affliction were adopted.

Serious degree: comprising the following two items:
1. Almost 100% assistance required to move, eat, eliminate
   and change clothes.
2. About 50% assistance required to perform the above; requires
   constant observation; dangerous if left alone.

Moderate degree: Includes the following two items:
3. Able to care for oneself, but cannot or find it difficult
   to engage in work; requires supervision in daily work.
4. Able to do simple work, but inefficient.

Mild degree:
5. Normal efficiency in the case of light work.
6. Almost no trouble in ordinary job.

Table 21 is the result of classification based on the foregoing criteria
of 275 Minamata disease patients of the Minamata district, following a general
examination. According to this table, 13.5% are rated as serious, 64.7% —
as moderate, and 19.61% — as mild cases. The percentage includes 6 cases
of congenital Minamata disease, i.e., 5 serious and 1 mild cases. Of the
43 Minamata disease patients of A community in Minamata district, 4 (9%) were
serious. 26 (61%) were moderate, and 13 (30%) were mild cases. As for
Goshonoura district, there were 2 serious, 26 moderate, and 7 mild cases;
in Ariake district, 0, 2, and 6, respectively.

TABLE 21. AGE AT ONSET AND DEGREE OF AFFLICTION AMONG MINA-
MATA DISTRICT RESIDENTS. FIGURES DENOTE ACTUAL NUMBER: FIGURES
IN PARENTHESES GIVE THE PERCENTAGE

Age	Serious	Moderate	Mild	Unknown	Total
0	5 (83)	–	1 (17)	–	6 (2.2)
1—5	2 (40)	3 (60)	–	–	5 (1.8)
6—39	7 (6.5)	67 (63.2)	30 (28.3)	2	106 (38.5)
40—59	11 (11.1)	67 (67.7)	20 (20.2)	1	99 (36.0)
60—	12 (24.0)	35 (70.0)	3 (6.0)	–	50 (18.2)
40—	23 (15.4)	102 (68.5)	23 (15.4)	1	149 (54.2)
0—5	7 (64)	3 (27)	1 (9)	–	11 (4.0)
Unknown	–	6	–	3	9 (3.3)
Over 0	37 (13.5)	178 (64.7)	54 (19.6)	6 (2.2)	275

Table 21 also clarifies the relationship of age at onset and degree of affliction. It shows that as the age at onset becomes lower than 5 (congenital Minamata disease for those whose age of onset was 0) and conversely rises, the greater the chance of the disease becoming serious; the younger the patient (up to age 6, however), the greater the likelihood of mild affliction. Moderate degree of affliction was noted equally in all age groups. The age classification used in this table took the following into account. Specifically, the mortality rate [16] of patients at the Matsuzawa Hospital, Tokyo, who died of malnutrition during World War II and that of the victims of CO poisoning in the Miiki Mine blast of November, 1963, was low for those aged 39 and under, and higher for those aged 40 or over — a significant difference, indeed [17].

12. Therapy

Table 22 reflects the decisions made concerning the treatment of Minamata disease patients following a general examination of Minamata district residents. Approximately 8% of the patients from the Minamata district were found to require hospitalization, and about 45% to require regular visits to the hospital for treatment and supervision, for a total of about 53% requiring medical observation. As for 43 Minamata disease patients from the A community

of Minamata district, 3 (7%) required hospitalization, 13 (30%) had to make regular hospital visits, 17 (40%) — periodically, and 10 (23%) required no therapy.

TABLE 22. CASES OF MINAMATA DISEASE DIAGNOSED AS REQUIRING OR NOT REQUIRING THERAPY; NUMBER OF CONGENITAL MINAMATA DISEASES CASES EXCLUDED: FIGURES IN PARENTHESES INDICATE PERCENTAGE

	Minimata district	Goshonoura district	Ariake district
Hospitalization required	·' (· ·)	: .·)·	
Regular hospital visits required	I.. (;·· ·)!	!! · ·!·	··
Periodic hospital supervision required	·) ·.!.·)!	!·· (I I)	··
Therapy virtually unneeded	!· (·i.7)!	· (·;)	
Therapy unneeded	!·· (I ·)!,	() (())	—
Total number examined	269	34	8

13. Designation

The number of individuals designated by Kumamoto Prefecture as having Minamata disease was 18 (also, 3 other congenital Minamata disease patients) during the period of December, 1956, through November, 1962; 1 in May, 1969; 16 (also, two other congenital Minamata disease patients) from January, 1970, through July, 1971. An additional 44 were designated during the period of this survey, from August, 1971, through March, 1973.

B. Congenital/Fetal Minamata Disease

Congenital Minamata disease patient from Minamata district numbered 2 males (born November 1958/April 1962) and 4 females (born August 1955, July 1956, October 1956, June 1957). The male child born April, 1962 was first uncovered during the general examination. He was stricken 45 days after birth with an onset of convulsions. As of February, 1973, he showed mild imbecility, disorders in the upper extremities and face, loss of coordination, breakthrough

141

of the θ-wave in the brain, and multiple higher/slower waves in the right side of the brain. He belongs to the mild category, probably congenital + posteriori Minamata disease. Both parents were designated as having Minamata disease, and application by the brother (age 19) for such a designation is pending.

Symptoms for the 6 cases were: mental disorder, 6 cases; primitive reflex, 4; inhibition of motion or involuntary movement, 5; cerebellum disorder, 4; seizures, 5. Degree of infliction: 5, serious; 0, moderate; 1, mild. Two required hospitalization, and 4 required hospital visits.

Of the six individuals, five have been designated by the prefecture as having Minamata disease.

Section 4. Probable Minamata Disease Cases

A probable Minamata disease case, with reference to Minamata district, means an individual who manifests only basic symptoms, such as sensory disorders or sensory disorder + field of vision constriction, and there are no other genuine Minamata disease patients in the family. In the case of Goshonoura district, those having a mild constriction of vision, or one good eye, even though the other eye had been lost, a cataract, or even old age, were counted as probable Minamata disease cases. The actual cases are enumerated in Part 3.

a. Epidemiological Matters

Year of onset: With respect to the 19 cases (of which 8 are males) scrutinized in Minamata district, onset of disease occurred between 1955 and 1972, with 11 being stricken between 1963 and 1969.

Outbreak in family: In the families of those with probable Minamata disease, 23.7% were afflicted in the Minamata district; the ratio in the Goshonoura district was lower, and none in the Ariake district.

Age afflicted: age 40 or over in the case of 10 persons, under 39
in the case of 9 persons, and unknown in one case.

b. Condition at Time of Examination

Various symptoms

All of the symptoms seen on the case diagnosed as Minamata disease were
observed on probable cases (Table 10)

Proprioceptive symptoms were identical to those of Minamata disease.
For example, they consisted of pain (16 cases), numbness (11 cases), weakness
of extremities (10 cases), aches (9 cases), tiredness (9 cases), forgetfulness
(9 cases), loss of strength (9 cases), fatigue (8 cases), falling down easily
(8 cases), cloudy vision (6 cases), and ringing in the ears (6 cases).

Sensory disorders also resembled those of Minamata disease, such as
serious disorders on the tip of the upper and lower extremities, disorders
around the mouth, islet-type disorders, disorders of the whole body, serious
disorders (loss of senses), and abnormal senses (Table 11). Loss of coordina-
tion as noted in various tests was also similar to that of Minamata disease
(Table 12). Several tremors were also noted in movement (Table 13).

A review of cases marked by unconsciousness and dizziness showed that
6 out of 19 persons from Minamata district, and 4 out of 31 persons from
Goshonoura district suffered from the same.

As for mental disorders, excluding those of the intellect and emotions,
there was 1 case involving neurological conditions (complaints of bodily and
mental disorders plus depression and anxiety) from Minamata district, and
another case involving a 59-year-old woman from Goshonoura who suffered from
occupational delirium and excessive sleep for about 8 years. As of February,
1973, the latter was unable to change body positions on her own power; had
delusions of a cat climbing on her buttocks and of working in the field;

143

constantly spoke to herself; lacked inhibition; excitable, serious disorder of mental functions, primitive reflex; loss of coordination and tremors as determined by finger-nose tests; sensory disorders of the mouth; serious vision impairment; excessive sleep, loquacity, and readily changing symptoms. Degree of affliction: 1; required hospitalization.

An x-ray test of the cervical vertebrae was conducted on 17 cases (Table 17). The results have been reviewed in the course of comparison with those of Minamata disease patients (Chapter 2, Section 3, VII, A, 7). In 3 of those cases, serious changes were also noted in the lumbar vertebrae. For example, the first case involved a 67-year-old woman characterized by a marked curvature of L_3 to the left; sensory disorders in the right half of the body; increase of muscular tension and of innate reflexes, and increase of roughness potential. Although such symptoms were serious, there was no great difference between the upper and lower extremities (Goshonoura). The second case involved a 64-year-old male with L_2 crushed in the frontal portion, moderate spondylosis; inability to tell left from right in movement or tremors; no difference was noted in the degree of disorder in upper and lower limbs with respect to muscular tension, innate reflex, and roughness potential (Minamata district). Thus, changes in the lumbar vertebrae did not appear clearly as neurological symptoms in these 3 cases. A mild spondylosis of the cervical vertebrae was also noted in these cases.

A biochemical test of the blood serum was conducted in 42 cases (Table 19).

Clinical picture

A review of the combinations of major symptoms making up the clinical picture showed they resembled those of cases diagnosed as Minamata disease (Table 20).

144

Among Goshonoura district residents, many cases embrace all of the symptoms. In one case in Ariake district, involving a fisherman born Nobember 21, 1896, the patient suffered from serious sensory disorder around the mouth and the tip of upper and lower extremities, and also had a loss of coordination, constriction of vision, moderate articulation disorders, imbecility, euphoria, and excitability. He succumbed on November 3, 1971, before he could undergo a second examination.

Degree_of affliction_and_relationship between it
and age at_onset_and_present_age

In Minamata and Goshonoura districts, those suffering from neuropsychological disorders numbered: serious degree, 0 and 3; moderate degree, 8 and 17; mild degree, 9 and 11, respectively. Out of the 19 Minamata district patients, 7 having a moderate degree of ailment were aged 40 or over at the time of onset, and 7 with a mild ailment — under age 39. As for present age and degree of affliction, 6 cases aged 60 or over were moderately affected, 4 cases aged 40-49 mildly affected, and 6 cases aged 39 of under were also mildly affected.

The rapy

In the two districts above, those requiring hospitalization numbered 0 and 1; those requiring regular hospital visits, 5 and 1; those requiring periodic visits, 5 and 22; and those not requiring therapy, 7 and 7, respectively.

Section 5. Deferred Diagnosis

Cases where diagnoses are deferred are those in which we cannot find reasons for completely denying the effects of organic mercury poisoning. For example, one patient in the Minamata area has a patient suspected of Minamata disease within his family, but he shows only a minor loss of balance and speech problem. There is another case where, although he has a Minamata disease patient in his family, he shows only a sensory disorder on the right

145

side of his body; a case where, although he shows a mild sensory disorder, he does not have any speech problem and does not have any Minamata disease patients in his family. Some examples of cases whose diagnoses are deferred in the Goshonoura area are: a case where a member of his family is a Minamata disease patient, but he has limited vision and hearing difficulties and does not show loss of balance; a case where, although limited vision is observed in his right eye, he is the only one who escaped loss of sight and a medium degree of hardening of the arteries at the bottom of this eye; no sensory disorder was observed; a case where sensory disorder, loss of balance, and noticeable limited vision are accompanied by deformation of retina pigments; a case where, although mild loss of balance and narrow vision are observed, the patient is over 80 years old. Cases of deferred diagnoses in each area are 0.7 - 1.7% of examined residents (Table 3). Occurrences of Minamata disease patients or suspected Minamata disease patients among members of families of diagnostic deferred patients are especially high in the Minamata area, and are 20% - 53.3% (Table 7).

Among these cases, similar symptoms seen in the cases which were diagnosed as Minamata disease were observed (Table 10). The frequencies of sensory disorders and hearing difficulty are high, and cases of narrow vision are also noticed. Many cases of sensory disorders exhibit a higher degree of disorder closer to the extremities of the four limbs (Table 11). Various kinds of loss of balance which are similar to those observed among Minamata disease patients are seen (Table 12). Biochemical examinations of serums were performed on 34 cases (Table 19).

As far as symptoms which are a combination of basic clinical symptoms are concerned, a few types of symptoms comprise a majority at the Minamata and Goshonoura areas (Table 20).

146

Section 6.   Other Diseases

Table 23 illustrates the number of patients of other diseases and their frequencies, observed in each of the investigated areas.

Neuropsychiatric disorders due to high blood pressure and hardening of the brain arteries are $6.60 \pm 1.17\%$, $14.70 \pm 1.27\%$, and $17.70 \pm 1.80\%$ in the Minamata, Goshonoura, and Ariake areas, respectively. The differences between the Minamata and Goshonoura areas $(\chi^2 \geq 18.25,\ P<0.001)$ and the difference between the Minamata and Ariake areas $(\chi^2 \geq 26.25,\ P<0.001)$ are both significant. On the other hand, the differences between the Minamata area and Heianza Island, Okinawa $(\chi^2 \geq 2.12)$, and the difference between the Goshonoura and Ariake areas and Heianza Island $(\chi^2 \geq 1.94)$ are not significant [15].

Idiotism in Table 23 is observed among people over 40 years old. These cases, from our experience, are mostly due to hardening of the brain arteries. If we include these cases, the frequencies of neuropsychiatric disorders due to high blood pressure and hardening of the brain arteries are $7.70 \pm 1.25\%$, $16.01 \pm 1.31\%$, $21.05 \pm 1.92\%$, at the three areas, respectively. The difference between the Minamata area and the Goshonoura and Ariake areas is significant $(\chi^2 \geq 17.52,\ P<0.001)$. The difference between the Goshonoura and Ariake areas is significant $(\chi^2 \geq ,\ P<0.05)$.

Two cases of progressive paralysis $(0.17 \pm 0.37\%)$ were discovered. This has been considered a rare disease in recent years. Both of them are in area mental hospitals.

Epilepsy is $0.55 \pm 0.28\%$, $1.31 \pm 0.32\%$, $1.15 \pm 0.40\%$ in the three areas, respectively. The value at Heianza Island is $0.11 \pm 0.12\%$. The difference between the Minamata area and Heianza Island $(\chi^2 \geq 324.88,\ P<0.001$; Yates' modification) and the difference between the Goshonoura area and Heianza Island are significant $(\chi^2 \geq 10.11,\ P<0.01)$. However, differences between the Minamata and the Goshonoura areas $(\chi^2 \geq 2.02)$, and between the Ariaka area and Heianza Island $(\chi^2 \geq 0.87)$, are not proven to be significant $(\chi^2 \geq 3.77,\ P<0.06)$.

147

TABLE 23. OTHER DISEASES

Disease	Minamata area			Goshonura area			Ariake area		
	Actual number of patients	Related number (age susceptible to the disease, years old)	Corrected frequency (%)	Actual number of patients	Related number	Corrected frequency (%)	Actual number of patients	Related number	Corrected frequency (%)
Neuropsychiatric disorder due to high blood pressure and hardening of brain arteries (a)	30	454 (> 40)	6.60	115	731	14.70	80	451	17.70
Idiotism (b)	5	454 (")	1.10	10	731	1.28	15	451	3.32
a + b	35	454 (")	7.70	125	731	16.01	95	451	21.06
Aftereffects of head injury	3	924 (> 0)	0.32	12	1,723	0.70	3	904	0.33
Progressive paralysis	2	428.5 (31—50)	0.47	0	—	—	0	—	—
Epilepsy	4	724.5 (5—30)	0.55	17	1273.0	0.55	8	697.5	1.15
Schizophrenic (c)	8	575.0 (16—40)	1.39	9	961.5	0.94	5	519.0	0.96
Suspected schizophrenic (d)	2	" "	—	6	—	—	0	—	—
c + d	10	" "	1.74	15	961.5	1.56	5	519.0	0.96
Depression	1	173.0 (21—50)	0.21	6	819.5	0.73	7	453.5	1.54
Nervous disease, hysteria	10	813 (> 10)	1.23	11	1446	0.76	6	744	0.81
Alcoholism	3	393.0 (31—70)	0.76	14	562.5	2.49	1	414.0	0.24
Weak mind	25	885 (> 5)	2.82	48	1592	3.02	35	855	4.09
Parkinsonism	1	924 (> 0)	2.71	10	1723	2.79	4	904	3.87
Deformation of spinal cord	13	—	—	55	—	—	31	—	—
Spinal cord disorder	6	—	—	7	—	—	5	—	—
SMON	1	—	—	0	—	—	0	—	—
Aftereffects of injury	4	—	—	6	—	—	1	—	—

148

TABLE 23. (continued)

Disease	Minimum and maximum for contrast (investigated area)
	Corrected frequency (%)
a	8.24 (Heianza Island, Okinawa [3])
b	–
a + b	–
Head injury aftereffects	0.13 (Heianza Island, Okinawa)
Progressive paralysis	0.33-0 (Ikebukuro, Tokyo - Miyake Island [9, 14])
Epilepsy	0.89-0.12 (Imazumi village, Saitama - Gokeso, Kumamoto [9, 14])
c	2.47-0.49 (Kurao, Saitama - Ikebukuro, Tokyo [9, 14])
d	–
c + d	–
Depression	0.87-0.14 (Komoro town, Nagano - Imazumi village, Saitama [9, 14])
Nervous disease, hysteria	0.39 (Heianza Island, Okinawa)
Alcoholism	0.71 (" " ")
Weak mind	2.38 (" " ")
" "	2.19 (" " ")
Parkinsonism	–
Spinal cord deformatn.	–
" " disorder	–
SMON	–
Injury aftereffects	–

Revised frequencies of schizophrenia are $1.39 \pm 0.49\%$, $0.91 \pm 0.31\%$, $0.96 \pm 0.43\%$ at the three areas respectively. These values are between the maximum and the minimum values for residents of other areas in Japan. The value at Heianza Island is $2.11 \pm 0.16\%$. The differences between the Minamata and the Goshonoura areas ($\chi^2 \geq 0.32$ and $\chi^2 \geq 0.13$), between the Goshonoura area and Heianza Island ($\chi^2 \geq 0.67$), between the Goshoura area and Heianza Island ($\chi^2 > 3.70$, $P < 0.06$), and between the Ariake area and Heianza Island ($\chi^2 > 2.01$) are not proven to be significant.

149

Among manic-depressive psychoses, only depression was found in this examination. Its frequencies are $0.21\pm0.21\%$, $0.73\pm0.30\%$, $1.51\pm0.58\%$ at the three areas, respectively. No significant difference $(\chi^2=1.12)$ is seen between the Minamata area and Komoro town, which is selected for comparison and whose frequency is $0.87\pm0.22\%$. Also, no significant differences are seen between the Minamata and the Goshonoura areas $(\chi^2>1.18)$ and between the Ariake area and Komoro town.

Alcoholism is $0.76\pm0.11\%$, $2.19\pm0.65\%$, $0.21\pm0.21\%$ at the three areas, respectively. Significant differences are seen between the Minamata and the Goshoura areas $(\chi^2=3.91,\ P<0.05)$, between the Goshonoura and the Ariake areas $(\chi^2=4.17,\ P<0.02)$, and the Goshonoura area and Heianza Island $(\chi^2=1.52,\ P<0.01)$. However, no significant difference is proven between the Minamata area and Heianza Island $(\chi^2\geq0.05)$.

Ratios of people who are mentally disturbed and are over 5 years old and total residents over 5 years old are $2.82\pm0.56\%$, $3.02\pm0.45\%$, $1.69\pm0.68\%$ at the three areas, respectively. Ratios of mentally disturbed people and total residents over 0 years old are $2.71\pm0.53\%$, $2.79\pm0.40\%$, $2.87\pm0.61\%$, respectively. The frequency of mentally disturbed people against total population is said to be 2 - 3%. The values for the Minamata area and Heianza Island are close. In the ratios of the population over 5 years old, no significant differences are recognized between the Minamata area and the Goshonoura and Ariake areas $(\chi^2>0.33)$, and between the Goshonoura and the Ariake areas $(\chi^2\geq1.97)$.

Among other diseases, x-ray examinations were performed on 23 cases, excluding inherent mental disease patients (Table 17), and biochemical examinations of serums were performed on 35 cases (Table 19).

There are 275 Minamata disease patients who have been recorded as a result of this investigation and 26 confirmed patients who died before the investigation, totaling 301 patients in the Minamata area. According to Table 24, 36 of them, i.e., 12%, were dead by the end of March, 1973. Among the

TABLE 24. DEATH OF MINAMATA DISEASE PATIENTS AND SUSPECTED MINAMATA
DISEASE PATIENTS

1) Numbers without parentheses are confirmed cases; numbers in
parentheses are unconfirmed cases who died between August,
1971 and to date.
2) Investigation up to the end of March, 1973.

Age	Minamata disease							Suspected Minamata disease		
	Minamata area			Unexamined areas				Minamata area		Ariake area
	M	F	T	M	F	T		M	F	M
2 - ?	1	3	4	1	2	3	7	—	—	—
? - 14	?	3	6	0	0	0	6	—	—	—
15 - 29	0	2	2	1	0	1	3	—	—	—
30 - 39	2	0	2	2	0	2	4	—	(1)	—
40 - 49	0	1	1	4	0	4	5	—	—	—
50 - 59	3 (1)	2 (1)	5 (2)	4	5	9	14	—	—	—
60 - 69	2	3 (2)	5 (2)	7	0	?	12	(1)	—	—
70 - 79	5	1	6	8	0	8	11	—	—	(1)
80 - 88	1	0	1	1	2	3	4	—	(1)	—
2 - ?	17 (1)	15 (3)	32 (4)	28	9	37	69	(1)	(?)	—

M — male; F — female; T — total.

dead, many were youngsters under 14 years old or elderly persons over 50.
This phenomenon is similar among patients in areas not investigated. As
stated before, 38 patients who showed Minamata disease-like symptoms were dead
before the investigation in the Minamata area. Also, three patients who were
suspected of Minamata disease were dead after the examination but during the
investigation. Therefore, 77 out of 342 patients, i.e., 22.5%, of the

Minamata disease and suspected Minamata disease patients from the Minamata area were dead between 1941 and March, 1973. During this 20-month investigation, 10 Minamata disease patients (6 are confirmed patients) and 3 suspected Minamata disease patients, totaling 13, were dead.

From the investigation of family history of residents of the Minamata area, 39 persons (24 males) were recorded dead before our medical examination; Minamata disease was unconfirmed, but they had Minamata disease-like symptoms. According to the description of these families, they showed symptoms such as numbness of hands and feet, shaking, speech difficulty, loss of strength, loss of balance, difficulty in walking, difficulty in moving hands and feet, and vision difficulty. Some reported that they even destroyed a paper sliding door by losing their balance and falling. During the investigation, 30 of them (79%) were proven to have 1 - 4 Minamata disease patients who lived with them. Two of them have suspected Minamata disease patients, as well as Minamata disease patients who lived with them. Also, five of them have suspected Minamata disease patients among members of their families who lived with them. The years of their deaths are: 32 between 1941 and 1971, 6 unknown; 27 between 1954 and 1971. Their ages at death are: 27 persons over 50 years old (71%), 5 persons — age unknown.

Section 8. Amounts of Mercury in Hair and the Disease

Measurements of amounts of total mercury in the hair of fishermen, primarily along the shores of the Shiranui Sea, were performed in 1960, 1961, and 1962 [11]. Among people who were examined at this time, 42 males and 27 females in the Minamata area and 2 persons (both females) in the Goshonoura area were measured for the mercury content in their hair at that time. At the examination, 35 persons had not had the Minamata disease or were not suspected of it; 5 persons became ill in the same year, and 18 persons already had the disease.

Among those examined, 34 males and 24 females had Minamata disease; 1 male and 1 female were suspected of having the disease, and 7 males and 4 females had other diseases.

A table was constructed in order to examine the relationships between amounts of mercury in hair and occurrences of the Minamata disease, degree of illness of the disease, and narrow vision (Table 25). The amounts of mercury were primarily obtained by measuring the materials collected in 1960. As is

TABLE 25. RELATIONSHIPS BETWEEN AMOUNTS OF MERCURY IN HAIR AND VISION. MINAMATA DISEASE, CONDITIONS OF MINAMATA DISEASE, AND POOR VISION NUMBERS WITHOUT PARENTHESES ARE FOR MALES, AND NUMBERS IN PAREN-THESES ARE FOR FEMALES

| PPM | Minamata + suspected Minamata disease | | | | | | | | | | | Other diseases | | | |
| | Total no. | Conditions | | | | | Poor vision | | | | Total no. | Conditions | | | Poor vision |
		1	2	3	4	5	++	+	+	−		2	4	5	−
−0	2 (1)	−	−	1 (1)	−	1	1 (1)	−	−	1	1	−	1	−	1
−0.5	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−1	(1)	(1)	−	−	−	−	(1)	−	−	−	−	−	−	−	−
−5	2 (6) 1	(1)	(1) 1		(1)	(3) 1 (2)	−	1 (1)	(1)	−	−	(1)	(1)
−10	3 (2)	−	1	(1) 2 (1)	−	−	3 (2)	−	−	1 (2)	−	−	4 (1) 4 (2)
−20	6 (5)	−	1 (2) 2 (2)	(1) 3		(1) 3 (1)	(1) 3 (1)	(1)	−	−	(1)	(1)
−30	4 (2) 1	−	(1) 3	(1)	−	1 (2)	−	2	1	1	−	−
−40	2 (2)	−	−	1 (1) 1 (1)	−	1	1 (1)	−	(1)	−	−	−	−	−
−50	8 (1)	−	−	3	3 (1) 2	3 (1) 2	−	3	−	−	−	−	−
−60	1	−	−	1	−	−	1	−	−	−	1	−	−	1	1
−70	1	−	−	−	1	−	−	1	−	−	−	−	−	−	−
−80	(1)	−	−	1	−	−	−	−	−	(1)	−	−	−	−	−
−90	3	−	1	1	−	1	2	−	1	−	−	−	−	−	−
−100	1 (1)	(1)	1	−	−	−	1	−	−	−	−	−	−	−
−150	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
−200	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
200−	1 (1)	−	(1) 1	−	−	−	1	(1)	−	−	−	−	−	−

Total 34(23) 2 (2) 3 (4)11(11)11 (4) 7 (2) 6 (6)14 (9) 1 (2)10 (4) 7 (4) 1 1 5 (3) 6 (4)

*
We could not prove poor vision for two cases of Minamata disease and one case of suspected Minamata disease, and for one case of the other diseases. Therefore, the number of cases for poor vision is 33 (21) for the former and 6 (4) for the latter. Underlined number — cases of suspected Minamata disease (2 cases).

clear from this table, 13 out of 18 males (72%) whose hair contained less than 20 ppm mercury, and 10 out of 13 (77%) females whose hair contained less than 10 ppm mercury had Minamata disease. Based on data by Ukita [4], it is

considered to be normal if a male has less than 20 ppm mercury in his hair, and if a female has less than 10 ppm. What can be observed from the next table is that 9 out of 11 persons who were diagnosed as having other diseases had less than 20 ppm mercury in their hair. Among those whose hair contained 0 - 10 ppm mercury, 7 out of 34 males (20.6%) and 10 out of 23 females (43.5%) had Minamata disease, and it is slightly higher among females $(\chi^2 \geq 3.43.$ $P < 0.07)$.

M.d :	55	Total 57	M.d: 33	Total 34	M.d: 22	Total	Other
SM.d:	2	cases,	SM.d: 1	cases,	SM.d: 1	23	diseases:
		M and F		male		cases,	11 cases,
						F	M and F
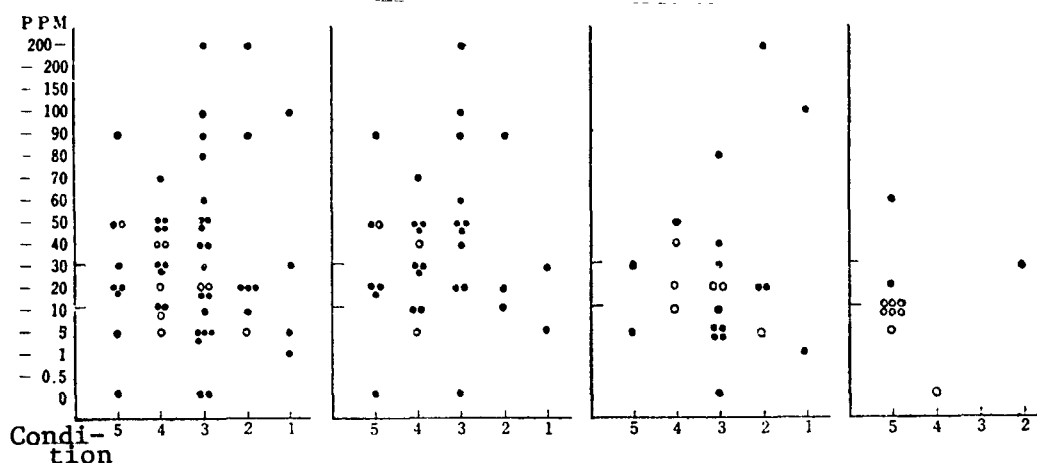
Figure 1. Relationships between amounts of mercury in hair and occurrences and conditions of Minamata disease:

● — materials in 1960; o — materials in 1961 - 1962; M.d — Minamata disease; SM.d — suspected Minamata disease; F — male; F — female

In order to facilitate the analysis of relationships between amounts of mercury in hair and conditions of the disease and poor vision, a graphical representation of the table is made (Figure 1). We cannot prove a fixed relationship between mercury content and conditions of the disease, even from this figure. However, the figure shows the various above-mentioned facts obtained from Table 25 more clearly.

Minimum and maximum cases of mercury content in hair

(i) The first case is a female born in Tsukinoura in March, 1951. She is a farmer, and her husband is an office worker. She ate fish 2 - 3 times a week. She started showing symptoms of twitching of her muscles in 1957, and of numbness of her hands and feet in 1960. The amount of mercury in her hair was 0 ppm in December 1960. Her condition on November 1, 1972 was: speech problem (+), loss of balance (+), poor vision (++), sensory disorders of all of her body, loss of sense of touch, aches, coldness, and warmness from her lower limbs to her feet (+++), hearing problem (+++), mental redardation (+), positive serum syphilis reaction (+); the condition of her disease: 3. Confirmed as having Minamata disease in April, 1973.

(ii) The second case was a fisherman (single rod fishing) of Yudo, born in March, 1914. He wife and 3 younger of his 6 children were Minamata disease patients. He showed symptoms of numbness in his hands and fever in April, 1954. He could not keep his slippers on in 1965. Loss of sight and a speech problem occurred in 1968. The amount of mercury in his hair was 302.5 ppm in November, 1960, 18.33 ppm in July, 1971, and 147.5 ppm in December, 1960. His condition on February 14, 1973 was: loss of balance (++), poor vision (+), sensory disorders (++), hearing problems (+), mental retardation (+), shaking of his body (++), weakness of muscles on the backs of his hands (2), deformation of spinal cord; blood pressure: 174/110; albumin in the urine (+); the condition of the disease: 3. Confirmed having Minamata disease in January, 1973.

PART 2

TREATMENT

Treatment by drugs was tried for subjective symptoms, which are one of
the worst sufferings among Minamata disease patients. This was done for the
patients who visited a mental hospital in the examined Minamata area and who
had illness conditions of 3 - 4. Ages and sex of the patients are: 30 - 39
years old, 0 (male) - 1 (female); 40 - 49 years old, 2 (male) - 1 (female);
50 - 59 years old, 1 (male) - 3 (female); 60 - 69 years old, 5 (male) - 10
(female); 70 - 79 years old, 4 (male) - 3 (female); total 30 persons (12 male).
The treatment periods were: 2 months, 11 persons (5 male); 4 months, 4 per-
sons (3 male); 6 months, 5 (1 male); 8 months, 3 (no males); 10 months, 6
(2 males); 12 months, 1 (male).

Table 26 summarizes the effect of treatment performed. According to the
table, some symptoms are relatively easily improved by the treatments: some
examples are insomnia, headaches, aching of limbs and body, and irritations.
On the other hand, the following symptoms are difficult to cure: forgetfulness,
hearing difficulty, poor vision, loss of strength, blackouts, etc.

The summary of the effects of treatments is shown in Table 27. According
to the table, 15 out of 30 examined cases showed improvement in their symptoms.
In the case of improvement, 8 cases showed partial reduction or partial im-
provement of subjective symptoms, and 7 cases showed improvements in every
symptom. Fourteen cases, which is a large number, showed improvement of some
symptoms and deterioration in other symptoms.

Finally, Table 28 illustrates drugs which are considered to be effective
for these symptoms. For insomnia, various drugs have proven effective. Among
them are tranquilizers (Dichlotride, Selpasel, Apresoline), Contamine, pheno-
barbital. These three drugs are also effective for headaches. Phenobarbital
and Contamine are effective for crow bend and aching of limbs and body, and
these two drugs and Contole are slightly effective for irritation.

156

TABLE 26. CHANGES IN SYMPTOMS DUE TO TREATMENT

	Subjective symptoms	Disappearance	Improvement	Unchanged, Deterioration
1.	Insomnia	13 (50.0) 1 (4.2)	11 (42.?) 12 (?)	2 (7.7) 1 (?)
2.	Headache			
3.	Crow bend			
4.	Aches in limbs and body	6 (23.1) 1 (3.4)	7 (26.9) 10 (34.5)	13 (50.0) 18 (62.1)
5.	Irritation	2 (7.4) —	8 (29.6) 4 (15.2)	17 (63.0) 18 (81.8)
6.	Depression			
7.	Shaking	1 (5.0) 1 (4.8)	2 (10.0) 2 (9.5)	17 (85.0) 18 (85.7)
8.	Muscle twitching			
9.	Difficulty in talking clearly			
10.	Numbness	—	1 (13.3) 4 (13.3)	25 (83.3) 26 (86.7)
11.	Loss of taste	—	2 (14.3) 3 (10.3)	13 (90.7) 25 (89.7)
12.	Loss of clear vision			
13.	Falling, staggering	—	3 (10.3) 1 (10.0)	27 (90.0) 9 (90.0)
14.	Abnormal feeling			
15.	Buzzing in ears	1 (4.8)	1 (4.8) 2 (7.1)	19 (90.5) 26 (92.9)
16.	Dullness of limbs and body			
17.	Difficulty in taking slippers off	—	2 (7.7) 1 (7.7)	25 (92.3) 12 (92.3)
18.	Faint			
19.	Difficulty in putting slippers on	—	2 (6.7) 2 (6.7)	28 (93.3) 28 (93.3)
20.	Early fatigue			
21.	Blackout	1 (4.3)	— (4.3)	22 (95.7) 22 (95.7)
22.	Laziness	—	1 (4.3)	22 (95.7) 22 (95.7)
23.	Loss of finger grip, dropping things	—	1 (4.0) 1 (3.3)	24 (96.0) 27 (96.7)
24.	Loss of strength			
25.	Poor vision	—	—	22 (100.0) 23 (100.0)
26.	Loss of sense of smell			
27.	Loss of hearing	—	—	28 (100.0)
28.	Forgetfulness			

157

TABLE 27. EFFECTS OF TREATMENTS

	Male	Female	Total
Improvement	6	9	15
Disappearance + better	2	6	8
Better	4	3	7
Partial improvement and partial deterioration	6	8	14
Unchanged	0	1	1
Deterioration	0	0	0
Total	12	18	30

TABLE 28. RELATIONSHIPS BETWEEN IMPROVED SYMPTOMS AND DRUGS

	Insomnia			Head-ache			Crow bend			Limb, body aches			Irrita-tion			Despres-sion		
	D*	I*	U*	D	I	U	D	I	U	D	I	U	D	I	U	D	I	U
	13	11	2	1	12	11	6	7	13	1	10	18	2	8	17	—	4	18
Nelbon	2	2	—	—	1	2	1	1	1	—	1	3	—	2	2	—	—	2
Phenobarbital	7	7	1	—	8	7	5	3	10	—	5	10	—	6	10	—	2	14
Netobal	1	2	—	—	2	1	—	1	—	—	—	3	—	1	2	—	—	1
Contamine	8	8	2	1	9	6	5	6	8	1	9	11	2	5	11	—	2	11
Horizon	3	—	—	—	—	2	1	1	1	—	—	3	—	1	2	—	1	—
Contole	4	6	—	—	6	3	2	1	7	1	2	7	2	5	4	—	2	6
Selnale	2	6	—	—	5	3	2	2	2	—	1	6	—	3	5	—	2	3
Triputanole	3	1	—	—	2	2	—	2	1	—	1	2	—	2	1	—	3	2
Calicrane	5	1	—	—	3	2	1	2	2	—	1	3	—	3	2	—	1	4
Hexanisset	6	2	—	—	3	5	—	5	6	1	3	8	1	4	6	—	1	5
Capilon	6	3	—	1	5	4	2	4	5	—	5	6	—	3	6	—	2	8
Pontal	6	0	1	1	2	1	—	3	3	1	3	3	1	1	4		1	5
Tranquilizer	10	5	1	1	9	5	3	7	8	1	6	12	2	6	8	—	3	11
Sularon	5	3	1	—	2	6	1	1	6	1	2	8	—	1	10	—	1	8
Neobitacane	2	1	—	—	1	2	1	—	1	—	—	3	—	—	3	—	—	2

*D — disappearance; I — improvement; U — unchanged.

158

Triputanole or tranquilizers are sometimes effective for
depression.

As a summary, phenobarbital, Contamine (chlorpromazine), and tranquili-
zers are, in general, effective for subjective symptoms of Minamata disease
patients.

PART 3

INVESTIGATION OF HEALTH CONDITIONS OF EMIGRANTS
TO OTHER PREFECTURES

Introduction

The health conditions of 22 persons who emigrated from the Minamata area
under investigation to other areas were examined neuropsychiatrically. All
of them were those who came back temporarily to the Minamata area for New
Years Day, Bon Festival, or memorial services. Years when they left Minamata
were as follows: 1950 - 1961, 11 persons; 1962 - 1968, 7 persons; 1970 - 1971,
4 persons. Their current addresses are: 2 in Tokyo, 2 in Chiba City, 6 in
Gifu, 1 in Nara, 6 in Osaka, and 5 in Kitakyushu City. Males (13 persons) and
persons under 39 years old (15 persons) are in the majority. Their current
occupations are: 4 plasterers, 4 construction workers, 3 businessmen, 2
drivers, 1 engineer, 1 carpenter, 1 clerk, and 5 housewives.

Twenty persons belong to 16 families in the Minamata area, and all of
these families have other Minamata disease patients within them.

Condition Before This Examination

Twenty out of 22 persons complained of symptoms, such as numbness, head-
aches, aches in joints, dizziness, insomnia, dullness, early fatigue, and
forgetfulness, and have received treatments at many hospitals. However, in

159

all cases, they were diagnosed as unknown or nervous disorders, and no cases were diagnosed as Minamata disease or suspected of it.

Fourteen persons experienced the subjective symptoms mentioned above before they left the Minamata area, and 6 persons did so after they left the area.

Results of Investigation

a. Overview of diagnoses

i) Minamata disease: 20 persons

ii) Suspected Minamata disease: 1 person

iii) High blood pressure: 1 person

b. Cases which were diagnosed as Minamata disease

1. Symptoms

The following symptoms: subjective symptoms, 20; speech problem, 16; loss of balance, 20; loss of strength, 13; poor reflexes, 3; shaking, 7; poor vision, 7; sensory disorder, 20; hearing difficulty, 16; olfactory disorder, 6; epilepsy, 1; dizziness and other disorders of the conscience, 15; mental disorder, 19; emotional disorder, 18; high blood pressure, 4; low blood pressure, 1.

2. Conditions of the disease, need for treatment

High: 2 cases; medium: 15 cases; low: 3 cases. Need to be hospitalized: 0; need to visit a hospital regularly: 7; need to visit a hospital occasionally: 10; no need for treatment: 3.

c. Case suspected of Minamata disease

The patient is a female, born in January, 1946, in the heavily mercury contaminated area, married in January, 1971, and moved to Chiba city. Although she showed a hearing difficulty in 1958, she did not show any subjective symptoms in May, 1972. However, she displayed dullness in the sense of touch, aches, and warmth in parts of her lips, right leg, disorder of vibration senses, loss of balance in knee bend test, and a medium degree of mental retardation. Both parents and two of her siblings are Minamata disease patients, and her other two siblings are suspected of the disease. This case can be actually considered as Minamata disease. The degree of disorder is light, and no medical treatment is considered to be needed.

d. Case of high blood pressure

The patient is a carpenter born in August, 1950, and moved to Osaka in April, 1959. In December, 1971, his blood pressure was 170/90, although no neurologically noticeable events were observed. His grandmother, with whom he lived before he moved to Osaka, is a Minamata disease patient, and his grandfather died from Minamata disease-like causes.

PART 4

MERCURY CONTENT IN HAIR OF RESIDENTS ALONG SHORES OF
THE SHIRANUI SEA AND IN FISH IN THE SAME AREAS

Introduction

This simultaneous examination brings up a question that mercury contamination in the Shiranui Sea may still affect the health conditions of residents today. This research was carried out to measure the amounts of total mercury contained in the hair of residents in the Minamata and Goshonoura areas,

where simultaneous examinations were performed, and in Kumamoto city for the purpose of comparison. Many residents in the Minamata and Goshonoura areas who were subjects of the measurment were either Minamata disease patients or suspected Minamata disease patients. Residents in Kumamoto city were selected randomly. Also, the amounts of mercury were determined in fish from Minamata Bay, the eastern short of Amagusa which includes the Goshonoura area, and the western shore of Amagusa which includes the Ariake area. These fish were obtained between February, 1972, and March, 1973.

Method and Data

The amount of total mercury was measured. Using instruments and methods described by AOAC [1], the hair was turned into wet ashes, all mercury was changed into inorganic mercury, reduced to metal mercury using tin monochloride, and measured by the mercury vapor ultraviolet absorption method (Hiranuma mercury gauge). Reagents used were either manufactured by Wakojunyaku or by Katayama Chemical for analytical purposes.

Approximately 100 - 200 mg of hair were used for measurement. The reason for choosing 100 - 200 mg of hair is that a full scale of the Hiranuma mercury gauge is 0.5 µg, and the amount of liquid required for each measurement is 100 ml. In order to use the central part of the scale for a measurement, it is appropriate to adjust so that the amount of mercury in 100 ml of test oxide liquid is about 0.25 µg. Theoretically, 100 ml of liquid for measurement is sufficient. However, it is difficult to keep the reaction liquid to 100 ml, considering the amount of oxidizing and deoxidizing materials used. Also, if we consider the possibility of measuring several times, using the same oxidizing liquid, 500 ml of oxidizing liquid would be appropriate. Calculating from the concentration described above, 1.25 µg of mercury is contained in 500 ml oxidizing liquid. In order to attain this value, about 150 mg hair containing 8 ppm mercury are sufficient. Eight ppm is close to the average amount of mercury in the hair of a Japanese, and major portions can be measured using the central part of the scale; thus, more precise values can be expected. For actual measurements, the amount of mercury in hair is

computed by subtracting a comparison value (using an equal amount of a re-
agent used in the experiment, the oxidizing liquid is obtained by performing
the identical operation without adding hair) from the actual reading. In
order to confirm the effectiveness of this method, the results from residents
in Kumamoto city measured by this method were compared statistically with
the results obtained in Tokyo in 1966 by Fukada et al. [4]. Both variances
$(P < 0.5)$ and averages $(P < 0.1)$ are similar, and no significant differences
are found. At the same time [3], a variance of the logarithm of amounts of
mercury in hair (9 subjects) which was taken from various parts of the same
person's head, and were measured by this method, is 0.0028. Considering that
amounts of mercury measured from one person vary, depending upon collection
places and locations of hairs on one's head, this variance is very small, and
the amount of mercury measured by this method is considered to be reliable.
Therefore, the amount of mercury in one person's hair, measured by mixing
the hair from different parts of his head and sampling 100 - 200 mg of it,
can be considered to represent an adequate measure [6]. For small size fish,
the entire fish is used, and for large size fish, 1 - 10 grams of primarily its
muscles are used. The mercury concentration is expressed as ppm (per wet
weight).

Results

1._ Amounts of mercury contained in hair

As illustrated in Table 29, information obtained from 16 males and 15
females who live in Kumamoto city is a maximum of 10.00 ppm and a minimum
of 0.91 ppm. Eighteen males and 17 females who live in the Minamata area
show a maximum of 19.40 ppm, and a minimum of 0.94 ppm. Nineteen males and
30 females from the Goshonoura area show a maximum of 19.69 ppm, and a mini-
mum of 1.10 ppm. It is known from a previous report [15] and from our in-
formation [6] that the amount of mercury in hair has a log-normal distribution.
Therefore, if a logarithm of mercury content in hair is taken, it will have
a normal distribution. Using this fact, the variance and average of mercury

163

content were computed. A statistical significance test was done by the variance analysis method [13]. In the following test, the amount of mercury in hair is indicated by a logarithm of mercury concentration (ppm).

In the previous literature [2, 4, 5, 11], mercury amounts are compared without segregating males from females. However, according to our study [6], the variances of amounts of mercury in hair grouped by sex are almost identical if subjects are in the same area. On the contrary, a very significant difference between male and female is found (P < 0.001) in terms of their average values. No significant differences in variance and average are observed when subjects are grouped according to their ages [6]. Therefore, it is considered necessary to group by sex, but not by age, in comparing amounts of mercury in hair.

Based on Table 30, which was produced based on the above observations, the variances in three areas are almost identical between male and female in each area, and no significant difference in average values is observed (P < 0.005).

Comparison of values for the same sex among these areas show that no significant difference of averages for females is observed. However, for men, as shown in Table 31, a significant difference is observed between Kumamoto and Goshonoura (P < 0.025). However, no significant differences are seen between Minamata and Kumamoto or between Minamata and Goshonoura.

2. Amount of mercury in fish

A total of 61 subjects were examined. Eighteen subjects were collected from Minamata Bay, 9 from the southern shore of Kyushu other than Minamata, 18 from the eastern shore of Amagusa, and 16 from the western shore of Amagusa (Figure 2, Table 32).

TABLE 29. AMOUNTS OF MERCURY (ppm) IN HAIR OF RESIDENTS IN THE KUMAMOTO, MINAMATA, AND GOSHONOURA AREAS

Kumamoto				Minamata				Goshonoura			
Male		Female		Male		Female		Male		Female	
Age	Hg conc.	Age	Hg conc.	Age	Hg conc.	Age	Hg conc.	Age	Hg conc.	Age	Hg. conc.
35	8.93	21	3.78	50	7.23	50	1.57	51	5.81	73	1.62
36	10.00	28	3.55	50	6.91	54	2.84	58	9.31	62	1.00
--	7.--	21	1.82	32	2.19	19	1.72	50	10.52	82	3.01
35	8.61	21	1.83	54	9.80	53	0.91	70	6.95	73	6.96
--	6.78	21	3.57	41	19.40	43	1.06	12	5.49	57	2.00
30	3.38	21	1.94	5	4.95	38	6.31	60	8.62	58	3.68
5	2.46	36	3.22	62	7.40	16	2.31	41	7.11	41	1.11
32	5.36	7	4.07	67	8.54	31	4.44	44	3.06	74	1.20
21	2.70	30	0.91	37	7.87	4	4.90	47	10.69	85	3.11
37	5.58	1	1.90	31	8.06	9	3.44	68	5.77	15	1.37
51	5.30	28	3.27	2	4.30	64	3.31	66	11.89	60	2.01
38	3.61	27	4.38	53	7.03	83	4.91	33	6.50	59	1.75
59	6.85	4	3.00	74	2.94	33	2.26	73	5.90	59	4.25
50	2.68	2	3.14	39	2.70	5	2.15	72	4.85	42	5.46
27	3.23	3	1.96	56	2.33	4	1.07	56	6.10	71	8.88
51	4.47			54	4.50	21	4.03	63	6.63	75	4.11
				25	1.89	25	1.15	68	1.91	50	2.02
				30	9.93			71	13.88	51	2.33
								53	10.87	58	1.95
										68	1.21
										61	6.45
										59	5.72
										84	1.91
										47	2.2
										74	2.13
										73	2.11
										15	1.10
										65	1.66
										61	3.83
										59	2.87

Note. ⊃ — Minamata disease; ? — suspected Minamata disease; with no mark — only healthy persons in Kumamoto, healthy persons and persons having other diseases in other areas.

A histogram of mercury contained in fish is illustrated in Figure 3, and from it, it can be estimated that the amount of mercury in fish is exponentially distributed. That is, it is expressed as [19]:

$$F(x) = \begin{cases} 1 - e^{\frac{x}{\lambda}} & , \ x \geq 0 \\ 0 & , \ x < 0 \end{cases}$$

Here, F(x) indicates the frequency of x (mercury concentration in fish); λ is the average value.

TABLE 30. STATISTICS OF AMOUNTS OF MERCURY IN HAIR OF
RESIDENTS IN THE KUMAMOTO, MINAMATA, AND GOSHONOURA AREAS

		Kumamoto	Minamata	Goshonoura
Male	Variance	0.030	0.073	0.037
	Average value (Converted to ppm)	0.717 (5.21)	0.743 (5.51)	0.875 (7.50)
	Number of data	16	18	19
Female	Variance	0.030	0.064	0.059
	Average value (Converted to ppm)	0.429 (2.68)	0.441 (2.76)	0.448 (2.80)
	Number of data	15	17	30

Note. Variance and average values are computed on the logarithm
of mercury concentration (ppm).

Let us assume that this equation holds. Then, if we plot the logarithm of frequency on the y-axis and the amount of mercury on the x-axis, log F(x) for its corresponding x should be a straight line. As shown in Figure 4, those points are approximately on a straight line. This result can be confirmed from the values in the thesis by Kitamura [8].

TABLE 31. A SIGNIFICANT TEST OF
MERCURY CONTENT IN MALE HAIR IN
KUMAMOTO, MINAMATA, AND GOSHONOURA

	Minamata	Goshonoura
Kumamoto	Insignificant (P<0.5)	Significant (P<0.025)
Minamata	--	Insignificant (P<0.5)

Table 33 illustrates the maximum, minimum, and average values of total mercury in fish from each area. In order to determine the significance of differences in those four areas, by letting λ_A / λ_B = L, a F-test is used to

166

TABLE 32. MERCURY CONTENT IN FISH FROM EACH AREA ALONG THE SHIRANUI SEA (IN ppm)

Minamata Bay		Western shore of Kyushu (excluding Minamata Bay)	
Subject name	Hg conc.	Subject name	Hg conc.
Bina	กำ	Bina	ก ดดด
Bina	0.ถ50	Bina	0.069
Hibarigai modoki	ก ดด	Clam	ก ดดด
Short-necked clam	0 ดีริ	Clam	0.000
Hibarigai modoki	ก ริ0	Clam	0.006
Hibarigai modoki	ก ก92	Bina	0.118
Hibarigai modoki	ก ดริ	Hibarigai modoki	
Bina	0.051	Bina	0 058
Crab	ก.ในก	Hibarigai modoki	ก 116
Sea bream	0.033		0.173
Prawn	0.231		
Garakabu	0.543		
Conger	0.326		
Flatfish	0.186		
Kuchiroko	0.356		
Gray mullet	0.013		
Bina	0 108		
Scabbard fish	0 046		

Western shore of Amagasu		Eastern shore of Amagasu	
Bream	ก กริริ	Bina	ก ดดด
Bream	0.ดดี	Bina	0.051
Halfbeak	ก.ก ก	Abalone	0 ดดด
Garakabu	0 ดี่1	Short-necked clam	ก ดใด
Short-necked clam	ก ด19	Gray mullet	ก ดดด
Bina	0.0ริ2	Bream	ก กริ5
Scabbard fish	0.02ริ	Sea bream	ก.017
Flatfish	0.ก11	Short-necked clam	0 ดริริ
Kurio	ก ดริริ	Short-necked clam	ด.ริใริ
Kawahagi	0.000	Bina	0.061
Stingfish	0.121	Bina	ก ด22
Turbot	0.ดริริ	Bina	ด.ดริริ
Kuroio	0.017	Garakabu	0.ดดริ
Bina	0.027	Lobster	0 ดใด
Garakabu	0.118	Octopus	0.016
Tilefish	0 15ก	Swellfish	0.065
Kusabi	0.1ริ0		
Unknown	0.063		
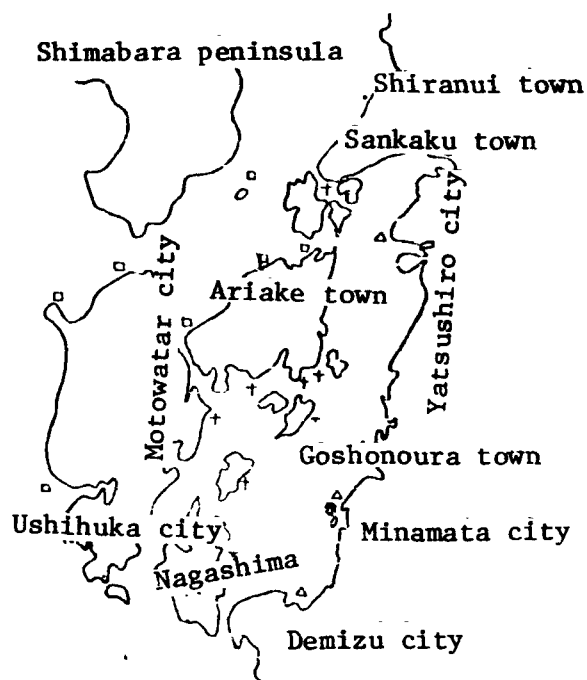
Figure 2. Fish collection sites:

o — Minamata Bay; Δ — western
shore of Kyushu; + — eastern
shore of Amagusa; ◻ — western
shore of Amagusa

Hg conc. in
fish (ppm)

Figure 3. Histogram of mercury con-
centration in fish (wet weight ppm)
along the Shiranui Sea (excluding
ones from Minamata Bay)
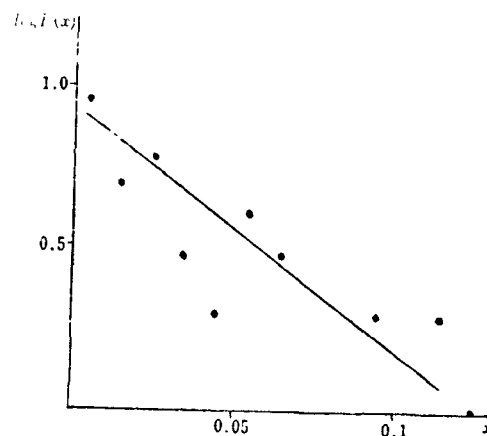
Figure 4. Mercury content in
fish and adjustment of their
frequencies to log [F(x)] =
Ax + B, where F(x) — frequency
of x, x — mercury content (ppm)

find whether L = 1, i.e., $\lambda_A = \lambda_B$, or

not [13]. Here, λ_A and λ_B are the average

mercury amounts in fish (wet weight) from
two areas respectively. Table 34 illus-
trates the result. From the table, sig-
nificant differences are recognized be-
tween Minamata Bay and three areas: the
western shore of Kyushu, eastern shore of
Amagusa, and western shore of Amagusa (P < 0.002). This result shows that the
mercury in Minamata Bay is still higher than that in other areas. No signi-
ficant differences are seen among other area.

TABLE 33. MAXIMUM, MINIMUM AND AVERAGE VALUES OF MERCURY
CONTENT IN FISH (WET WEIGHT ppm) FROM EACH AREA ALONG THE
SHIRANUI SEA

	Mina-mata Bay	Kyushu western shore	Amagusa eastern shore	Amagusa western shore
Maximum value (ppm)	3.50	0.173	0.331	0.096
Minimum value (ppm)	0.013	0.000	0.000	0.000
Average value (ppm)	0.175	0.060	0.066	0.033
No. of examined fish	18	9	19	16

TABLE 34. A SIGNIFICANCE TEST OF MERCURY CONTENT IN FISH (NET
WEIGHT (ppm) FROM EACH AREA ALONG THE SHIRANUI SEA

	Minimata Bay	Western shore of Kyushu	Eastern shore of Amagusa
Western shore of Kyushu	Significant (P<0.02)	—	—
Eastern shore of Amagusa	Significant (P<0.02)	Not significant P<0.5	—
Western shore of Amagusa	Significant (P<0.05)	Not significant P<0.1	Not significant (P<0.05)

It should be noted that the mercury contained in Bina fish caught from the Ariake shore in the Ariake area was nearly zero. This fact was previously stated in Chapter 2, Section 3, II.

Observations

The amounts of mercury in hair do not indicate clear differences from one area to another, as long as hair of the same sex is compared. The dif-ference in the amounts due to sex is larger than the difference due to areas.

The mercury content in hair of Minamata disease patients, reported by Kita-
mura [5] in 1960, also shows a significant difference in the average values
between the sexes. It is under investigation whether the reason for this dif-
ference is food, especially the consumption of fish, between male and female,
or whether it is related fundamentally to the difference of sex [6]. The in-
vestigation of the relationship between the difference in the amounts of mer-
cury contained in male hair and female hair and the disease rate of the Mina-
mata disease between males and females may provide a clue for the structure of
occurrences of the Minamata disease or its treatment.

The amount of mercury in fish from Minamata Bay is significantly higher
$P < 0.002$, compared with that from other areas. However, compared with the
value of 14.47 ppm (average value)[8] reported by Kitamura in 1960 on the mer-
cury content of fish from Minamata Bay, the current value can be said to be
remarkably lower. The current value is equal to 0.5 ppm, which is the standard
set by the FDA.

Summary

The amounts of mercury in fish and in the hair of residents in the sea-
shore areas of the Shiranui Sea were discussed. The mercury content of fish
from the Minamata area showed a higher value, compared with that from other
areas. Goshonoura residents (males) showed a slightly higher mercury content
in their hair.

PART 5

EXPLANATION

From August, 1951, to March, 1973, in order to determine the effects of
organic mercury poisoning on human health conditions, direct neurological
and psychiatric examinations were performed on a total of 3589 persons, 965
from the Minamata area whose bay was heavily contaminated by organic mercury,

1723 from the Goshonoura town, Arashiguchi area, which was lightly contaminated, and 901 from Ariake town, Amagusa county. Moreover, direct examinations of people who moved out of the prefecture from the Minamata area were undertaken, and the mercury content in fish from the nearby sea and in the hair of residents in the above-examined areas was measured. Major conclusions obtained and worth noting are as follows: establishment of percentages of Minamata disease patients (disease rate) among residents of examined areas; confirmation of the existence of Minamata disease patients in the Ariake area; establishment that high blood pressure and blood circulation problems will occur from organic mercury poisoning; discovery of a high ratio of the suspicion of liver problems among residents in the Minamata area; findings of many Minamata disease patients among people whose hair contained 20 - 0 ppm mercury in 1960; findings of frequency of patients who need to enter hospitals or to visit hospitals for medical treatment.

I. DISEASE RATE

1. Percentages of people who have neurological and psychiatric disorders in each area are as follows: Minamata area, 59 48±1.58%; Goshonoura area 27.63±1.08%; Ariake area, 31 29±1.51%. It should be noted that nearly 60% of the residents in the Minamata area have some sort of disorder.

2. The number of Minamata disease patients and the number of suspected Minamata disease patients plus Minamata disease patients (in parentheses) are as follows: community A in the Minamata area, 44 (49); Tsukinoura, excluding community A, 56 (69); Yudo, 130 (141); Detsuki, 45 (54), Goshonoura area, 34 (65); Ariake area, 8 (10). The frequencies of Minamata disease patients relative to the total examined residents are, respectively: 71 , .5 67%% 26 17 ±3.00 % 32 31 ±2 33 15 52 ±2.13%, 1.97±0.33%, 0.89±0.51% . The frequencies of both Minamata disease and suspected Minamata disease patients are: 83.05± ! 88%, 32 21±3.29%, 35 07±2.38%, 18 62±2.29 %, 5 77±0. 16%, 1.11±0 35% , respectively. However, in this investigation, there is a possibility that some cases of organic mercury poisoning, which show few basic symptoms for clinical judgment, may be overlooked. This is based on our observation, as previously

mentioned (Part 1, Chapter 2, Section 3, VII, A, 10), that the majority of cases which are diagnosed as Minamata disease display many basic symptoms.

Community A is located closest to the sea, which is contaminated by mercury. Residents, fishermen or non-fishermen, are — in general — poor, and they have no choice but to eat only the fish that they catch. Detsuki is close to the shore of community A, but does not face the sea, and many people there engage in non-fishing work, such as farming. The other two areas in the Minamata area are located between these two areas. The Goshonoura town, Arashiguchi area, is 16.5 km away from the source of mercury contamination, and is located on the other side of the mercury contaminated source relative to the Shiranui Sea. Sixty to seventy percent of the residents engage in fishing. The Ariake area faces the Ariake Sea, and contains a number of fishermen. The area is selected for the purpose of comparing it with the examined areas mention above.

In the Minamata area examined, 301 Minamata disease patients have been confirmed to date. The number of patients recognized by Kumamoto Prefecture was 40; 21 up to 1962; 0 from 1963 to 1968; 1 in 1969; and 18 from 1970 until this examination. Until this examination, the remaining 261, with some exceptions, already had the disease, but were not detected. The situation is similar among patients in the Goshonoura area. Many reasons can be considered for this situation: for instance, a lack of knowledge among residents, including patients, from a hygienic standpoint, reserved attitude of people toward the disease, poverty which prevented the treatment financially. At first, among doctors — including us, the difficulty in precisely diagnosing the disease was also one of the most important factors. The lack of cooperation from the government, local society, fishermen, and related enterprises in finding patients and in simultaneously examining residents was also important in creating the above situations.

In the Minamata area, 38 persons who exhibited Minamata disease-like symptoms were recorded dead prior to this examination.

172

II. MINAMATA DISEASE PATIENTS FOUND IN THE ARIAKE AREA

In the Ariake area, 9 patients whose symptoms could not be distinguished from those for Minamata disease and 1 patient suspected of the disease were found. One of them is considered to be a patient of organic mercury poisoning from fish in the Shiranui Sea. However, the fishing grounds for other patients are the Ariake Sea. Therefore, it is necessary to investigate the source of organic mercury poisoning for the sea in this area. This seems to suggest that we should consider whether the effects of mercury contamination on the health conditions of residents can be seen not only in the Ariake seashore, but also in Japan's inland seas and bays beside which factories are built. In this examination, the amount of mercury in shellfish from the seashore of the Ariake area was nearly zero ppm.

III. MINAMATA DISEASE PATIENTS FOUND AMONG PEOPLE MOVING
TO OTHER PREFECTURES

Nineteen Minamata disease patients and one suspected patient were found among 21 persons who moved to other prefectures, such as Gifu and Osaka, from the Minamata area, which was under this investigation. These patients were not diagnosed properly at medical facilities in their new areas, or were diagnosed as having nervous disorders and treated accordingly.

Many residents along the shores of the Shiranui Sea also moved to other prefectures, and it it estimated that many of them might be Minamata disease patients. We should consider methods of diagnosing them, and plans for helping them.

IV. EFFECTS OF ORGANIC MERCURY ON HUMAN HEALTH

This investigation has proven that, among organic mercury poisoned patients, almost all of the cases showed not only various types of neurotic conditions due to peripheral nerve, cerebellum, and thalamus damage, but also various kinds of mental disorders. It was also proven that this poisoning

could be a cause of convulsive and neurotic conditions due to high blood pressure, damage to the heart vessels, and blood circulation problems. The high frequency of abnormal values of GOT and GPT, which indicate the suspicion of damages to the liver among residents in the Minamata area, shows a significant difference from that in the Ariake area. As a summary, it can be said that the number of types of symptoms for organic mercury poisoning is remarkably increased from what the number used to be.

V. DIAGNOSIS OF ORGANIC MERCURY POISONING

Diagnosis of organic mercury poisoning is made from the combined judgment of the following items: existence of contaminated areas, outbreak of the disease within a family, basic symptoms — such as sensory disorders, loss of coordination, mental retardation, hearing problems, speech problems, and centric vision constriction. When the area organic mercury contamination is light or unknown, if there is no patient in a family, then the diagnosis of the disease must be done cautiously, even if the various symptoms mentioned above are observed. On the other hand, if many members of a family are Minamata disease patients and a subject is young, sensory disorders alone are sufficient to diagnose the subject as a Minamata disease patient.

Conditions of organic mercury poisoning may be controlled by conditions such as development of high muscle tension in the lower half of the body, sensory or motion paralysis on one side of the body, epilepsy, mental retardation, nervous disease, nervous breakdown, illusions, and fantasies. Therefore, these conditions must be brought to mind in diagnosing the disease.

We examined 17 old men aged between 65 and 80 (7 males) living in Kumamoto city for the purpose of comparison. According to this, it was observed that some of them also had symptoms such as sensory disorders at the tips of the extremities, loss of balance, mental retardation, hearing problems, speech problems. According to Kameyama [7], 118 out of 190 persons over 60 years old were found to have abnormal degeneration in the spinal cord. Also, according to Tog [19], the density of the total nerve fibers in intestinal

nerves rapidly decreases until the age of 10, and then gradually decreases. For the purpose of comparison, we examined ¥ (7 males) healthy persons aged 20 - 50, primarily between 20 and 30, living in Kumamoto city. We could find neither any loss of balance nor sensory disorders. However, for the sense of touch and pain, 5 and 12 subjects, respectively, showed low sensitivity around their mouths and at the extremities of their four limbs. In order to diagnose Minamata disease, we must take the observations mentioned above into consideration.

Many patients who were diagnosed as having Minamata disease showed an abnormality in the spinal cord. However, the possibility that the spinal disease may be diagnosed as Minamata disease has not been disproven. At the same time, no proof has been obtained that the spinal disease will significantly affect the Minamata disease. If we review Minamata disease patients having spinal abnormalities, it is revealed that the diagnoses of the Minamata disease were based on the following symptoms which were not related to the spinal abnormality: sensory disorders around the mouth, speech problems, poor vision, other patients in a family. However, in the case of changes in the lumbar vertebrae, about half the subjects showed sensory disorders and muscle tension in the lower limbs more than in the upper limbs.

VI. PLANS

1. Relief of Patients

Relief of patients has already partially started. However, it is desirable to provide not only medical assistance, but also assistance in living, occupation, and education. Its implementation must be carried out quickly. Among Minamata disease patients, many are old and their death rate is high.

2. Medical Treatment

   a. Creation of environment for treatment in
       area society

   Minamata disease patients not only have physical disorders, but also
mental disorders. Many of them especially show mental reactions such as
anxiety, melancholy, and offensiveness. In general, in order to examine
patients such as these, it is most important to create an atmosphere such that
the family and area society cooperate with the patient's treatment. The fol-
lowing are considered to disturb such an atmosphere for treatment: adminis-
trative, social, and industrial prejudice against patients, heretic treatment
of patients, criticism, jealousy, difference of opinion among patients. It
is necessary for the administrative staff and the medical staff to correctly
understand Minamata disease patients in the same way as patients of other
diseases, and guide the area society in creating an atmosphere for promoting
and assisting medical treatment of patients. The reason that many Minamata
disease patients show nervous conditions, depression, anxiety, melancholy, and
offensiveness is considered to be because of their disease and the disease
among their families, as well as because of abnormalities in the environment
surrounding them.

   b. Establishment of a center for medical treatment

   Since there are many patients, it is desirable to establish centralized
facilities which can accomodate diagnoses, treatment, and rehabilitation of
patients primarily for this disease.

   c. Methods of treatment

   Eighty percent of Minamata disease patients are considered adequately
cared for by merely having them visit hospitals. Therefore, methods of treat-
ment should focus on treatment by visiting hospitals. On the other hand, the
rate of patients who need to be hospitalized increases with the patients's age,

and thus increases in the future. Families just consisting of old patients whose children have moved to other prefectures are sometimes seen.

Currently, many patients receive treatment from physicians practicing nearby. In the future it will be necessary to obtain the cooperation of local medical facilities in treating the patients. Minamata disease patients require not only neurological treatment, but also treatment from many directions, such as from a psychiatric view, from the standpoint of the heart, blood vessels, and liver diseases.

From actual experience,some of the subjective symptoms, such as insomnia, headaches, crow bend, body aches, and irritation, are improved fairly well by drugs such as phenobarbitol, chlorpromazine, and tranquilizers. However, many patients still rely on moxa cautery to cure subjective symptoms. A systematic study on treating the disease is seen to be difficult at present.

3.   Prevention Plans

a.   Prohibition of fishing in the Shiranui Sea

Amounts of mercury in fish from the Minamata Bay and in the hair of residents in the Goshonoura area still showed high values in this investigation. Fifty-five percent of the patients described their condition as deteriorating and, in some cases, deterioration of the patient's condition was confirmed. In 1972 and 1971, new patients suddenly appeared. Also, on May 19, 1973, a 34-year-old housewife from Morimichi exhibited a violent attack of symptoms during her examination. Sudden sensory abnormalities which are hard to describe, such as bone grinding aches, numbness, no feeling, heavy feeling, dullness, and loss of muscle strength, occurred in the lower half of her body beneath her waist. She could not even sit in a chair. She lay on the floor, supporting her upper body with her hands, stretching her lower limbs, and suffered from pains in the four limbs, joints, and bones. After about 20 minutes, she left the examination room, held by her husband. From the violent reaction to conditions, the phenomenon mentioned above is not due to sequela

177

of poison invasion of her body in the past, but is assumed to be due to the poison invasions of her body which she still had at the time of the examination. The family occupation was fishing, and the main dishes were fish, and no changes had been observed until then. The floor of Minamata Bay also contains a large amount of mercury [10]. From the above, it cannot be denied that fish in the Shiranui Sea are still possibly poisonous. Therefore, we should consider prohibition of fishing in the Shiranui Sea. In this case, unless those who are affected by the prohibition of fishing are compensated for their loss, it is difficult to execute this effectively. For example, those whose only income is from fishing may fish for themselves and for sale, even if fishing is prohibited.

b. Avoidance of consumption of food containing mercury, especially among pregnant women, infants and the aged

Those younger than 5 years old, especially zero years old (i.e., hereditary or fetus Minamata disease), or those older than 40 years, and especially over 60 years, have a higher frequency of serious illness if they become patients. There is a high possibility that the brain of such a patient will be damaged by organic mercury. Therefore, pregnant women, infants under 5 years old, and old persons should avoid consuming such food, even though the mercury content is low.

c. Mercury content in the hair of many Minamata patients is 20 - 0 ppm

Thirteen out of 19 men whose hair contained less than 20 ppm, and 10 out of 15 women whose hair contained less than 10 ppm, in 1960, were diagnosed as having Minamata disease. That is, small mercury content in the hair does not preclude occurrences of Minamata disease. According to an outline for plans against environmental contamination by mercury issued by the Ministry of Public Welfare (August 17, 1968), normal mercury content in human hair is, in general, less than 20 ppm. Mercury content in the hair of residents in the Minamata area

178

decreased remarkably in 1961, as compared to 1960, and further decreased in 1962 [11]. On the other hand, it is reported that a resident in an area not included in this examination, whose hair contained 630 ppm mercury in 1961, does not currently show any sign of symptoms. In either case, it suggests the necessity of reexamining the significance of the mercury content in hair.

4. Health Examination of Residents in Other Areas Along Shores of the Shiranui Sea and the Ariake Sea

From the illness rate stated at the beginning, it is estimated that many unconfirmed Minamata disease patients exist among residents along the Shiranui Sea. We often found that many patients were unaware of symptoms, although they showed symptoms, such as a high degree of sensory disorder and poor vision. Therefore, unless we examine each resident one by one directly, an investigation of health conditions of residents cannot be said to be complete. It will take time to do an investigation of health conditions of all residents. Before doing so, it will be necessary to investigate the health conditions of residents in urban areas of Minamata city, its suburbs, Tsunaki town, Taura town, Otabi, Amagusa county. From the illness rate of the Minamata disease obtained from the investigation, we can estimate the approximate number of Minamata disease patients among residents along the shores of the Shiranui Sea.

In a similar manner, an investigation of health conditions of the residents along the shores of the Ariake Sea should be planned.

5. Examination and Treatment of Emigrants to Other Prefectures

A number of people moved to other prefectures from the shores of the Shiranui Sea, and many of them are estimated to be poisoned by organic mercury. Methods of treatment and plans for their relief are an important future problem.

179

6. Medical Studies on Organic Mercury Poisoning

From the results of this investigation, the following are considered to be important in treating and preventing this disease: methods of treatment, development steps of subjective symptoms seen among organic mercury poisoned patients; mechanisms of symptom development; relationships between organic mercury poisoning and blood pressure, heart-blood vessel system and blood circulation, stages of developing diseases due to organic mercury poisoning — such as epilepsy, sensory disorder, and half body paralysis, relationships between organic mercury poisoning and organ functions — such as the liver, stages of developing mental diseases, relationships between the mercury content in hair and appearance of poisoning symptoms, effects of mercury passed from the mother's body to the brain of a fetus, relationships between occurrences of organic mercury poisoning and age.

7. Establishment of a Medicaland Research Center for Organic Mercury Poisoning

The establishment of such a center has been already planned by the Environmental Bureau. From out experience in this investigation, we consider such a facility essential, and its realization most urgent. Its functions include: treatment of patients; guidance for patient rehabilitation to society; guidance for life and occupation, and information gathering of patients staying at home; examination of confirmed patients; simultaneous examination of people in mercury contaminated areas; communication with related medical, welfare, and government facilities; basic research, such as treatment and prevention of diseases caused by poison; and elucidation of outbreak stages. It is necessary to have a staff not only of doctors and nurses, but also many paramedical staff members and researchers in areas other than medicine. It is desirable that the center be nationally administered, and that it be located in the Minamata area. Considering the great number of patients, wide functional field, nearly permanent lasting problems, and international responsibility, the plan should proceed on a broad scale.

VII. OTHER DISEASES

In order to simplify the discussion, the following abbreviations are used: Minamata area — M, Goshonoura area — G, Ariake area — A, Heianza Island [3] — H. >> indicates a significant difference from the statistical estimate standpoint, > — a minor difference, = — no significant difference. What is striking in comparing frequencies of each disease in examined areas is that no significant differences are seen among the areas for diseases, such as mental diseases for internal causes. That is, for nervous breakdowns, M = G = A = H; for depression — M = G = A = Komoro town, Nagaro [9, 14]; for epilepsy, M = G = A = H; and for mental disorders, M = G = A = H. However, if neuropsychiatric disorders are related to these diseases, then considerable differences in values among these areas become apparent. For example, for the total number of patients who have some sort of neurological or psychiatric symptoms: M > A ≥ G >> H; for the Minamata disease: M > G = A for the Minamata disease and its suspects: M >> G > A; for other diseases: A > M = G. By assemblyng the facts described above, we can conclude that a significant effect of organic mercury on health conditions of residents in the examined areas, especially the Minamata area, is proven from the values mentioned above.

However, for alcoholism, G > M = H > A, and for high blood pressure and neuropsychiatric disorders due to hardening of the brain arteries — A > G > M = H. For the latter, it is possible that cases of high blood pressure and hardening of the brain arteries may include some which are related to organic mercury poisoning.

PART 6

SUMMARY

The following results are obtained from direct examination of 3589 persons, mainly the residents along the Shiranui Sea, held from August, 1971, to March, 1973.

1. Occurrences of neuropsychiatric disorders among the examined residents of Minamata, Goshonoura, and Ariake were approximately 59%, 28%, and 31%, respectively. They are remarkably high compared with the 10% at Heianza Island, Okinawa. However, if we exclude the cases of organic mercury poisoning and its suspected cases from them, then the occurrence of other diseases, especially of inherent mental disorders and mental breakdown, is similar to those in other areas of Japan in these three examined areas.

2. Among the examined residents along the Shiranui Sea, the percentages of Minamata disease patients in the examined areas are as follows: $71.58\pm5.67\%$, 24.17 5.00%, 13.52 ± 2.13 If suspected Minamata disease patients are added to them, then these figures become approximately 83%, 35%, 32%, 19%, and 4%, respectively. Figures for people in other areas will be somewhere between or close to these figures. Frequent occurrences of patients are seen in areas and among families that have the following conditions: their homes are very close to the sea which is close to the contaminated area, shellfish can be caught at the seashore, they are dependent only upon fishing, and they are poor.

The Minamata disease remarkably displays the tendency of concentrated outbreak not only in geographical areas, but also in families, especially in the Minamata area.

3. Even in the Arike district, 8 patients, about 1% of the examined people, showed symptoms which could not be distinguished from those of Minamata

182

disease. It is necessary to investigate their cause or the source of mercury contamination.

4. Twenty-one out of 22 persons who moved out of the prefecture, away from the organic mercury contaminated Minamata area, and who were examined when they came home temporarily, either had the Minamata disease or were suspected of having it. There are many people who moved from the Minamata area, and many of them are considered to be Minamata disease patients. We should plan methods of examining and treating them.

5. Minamata patients show not only nervous diseases, but also various kinds of mental disorders. High blood pressure, half body paralysis, epilepsy, and other sensory disorders may also result from organic mercury poisoning. Liver problems are also considered to result from organic mercury poisoning.

6. Diagnoses of the Minamata disease are with combined judgements, reached from medical views, as well as patients in the family, a combination of of basic symptoms, and characteristics of sensory impediments. It must be taken into consideration that Minamata disease is similar to other kinds of nervous disorders and mental diseases, and that aged persons sometimes show similar symptoms, even if they are healthy.

7. From the results of this examination, it becomes necessary to examine directly all the residents in areas along the Shiranui Sea and the Ariake Sea which were not examined.

8. Relief for Minamata disease patients should proceed, as it does now, not only from a medical standpoint, but also from living, occupational, and educational viewpoints. Since many patients are old and the death rate among them is high, it is necessary to speed up relief plan. As a medical treatment plan, it is necessary to form an environment suitable for treatment in these area societies: medical treatment by patients visiting hospitals and by visiting patients in their homes.

9. We cannot deny that there is still a possibility that fish in the Shiranui Sea are poisoned. Therefore, fishing in the Sea should be forbidden.

10. Effects of organic mercury poisoning are especially heavy on fetuses, infants under 5, and the aged. Consumption of food containing mercury should be avoided among people in these age groups, including pregnant women.

11. Many people whose hair, measured in 1960, contained mercury less than the normal value — i.e., 20 ppm determined by the Ministry of Public Welfare, or less than 20 ppm for males and 10 ppm for females, determined from the data by Ukita [16] — were diagnosed as having Minamata disease. The rate of Minamata disease patients having less than 10 ppm was higher in females than in males. From these observations, we cannot assume that small amounts of mercury in hair indicates less outbreak of the disease. In the examination, no male Minamata disease patients and suspected patients showed over 20 ppm total mercury content in their hair. The majority of male and all female patients was less than 10 ppm (refer to Chapter 6).

12. We consider the following research subjects important for the treatment of, and the prevention of, this disease: establishment of systematic treatment methods for Minamata disease; elucidation of outbreak steps for subjective symptoms, occurrences of sensory impediments, symptoms on only one side of the body, and mental disorders; effects of organic mercury on infants and brains of fetuses; and the relationship between organic mercury and blood pressure and heart-blood vessels.

13. Approximately 22 - 30% of the residents examined in the areas had neuropsychiatric disorders unrelated to organic mercury poisoning. It is equally important to medically treat these patients as we do Minamata disease patients. Unbalance in medical treatment for these two groups of patients will disturb the environment for medical treatment in these areas.

14. As is understood from the above descriptions, there is a wide range of important problems concerning the Minamata disease, such as its treatment, simultaneous examination of resident, examination of applicants for diagnoses, and research on its treatment and prevention. In order to cope with these problems, the need for facilities for the center of these activities is urgent, and its prompt implementation is desired. The site of the facilities should be in the Minamata area.

REFERENCES

1. AOAC; 9th Edition, 1960, p. 438.

2. Hiroshi, Aoki. Studies on Environmental Pollution by Mercury, Report No. 3; Japan Hygiene Journal, Vol. 24, Nos. 5, 6, 1970.

3. Hirayasu, Tsunetoshi. Epidemiological and Social, Psycho-Medical Studies on Psychoneurotic Patients on a Remote Island in Okinawa. Journal on Psychoneurosis, Vol. 71, 1966, p. 466.

4. Hoshino, Otomatsu, Keiko Tanzawa, Yoshinari Hasegawa, Tadanoshin Ukita. Differences in Mercury Level in Hair of Healthy Persons Due to Residential Environment. Hygiene Chemistry, Vol. 12, 1966, p. 90.

5. Hoshino, Otomatsu, Keiko Tanzawa, Mitsuo Terao, Tadanoshin Ukita. Determination of Mercury in Hair thru Radiochemical Analysis; Hygiene Chemistry, Vol. 12, 1966, p. 94.

6. Inoue, Takeshi, Aoi Teraoka, Shozo Egami. Unannounced Essay.

7. Kameyama, Masakuni, Eiichi Otomo, Shoichi Maruyama, Toru Mannen, Kazuo Takahashi. Degeneration of Posterior Fibers in the Spinal Cord of the Aged. I: Its Frequency, Origin and Clinical Significance; Clinical Neurology, Vol. 5, 1965, p. 709.

8. Kitamura, Seija, Kyoji Ueda, Jitsuko Niino, Irei Ujioka, Hikoji Misumi, Toshiyuki Kakita. Results of Probe into Chemical Toxins relative to Minamata Disease (Report No. 5); Kumamoto Igakusha Journal, Vol. 34, Supplement 3, 1960, p. 593.

9. Public Hygiene Bureau, Ministry of Welfare. Present Conditions of Psychiatric Patients in our Nation; Survey of the Mental Hygiene Situation for 1963; 1965.

10. Kumamoto Prefecture. Pollution White Paper; 1972 Edition.

11. Hygiene Research Institute, Kumamoto Prefecture. Survey of Mercury
    Level in Hair Relative to Minamata Disease; Report No. 1 of May 1961;
    Report No. 2 of May, 1962; Report No. 3 of May, 1963.

12. Masuyama, Motosaburo. The Road to Sampling. Tokyo University Publica-
    tions Association, 1957.

13. Masuyama, Motosaburo. The Method of Consolidating Minority Cases;
    Takeuchi Bookstore, 1964.

14. Muramatsu, Tsuneo. Mental Hygiene, 6th Edition, Nanzando, 1960.

15. Noushi, Hiroshi, Taichiro Nishima, Uko Tada, Mamoru Nagasaki.
    Mercury, Lead and Cadmium Levels in the Hair of Tokyo City Residents.
    Japan Hygiene Journal, Vol. 27, No. 1, 1972, p. 50.

16. Tatezu, Seijun, et al. The Wartime Mortality Rate of Inmates of
    Matsuzawa Hospital. Journal on Psychoneurosis., Vol. 60, 1958, p. 596.

17. Tatezu, Seijun, Akatsuki Azumaya, Koichi Mimura, Masazumi Harada,
    Tsuyoshi Tsukayama. Clinical Studies, Based on Follow-Up Surveys of
    Mass Carbon Monoxide Poisoned Persons as a Result of Mine Explosion
    Covering a Four-Year Period. Progress in Neurology, Vol. 13, 1969,
    p. 11.

18. Tatezu, Seijun, et al. Psychoneurotic Studies on Minamata Disease and
    Clinical Epidemiological and Symptomatic Studies on Minamata Disease.
    Post 10-year Minamata Disease Research Team of Kumamoto University
    Medical Department. Epidemiological, Clinical and Pathological Studies
    on Minamata Disease at the End of 10 Years. 1972, p. 41.

19. Togi, Hideo. Metrologic Studies on Age Differences in the Gastrocnemius
    Nerve of a Normal Person. Clinical Neurology, Vol. 12, 1972, p. 484.

20. Omura, Taira, Naotaka Imada. Fortran of Conjectural Statistics.
    Omusha, 1972.

5. HEALTH SURVEYS IN THE AREA OF PEDIATRICS, RESULTS OF EXAMINATIONS OF MINAMATA DISEASE-STRICKEN CHILDREN, AND CHROMOSOMES OF CONGENITAL MINAMATA DISEASE STRICKEN CHILDREN

Department of Pediatrics, Research Institute of Internal
Medicine, Kumamoto Univeristy

Team member: Yoshitaka Harada
Research assistants: Ryoko Miyamoto, Hiroyuki Moriyama,
                     Midori Takahashi, Sachio Takenaga,
                     Toei Ishizu, Nobumasa Suetsugi

Preface

The majority of congenital Minamata disease-stricken children were born prior to 1960. No surveys or examinations have been performed on their brothers/sisters born subsequent to that year. Not a few Minamata disease-stricken patients have subsequently given birth to offspring. It is unknown whether the effects of organic mercury are confined solely to the patient or continues to persist in succeeding generations. The purpose of this study was to conduct various surveys and health examinations in conjunction with efforts to clarify the correlation of chromosomes and Minamata disease through an examination of chromosomes of congenital Minamata disease-stricken symptoms, neurological abnormalities, in particular, of children subsequently born to patients and of brothers/sisters of congenital Minamata disease-stricken children.

Survey of Households of Minamata Disease-
Stricken Patients

1. Object of survey

Examination of record of growth and determination of bodily disorders,
especially neurological abnormalities, of children of 29 households or
patients born subsequent to the affliction of disease, and of brothers/sisters
of 11 cases of congenital juvenile patients.

2. Results

Of a total of 76 individuals, above, 36 cooperated and 36 consented to
submit to examination, the results of which are indicated below. Table 1
reflects the age group, sex, and the number of abnormalities.

TABLE 1

Age group (years)	Male	Female	Total	Abnormalities
0 ~ 5	1	4	5	0
6 ~ 10	8	4	12	7
11~15	8	9	17	4
16 ~		2	2	2
Total	17	19	36	13

The heading "abnormalities" includes those individual with proprioceptive
symptoms, a suspicion of disease by family members, or even the slightest
indication of abnormality, exteroceptively, in movement, in behavior, or in
perception. It does not include cases where there is no physical abnormality,
despite deterioration of mental faculties.

The number of persons in the above category (those subjected to examination) totalled 13 out of 36. The 13 persons adjudged abnormal were classified according to proprioceptive symptoms or primary grievance, perception, movement, behavior, and intellect to indicate abnormality or disorder, if any. This is shown in Table 2.

TABLE 2

Case no.	Age	Proprioceptive symptoms or primary grievance	Exteroceptive symptoms				
			Perception abnormality	Impairment of movement	Abnormal behavior	Intellect abnormal.	Remarks
1. Ishi-	10	Perception	–	–	–	–	
2. Ishi-	17	Perception	+	–	–	–	
3. Ina-	7	–	–	+	–	–	
4. Shina-	18	Intellect, movement	–	–	–	IQ = 89	
5. Fuku-	8	Perception	+	–	–	–	
6. Sa-	14	Perception	+	–	–	–	
7. Sa-	7	Perception	+	–	–	–	
8. Kawa-	14	–	–	+	–	–	
9. Ta -	8	Intellect	–	–	–	IQ = 99	
10. Yama-	15	Movement	–	+	+	–	polio
11. Ta-	13	Intellect	–	+	–	–	
12. Ta-	10	–	–	+	–	–	
13. Oni-	9	–	+	–	–	–	

Excluding the cases of abnormal perception without any exteroceptive symptoms (No. 1), mental deterioration amid perfectly normal bodily conditions (No. 4), alleged mental deterioration but no particular disability (No. 9), and clear diagnosis (No. 10), there were 9 cases involving abnormality of perception and impairment of movement. In case no. 8, involving impairment of movement, disability consisted of flaccidity of the lower left leg, characterized by slight spasmatic resistance, without any other movement disability. Case no. 12 could not move her tongue rapidly, but had no difficulty in speech or articulation, nor did she have any difficulty while being nursed as an infant. Thus, a total of 7 cases were considered to pose a problem: five cases of impairment of perception, one of movement, and another of both intellect

189

and movement. These cases are briefly described according to symptoms, as follows.

Case 1 (No. 2): Ishi-, 17 years of age, female.

Development from the period of conception, birth, suckling, and childhood was normal. Complained of fatigue in the right shoulder from around the age of 12 (1967). Began to experience pain in right forewrist and twitching of muscle from one year ago. Pain alleviated by massage. About the same time, began to sense numbness in the area below the right lower thigh; numbness still persists to date.

Deterioration of sense of touch, pain, and temperature was observed in the right upper limb. Weakening of the sense of pain and temperature was noted in the left hand and on the left lower thigh as well. Weakening of the sense of temperature was seen in the left and right lower thighs. Weak sense of sensation; normal physical function in upper and lower limbs; abnormality involved only perception.

Case 2 (No. 3): Ina-, 7 years old, male.

Normal development from time of conception, birth, and suckling. No complaints by individual. Tensile tremor noted on left hand. Maladroitness in movement of fingers (finger test), heels (knee test), and in changing positions were noted. Writing ability was relatively satisfactory.

Case 3 (No. 5): Fuku-, 8 years of age, male.

Development through the periods of conception, birth, and suckling was unknown, but thought to be almost normal. Has complained since last year of of fatigue and pain in right upper shank. These symptoms were included in the column on abnormality of perception, but they are not abnormality of perception as such — rather, a case of muscular pain.

<u>Case 4</u> (No.6): Sa-, 14 years of age, male.

Development through the periods of conception, birth, and suckling unknown, but believed to be almost normal. Has complained of numbness in the left hand since age 12 (1970). Some weakening of perception was noted following examinations to determine touch and pain reactions, but there was no dullness or loss of such perception. Movement functions were absolutely normal.

<u>Case 5</u> (No. 7): Sa-, 7 years of age, male.

Development through the periods of conception, birth and suckling was unknown, but is believed to be normal. Complained of abnormal sensation since around age 3 in left lower limb, at times accompanied by pain. Abnormal sensation and pain in entire left lower limb, frequently during middle of night or after a noon nap. Occasionally quivering of the same area.

Senses of touch and pain differed between left and right sides of the body. No loss of sensation was noted. Hypersensation was observed in the right limb at the upper and lower shank, while a moderate degree of hypersensation was noted on the left limb at the instep. No abnormality was detected in movement functions and deep sensation.

<u>Case 6</u> (No. 11): Ta-, 13 years of age, female.

Development through the periods of conception, birth, and suckling was normal. Intellectual deterioration was noted, with IQ of 62. Some maladroitness was observed in the finger test. However, ability to change body position was normal, and handwriting was normal.

<u>Case 7</u> (No. 13): Oni-, 9 years of age, male.

No abnormality since the period of conception and birth. Was slightly underweight at time of birth, but development during the period of suckling

was normal. No proprioceptive symptoms or grievances from family members. No dullness or loss of senses of touch or pain in area below upper part of both limbs, although some deterioration was noted.

3. Remarks

Of 76 individuals who were the objects of the survey, 36 were examined. While it was difficult on the basis of these results to calculate the effects, if any, of Minamata disease on posterity, 13 or 36.1% complained of or suffered from some physical abnormality. However, inasmuch as these included individuals who could not be judged as normal, the frequency of appearance of abnormality could not be determined. We cannot leave the realm of conjecture because, as can be noted in the aforementioned cases, the guardians of patients failed to closely observe the latter's record of development through conception, birth, and suckling, and because of deficient background data relative to the guardian's knowledge of child rearing or his livelihood. What is certain is that three cases involved abnormal perception, proprioceptive and exteroceptive, devoid of any marked changes such as total loss or dullness of perception. A further monitoring of such cases is probably required.

In such movement disability as seen in Cases 2 and 3, the patients, because of their ages, were unable to fully respond to directions. This inability to comply may be due to maladroitness due to tension of the initial experience, or weak movement capability due to intellectual deterioration. At any rate, inability to effect coordinated movement, while not marked, was observed during the finger test. In other coordinated actions, such as buttoning clothes, no abnormality was seen. A definite finding probably cannot be reached unless studies are based on repeated actions. Even though abnormal perception and physical movement disability were slight, they were discernible in the cases examined. It cannot be established whether they are linked to Minamata disease or whether they were transmitted through Minamata-disease-afflicted parents or congenitally. Today, when there is no outbreak of a typical case of Minamata disease, no conclusion can be reached on the basis

192

of only one survey. It is apparent that further studies of yet unexamined cases and monitoring of cases cited in this report are required.

Conclusion

1. A survey was conducted among children of Minamata disease-afflicted patients and brothers/sisters of children stricken by the same disease. The target of the survey was 76 individuals, but only 36 were diagnosed and examined.

2. Of the 36 individuals examined, 13 showed some form of abnormality and were either conscious or unconscious of the symptoms or the symptoms had been pointed out to them by family members.

3. In most cases, the symptoms of which the patients were conscious were dominant.

4. Those requiring additional studies were three cases of abnormal perception, proprioceptive or exteroceptive, four of impairment of movement — though of moderate degree, and one of exteroceptive perception abnormality.

Chromosomes of Congenital Minamata Disease-Afflicted Children

Chromosome examinations were conducted in seven cases of congenital Minamata disease-afflicted children.

1. **Method of examination.** The peripheral blood leucocyte culture method was used. Specifically, 6 - 8 ml of peripheral blood was drawn with a 10 ml hypodermic syringe containing 0.1 - 0.2 ml heparin sodium. After being put aside for several hours under sterile conditions, the isolated leucocytes, along with blood plasma, were removed and cultured for 72 hours, using culture solution 109. At the same time, 0.2 ml PHA-M was added to the total volume of 10 ml. Twelve to 15 hours prior to completion of the culture, 2 or 3 drops

193

of korusemido* [sic] were added, upon completion of the culture was centrifuged, a hypotonic solution added to the sediment, and water treated. Following 15 minutes of water treatment, it was again centrifuged, fixed with karunoa* [sic] for 15 - 20 minutes, which process was repeated three times, after which a preparation was formed. The preparation was produced by flame fixing and by crushing, using the rapid drying method. After it was completely dry, it was dyed with gimuza* [sic], and examined with a microscope.

2. Results. The results are shown in Table 3. The total number of cells examined was 301, and the karyotype — 46XY or 46XX. Thirteen reflected the number of chromosomes as 45, and 4 — as 44, an artificially abnormal number in producing the preparation. No specific abnormal cell structure was observed. Three were abnormal chromosomes, revealing a chromatid separation. Such abnormalities did not show any relationship with the gravity of Minamata disease symptoms.

3. Comments. When considering the effect of organic mercury on chromosomes, it can readily be stated that there is presently no effect on the number of chromosomes. It is clear from animal experiments using organic mercury that the latter passes through the mother's body to the placenta and affects the fetus. Although partial physical abnormalities such as hydrocephalus can occur, there has been no case of abnormality of the entire body attributed to chromatic changes or so-called physical, multiple deformities apparent in fetuses undergoing miscarriage, premature birth, or stillbirth. In fact, deformity seldom occurred, despite the high content of mercury in the fetus. Even among clinical symptoms such as serious cranial paralysis in infantile Minamata disease-afflicted children, no physical deformity has been observed, even though the paralysis either could not be physiologically categorized, or disclosed mixed symptoms. And since there were no symptoms to suggest that they were the result of changes in the number of chromosomes, it was not possible to expect them to reveal any changes in the chromosome count. It can be said that no abnormal count was detected in the examination of 301 cells in seven cases.

*Translator's note. This is a Japanese compound whose exact English counterpart is unknown.

According to reports available up to now, morphological changes in chromosomes have been brought forth by various biological chemical and physical stimulations. Such changes consisted of chromatid separations, chromatid gaps, fragments, dicentric portions and rings, while in animal experiments

TABLE 3. CHROMOSOMES OF CONGENITAL MINAMATA
DISEASED CHILDREN

Name	Degree of serious- ness	Chromosome count					
		44	45	46	47	Total cell count	No. of abnormal cells
NAKA, Chi	5			23		2:	1
IWA, Sue	4	2	2	52		56	
HAN, Kazu	4	1	2	41		1:	
KANE, Mesu	3		2	55		57	
ONI, Yu	3		1	1'		11	
NAGA-	3		2	2:		26	2
KA, Kiyo	2	1	4	43		48	
Total		4	13	234		301	3
%		1.3	4.3	91.4		100.0	0.9

using organic mercury, morphological abnormalities observed were separation, gap, and fragment. These changes, however, are known to undergo marked decrease as time progresses, following administration of organic mercury.

In our study, we observed three cells with a separation among the 301 cells that we examined. This was a frequency of 0.9% of the whole. It cannot be affirmed that such a change was evident until the present time as a result of the effect of organic mercury. It was not possible from our experiment to draw sufficient data to compare the frequency of appearance of abnormality under normal conditions, that is, a comparison with the frequency of appearance of artificial abnormality, to consider the differences. Based on these results alone, it can probably be stated that the chromosomes of such stricken children are completely normal at this time.

195

When discussing these problems on the basis of only a few discernible chromosomes, there is an apparent danger that some uncovered abnormalities are still lurking in the background. As for the problem of congenital abnormality attributed to chromosomes, it is considered necessary to conduct sufficient examinations of the offspring of Minamata disease-stricken patients, and to monitor conditions of such offspring, including brothers/sisters of stricken children, over a prolonged period.

Conclusion

1. Chromosome examination of seven cases of congenital Minamata disease patients were conducted.

2. The peripheral blood culture method was utilized for the chromosome preparation.

3. The cell count of chromosome preparation examined was 301. In the chromosome count, all indicated a normal karyotype, with no sign of abnormality.

4. As for morphological (structural) changes, three cells revealed separations, but not of a frequency to suggest the development of abnormal chromosomes.

5. No chromosomatic abnormalities were detected in the current examination; hence, it can be stated that the chromosomes were normal.

6. NEUROOPHTHALMOLOGICAL STUDY OF MINAMATA DISEASE (Part 2)

1) On Patchy, Dark Scotomas Observed in Minamata Disease

Ophthalmological Seminar, Department of
Medicine, Kumamoto University

Team member: Jun Tsutsui
Research assistants: Hiroyoshi Ogata
Fumio Miyamoto

I. PREFACE

Patchy, dark scotomas represent a phenomenon in which numerous
minute dark spots are found in the residual center of field of vision narrowed
by Minamata disease. The development of centripetal field of vision constric-
tion through organic mercury poisoning has been known since the days of
Hunter-Russel. With respect to Minamata disease, however, only centripetal
constriction of the surrounding field of vision has been emphasized, and not
enough research has been completed on the quantitative or static measurement
of the field of vision.

In Minamata disease, a biopsy of cases revealed that patchy fallout of
nerve cells of the brain cortex, especially of the optic area, constitutes
the most unique change. Therefore, a certain kind of statical, quantitative
method was used to carry out a new form of field of vision measurement, known
as patchy, dark scotoma measurement.

II. METHODS AND PATIENTS

Thirty-one cases of confirmed Minamata disease patients were examined.
Excluded were those who, at the time of field of vision tests, evidenced a

197

lack of understanding or a poor state of fixed vision, which could lead to unreliable findings. Also excluded were cases where there was a probability of patients developing a field of vision constriction.

A Goldmann Perimeter 940, manufactured by Haag-Streit, was utilized. The nature of the test was described to the patients, and checks were made for abnormality of vision and of the Marriott's blind spot.

Then, 3/0 optometer charts were posted at several places around a fixed spot, and after making sure that they were visible, a field of vision of 10° or less at the center (5° or less in some cases) was measured at a total of 40 places. The scotoma area was then counted, and the extent of patchiness of micro-dark spots was sought. 3/0 was equal to around 2.8' in the angle of vision, projecting the functions of about 8 optic cell units in the center where the cones were supreme.

III. FINDINGS

Analysis was conducted on the following five items.

1. Relationship with the degree of field of
   vision constriction

Generally, the degree of patchiness increased as the constriction of the field of vision advanced. Out of 31 cases examined, seven cases (22.6%) disclosed no evidence of patchy, dark scotomas, this group having a field of vision of 50° - 70°. A high maximum degree of 62.5% was noted in cases where there was no extensive constriction of the field of vision, or where the average field of vision was 50° or more. Consequently, a relatively wide field of vision does not mean that functions in the remaining field are complete. Herein lies the significance of patchy, dark scotomas and the gravitation of the field of vision as explained below.

2. Relationship with gravitation of field
   of vision

Observation of the relationship between gravitation of the field of
vision, as measured with a 2/1 Isopter, and patchy, dark scotomas detected
a parallel relationship between both. The greater the gravitation of the
inner Isopter, the more conspicuous was the rise of the degree of patchiness.
Specifically, they constituted both the frontal and rear aspects, revealing
the functions on the same plane.

3. Relationship with visual power

While no definite relationship was noted, the visual power was relatively
sound in most instances where the degree of patchiness was 0.

4. Relationship with degree of seriousness

A direct relationship was not noted when the degree of affliction of
Minamata disease was classified into grave, moderate, and light, and compared
with patchy, dark spots. Patchiness was not necessarily high in patients
with total bodily symptoms. This is only natural, because, in the case of
Minamata disease, perception or hearing disabilities which are one of the
major afflictions do not appear to be attributable to impairment of the
occipital lobe brain cortex alone.

5. Control cases

Virtually no patchy dark scotomas were detected in cases of about
the same age group having no neurological or ophthalmic abnormalities. A
slight degree of patchiness was detected in cases of centripetal retinitis
and in changes affecting the sclera. It can be distinguished from that in
the occipital lobe through visual examination.

IV. COMMENTS

Numerous micro-dark spots called patchy, dark, scotomas were found in great frequency in the residual area of the field of vision. This was a phenomenon newly found by us as a type of abnormal field of vision due to organic mercury poisoning. In this latest study, we avoided cases of which we possessed little knowledge, carrying out repeated measurements through micro-optometric charts equal to 2.8' in the angle of vision. The phenomenon is believed to be valid because of the close relationship with field of vision constriction and the state of gravitation. Hitherto, these micro-dark spots were utilized for the detection of vascular dark spots in the retina, involving primarily the area surrounding the head of the optic nerve, but it is said to be difficult to uncover such dark spots within 10° of a fixed visual point. They virtually do not appear in healthy individuals. These dark spots were detected in some individuals suffering from retinitis, but they can be clearly distinguished from Minamata disease through eye examinations. In pathological examinations of the brain relative to Minamata disease, fall-out of nerve cells, unlike that in the field of vision constriction, was identified at the tip of the optic center of the occipital lobe. It is possible that an equivalent will turn out to be patchy dark scotomas. We hope to continue the study of not only organic mercury poisoning, but also pathways of vision and optic center deformities.

V. CONCLUSION

Using Goldmann Perimeter 3/0 Isopter with an angle of view of 2.8', numerous micro-dark spots were detected in the residual area of the central field of vision in the centripetal field of vision constriction due to Minamata disease. This occurred in 77% of the cases of Minamata disease involving constriction of the field of vision, and the presence of many micro-dark spots was confirmed.

6. 2) Peculiarities of Strabismus in Minamata Disease

Ophthalmological Seminar, Department of
Medicine, Kumamoto University

Team member: Jun Tsutsui
Research assistants: Sakuko Fukai
Hiroyoshi Ogata

I. PREFACE

There is a high rate of occurrence (over 50%) of strabismus in fetal Minamata disease. This ailment is extremely distinct through cerebral pathological examination, and can be compared with other clinical symptoms from a pathological standpoint. The following are the findings as a result of extensive analysis of fetal Minamata disease accompanied by strabismus.

II. METHOD AND CASES

Neuroophthalmological examinations, as well as an analysis of constituent factors of strabismus, visual corrections before, during, and after surgery, and drug treatment methods, were conducted over different periods. Example: M.T. age 16, male, IQ 61 (Vinnie Tanaka method), Family record: grandmother, father and mother are recognized Minamata Disease patients.

III. RESULTS OF TREATMENT

The results of treatment were studied on the basis of three different periods.

First period: Presurgery (July 18 - August 2, 1972). Strabismus was detected about 6 months after birth. Visual capacity changed greatly, with OD-(0.4) - (0.5) OS-(0.4) - (1.0). The field of vision was only outer isopter, with both eyes having a constriction of 5° - 13°, and disclosing gravitation. The angle of strabismus was 15△XT′, 15△XT , at P.C.T.,

201

characterized by common, alternative outward strabismus. Visual functions
in both eyes were impaired, and some suppression was noted with respect to
alternativeness. In a reaction test, normal conditions were noted (receipt
of afterimage, rod light test). In the afterimage test, an afterimage was
observed, extending to the affected part of the field of vision. The con-
vergence point was 40 - 50 cm or farther, depending on the day. A double
vision perception method was attempted for purposes of pre-operative visual
correction, but was not successful. Cosmetic surgery was then performed, as
the symptoms resembled strabismus which appeared in cerebral infantile
paralysis.

Second period: (August 3, 1972). With respect to ธ△XT (cc) , the
muscle in the right eye was shortened 6 mm, and that in the left eye — by
the same length. The remaining angle was at a normal position at P.C.T.,
Alt. P.C.T. A fusion of the image was achieved dramatically with the S.G.
test. The S.G. test was further employed for convergence training during
the surgery. A convergence point of 30 cm was attained, giving hopes for
visual corrections following surgery.

Third period: Post-operative progress (August 4 - October 1, 1972).
One week after surgery, the patients began to complain of insomnia and ner-
vousness, as well as headaches. This was believed due to eye corrections.
Central nerve suppressants were used for various nervous conditions. As such
symptoms decreased, vigorous suppression-removal training was conducted. The
method consisted of one week of stimulation of the mid-regions of the objec-
tive angle of strabismus, followed by kinetic biretinal stimulation through
the use of visual slides, which, after five minutes, led to vomiting, head-
ache, and nervousness on the part of the patients. Since the previously de-
scribed symptoms of nervousness reappeared, the administration of central
nerve suppressants and orthoptics was suspended. After an active agent only
was used for a while, the field of vision enlarged, and the inner isopter
emerged. There was also a period, following surgery, of complaints of double
vision outside the area of Panum's image fusion. This, however, was put to
counter use and applied for indoor training. Orthoptics was performed as the

202

symptoms of nervousness diminished. A day later, a fusion function appeared
and vigorous training was performed to enhance it, resulting in a reading of
+10° - -4°. Convergence point also improved to 10 cm - 7 cm. Monitoring
is being continued at present.

IV. COMMENTS AND CONCLUSION

Minamata disease is a typical affliction responsible for organic changes
of the brain, and produces fallout, obliteration, or degeneration of nerve
cells. Some activity of degenerative cells is believed to have occurred in
the cases we have examined. This is thought to be a case of outer strabismus
attributed to congenital fusion deficiencies. The fact that orthoptics,
central nerve activating agents, and surgery for strabismus have led to some
improvement of functions in both eyes should revise the opinions that these
ailments are not in the realm of orthoptics because of their organic nature.

6. 3) Cerebral Pathology of Visual Association Area of Minamata Disease

Second Pathology Seminar, Department of Medicine, Kumamoto
University
Team member: Tadao Takeuchi
Research assistant: Mitsuaki Eto

Ophthalmological Seminar, Department of Medicine, Kumamoto
University
Team member: Jun Tsutsui
Research assistants: Kimiko Mayuzumi, Fumio Miyamoto,
Hiroyoshi Ogata, Junko Takenaka

I. PREFACE

Centripetal constriction of the field of vision has been known since the days of Hunter-Russell to be a major neuroophthalmological symptom of Minamata disease. We, a group at neuroophthalmology of Kumamoto University, have successively reported on findings not previously known, such as abnormality of eye movement in both eyes, reflex disturbance of pupils, and on the residual field of vision and patchy, dark scotomas. Accordingly, it was necessary to confirm our findings through a comparison of cerebral, pathological findings with neuroophthalmological symptoms. We were able to direct our studies to an appropriate specimen derived from numerous anatomical studies of Takeuchi and his associates of the Second Pathological Seminar, Kumomoto University. While it would be necessary ultimately to delve into the aspects of the brain related with visual functions, our latest study was centered on the occipital lobe, which showed the greatest changes. Since the reports of Takeuchi, Eto, and their associates are available concerning falx cerebri, we shall turn our attention to the visual association area, as it has not yet been reported on in detail.

II. METHOD AND MATERIALS

Five specimens were selected from among the cerebral specimens pre-
served at the Second Pathology Seminar, Kumamoto University, for microscopic
examination. A series of optical microscopic photographs, taken from one
these specimens, was pasted together (for a panoramic view) to conduct a study
of extensive cell disorders.

The case under study was stricken on October 7, 1960, and after having
gone through chronic Minamata disease, died on January 15, 1965, of dysphagic
pneumonia at the age of 79. Neurological disorders included those of surface
perception, handwriting difficulty, tremors, and of hearing. Ophthalmologi-
cally, a moderate 30 - 50° of centripetal constriction of the field of vision
was noted. It is to be noted that no minute examinations of the eyeball
movement or pupil reflex were made.

The specimens were cross sections of the forehead, obtained by slicing
at three places, approximately 6, 10, and 21 mm from the occipital pole of
the lobe. Staining was by the Kluver-Barrera, Haematoxylin-Eosin method.

Used as control was a brain specimen of a 69-year-old without any dis-
orders, which was close to the histological image described in previous
reports.

III. FINDINGS

In the falx cerebri of Minamata disease, there was fallout of nerve
cells, as well as contraction and hardening. Also noted were an increase of
glia and changes or loss of medullary sheath. Even in areas where the dis-
orders were relatively light, changes in the II-IV layers were conspicuous.
As for these layers, layer I evidenced a slight roughness, whereas layer II
evidenced great fallout of nerve cells and sometimes appeared to be spongy
when observed under an optical microscope. In layer III, there was patchy

fallout of 50 - 80% of nerve cells as opposed to a comparable increase of glia, with the remaining nerve cells contracting and hardening. Observed in phagocytes were chromosome sedimentations, thought to be destructive by-products of nerve cells. In layer IV, there was a great fallout of granular cells, and it was difficult to distinguish IV a - c layers. Isolated cells in the IV b layer were changed, and included within their bodies granules stained red by He. Conical cells also evidenced regressive changes. In layer V, 80 - 100% fallout was observed in concial cells, which were being replaced by glia cells. Layer VI also had fallout of nerve cells, resulting in indistinct layer structure. When observed under a medullary sheath stain, nerve fibers generally appeared rough and undergoing degeneration. This was believed due to disorders of the cortical nerve cells. On the other hand, a comparison with the specimen which had been slided at the forehead about 6, 10, and 21 mm from the occipital pole showed that changes in the 21 mm section were greater than in the other two sections. Also, the ridge was affected more than the apical area, and fallout of nerve cells was noted.

Next, in Area 18, changes were evident from layer I, there being roughness as well as corpora amylacea. In layer II, there were, as in the case of Area 17, fallout of granular cells and multiplication of glia, which accounted for an indistinct layer structure. Conical cells in layer III had undergone a fallout, with the remaining cells in disarray or change. The indistinct structural layer of layer IV had induced abnormal positions of large conical cells. In layer V, the original nerve cells were few and in disarray or change, with some presenting the appearance of Betz macro cells seen in the precentral area. Layer VI and the medullary sheath were virtually identical to that of Area 17.

In Area 19, the layer structure was in relatively good shape, but changes were noted in all remaining nerve cells. Fallout of cells was slight as compared to Areas 17 and 18, with almost no difference between the ridge and the apex. Layer I was also in fairly good shape, with little fallout of cells. Layer II had a 50% fallout of nerve cells. Layer III showed a fallout of

conical cells, as in Area 17, but the remaining nerve cells showed only slight change. Layers IV - VI were almost identical to that of Area 18.

IV. COMMENTS

Many points remain unclear with respect to the relationship of cell structure and functions in the occipital lobe. This area is said to hold the answers to many questions, ranging from simple vision to such complex visual functions as mental reflex, eye movement reflex, and preservation of sight functions in both eyes. According to notes of Duke-Elder, Walsh, Crosby, et al., the centripetal impulse above the pathway of vision commences with optic radiation and ends in the vicinity of layer IV b of Area 17, with the end cell branches forming a synapse with granular cells of layer IV a, c, where-upon amplification of impulses and production of transmissible substances to Meynert cells begin. The impulse is also transmitted to identical cells, identical layers, to conical cells in layer III, and to Martinotti cells in layer V. It is further transmitted to layers I and II through the dentrite from conical cells of layer III, with fibers in layer I transmitting the impulse in all directions. On downward movement, Golgi type I cells of layer III are said to be related with the corticofugal fiber, and Golgi type II cells — with the association fiber. Upon moving further downward to layer V, the impulse proceeds from the Meynert cells downward. In the meantime, there are messages from Areas 18 and 19. The impulse from Area 18 is thought to be transmitted to Areas 18 and 19 on the opposite side via the corpus callosum. However, there is no direct transmission path from Area 17 to Area 19. The downward pathway from the Meynert cells of layers V of Areas 18 and 19 passes through the Internal Sagittal Layer, rising in the Pulvinar vicinity, and running lengthwise in the inner side. The pathway of optic motion is thought to exist along this route.

The pathological studies of the brain by Takeuchi and his associates, relative to Minamata disease, can be summed up as follows: Brain damages from Minamata disease are due to circulatory disorders brought about by the initial cranial vascular disorders and regressive changes of the cortex of

207

the cerebrum and cerebellum. As the chronic condition progresses, fallout of nerve cells and related changes occurs, affecting nerve fibers. Of the brain cortex, those areas especially affected are falx cerebri of the occipital lobe, front center, rear center, and parietal lobe. Localization is hardly seen in cases of juvenile or fetal Minamata disease, and shows a trend of extensive cortical disorders. Also, in the same cortex, there are great changes in the pit of the cerebral fissure. In the cerebellum, disorders of the granular cells are prominent, but changes of Purkinje cells are slight. However, relatively weak changes were noted in neurons in the diencephalon, with little damage on the base of the brain and spinal cord. In the spinal cord, secondary changes were noted in the lateral and posterior fibers when degenerative changes were great in the front center. In the peripheral nerves, the sensory nerve was relatively susceptible to injury, with changes in posterior fibers being noted around this time. No prominent degenerative changes were noted in the apical region, optic nerve and the retina. In considering the correlation of the histological image of Minamata disease in Areas 17, 18, and 19 in the occipital lobe with functions, it can be observed that there is a fallout of nerve cells in all cases and a complete severance of neurons, except for a few. Thus, we believe that in Minamata disease, the primary cause of abnormalities of occipital lobe-type eye movement, myopic reflex of pupils, centripetal constriction of field of vision, and patchy dark scotomas in the central, residual field of vision can all be traced to cerebral changes in the occipital lobe. Generally, disorders of eye movement and pupil reflex are considered to be due to disorders of the brain stem, but in the case of Minamata disease involving the brain stem, eye abnormalities of this type develop only in relatively serious cases. Thus, we believe that our position is correct.

V. CONCLUSION

Neuroophthalmological symptoms observed in the case of Minamata disease are patchy dark scotomas, constriction of the visual field and of the residual region, inability to perform coordinated movement, myopic reflex disorders of the pupil. Microscopic studies of the visual association area were conducted to compare such clinical findings with cerebral, pathological findings. Patchy fallout of nerve cells was prominent in layers II, III, and IV of Area 17. A comparison of Areas 18 and 19 with Area 17 showed that both 18 and 19 also had great, patchy fallout of nerve cells throughout the whole layers, with many neurons being severed. Thus, the reason for disorders in the relay of messages from the optic sense to the motor fiber and in the myopic reflex of the pupil is believe to lie extensively in the occipital lobe.

Report to the Tenth Neuroophthalmological Society
(September 7, 1972). Proposed for publication in
Volume 67, No. 6, of Ophthalmological, Clinical
Medicine Bulletin

6. <u>4) Development of Visual Field, Eyeball Movement Meter for Use in Mass</u>
<u>Examination</u>

Opthalmological Seminar, Department of Medicine,
Kumamoto University

Team member: Jun Tsutsui
Research Assistants: Hiroyoshi Ogata, Fumio Miyamoto,
Sakuko Fukai, Kimiko Mayuzumi

I. PREFACE

In neuroophthalmological disorders, examinations of the visual field and eyeball movements are particularly vital. Speedy examinations are required in these days of such pollution diseases as organic mercury poisoning and pesticide poisoning. Therefore, a new device enabling the examination of both the field of vision and eyeball movements was developed.

II. METHOD

This device is basically a muscle trainer, on which an arm and a light were attached to serve as a visual field meter. The index comes in two types for use with outer or inner isopters, and can measure the visual field in all directions. With respect to eyeball movement, the device permits observation with the naked eye or can record EOG simultaneously. The speed of the visual chart is 0.25 - 0.5 c.p.s.

III. RESULTS

1. <u>On normal persons</u>: A comparison was made with the visual field registered by the Goldmann perimeter. The outer isopter corresponded to 4/v of the Goldmann perimeter, whereas the inner isopter showed almost 3/1. In the case of EOG, normal, coordinated movement of the sinusoidal wave-type
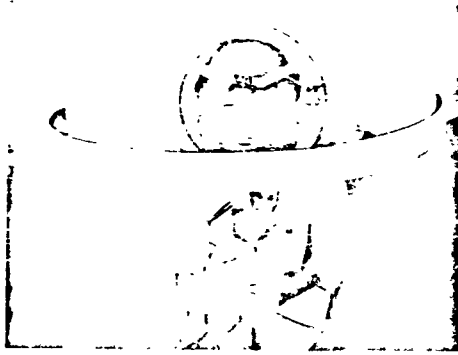
Figure 1
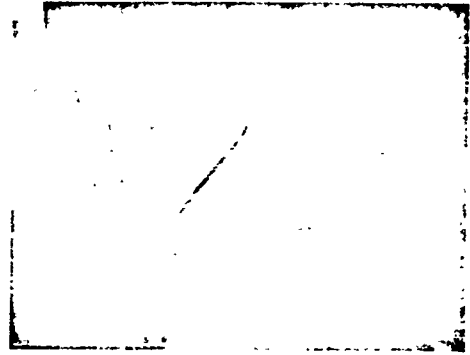
Figure 2

was measurable.

2. For mass examinations: In the screening
test, abnormalities of the visual field and of
eyeball movement were detected in about 6 - 8 min-
utes per case. When the device was actually used
for Minamata disease examination, the results
relative to constriction and gravitation in the
visual field were identical to those observed with
the Goldman perimeter. Examination of eyeball
movement with the naked eye achieve the same
results as the EOG.
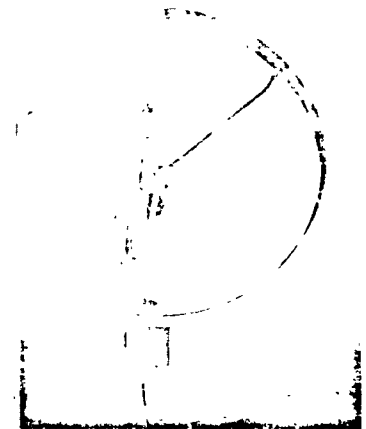
Figure 3

IV. CONCLUSION

Reliable screening was possible with our visual field, eyeball movement
meter for use in mass examinations from the standpoint of tests of visual
field constriction, gravitation, and coordinated reaction movement. It per-
mits examinations of about 8 - 10 cases per hour. Visual field readings were
almost commensurate with 4/V, 3/1 registered by the Goldmann perimeter. The
rate of detection of abnormality of coordinated reaction movement by the naked
eye was identical with the EOG. It was also an outstanding screening device
for use in everyday examinations. It was tentatively named Kumamoto Univer-
sity type neuroophthalmometer.

Report to the general meeting of Japan Ophthalmological Society on May 26,
1973

211

7. OTOLARYNGOLOGICAL STUDIES RELATIVE TO MINAMATA DISEASE (Part 2)

Otolaryngological Seminar, Department of Medicine,
Kumamoto University

Team member: Yasuji Nozaka
Research Assistants: Takezo Kiyofuji, Yoshimi Mioki,
Kosei Shimoyama, Yoichiro Ochiai,
Kenjiro Tuschio, Shinya Araki,
Hidetoshi Iwasaki

Since 1957, we have periodically announced the results of our otolaryngo-logical studies relative to hearing, equilibrium, taste, smell, and speed im-pairments attributed to Minamata disease. Also, in 1959, we reported finding an improvement in the hearing power and vestibular, equilibrium functions in Minamata patients 10 years later.

Our principal interest in Minamata disease is in the area of hearing difficulty, which is one of the major symptoms. We touched on this matter in our previous report, but now wish to discuss this more in detail. We also wish to discuss the results of examinations of patients visiting our hospital on suspicion of having contracted Minamata disease.

The chief aim of our research was as follows.

1. Experimental study on the sequence of development
    of hearing difficulty

In the study of hearing difficulty, it was not only necessary to conduct clinical hearing tests but also biochemical and electrophysiological obser-vations of the inner ear fluid, including general and histological observa-tions of the auditory nerve pathways and the inner ear. In our latest report, we have covered pathological and histological studies on the mercury content

in the inner ear, cochlear reaction, hearing nerve movement potential, and the inner ear.

2. Test results of patients suspected of having Minamata disease

1) Hearing impairment

The object of this study was 96 individuals (52 males, 44 females) on whom hearing tests were conducted from among those requiring such tests on the basis of the second questionnaire survey conducted by the Minamata disease research team of Kumamoto University. A breakdown by locality showed 40 from Minamata, 34 from Goshonoura, and 22 from Ariake, ranging in age from 12 to 80, but mostly in the 40 - 60 age group. Tests consisted mainly of pure sounds, word sounds, recruitment, T.T.D. and T.T.S. Results of the test are as indicated in Table 1.

TABLE 1. LIST OF HEARING IMPAIRMENTS

	Minamata district	Goshonoura district	Ariake district	Total
I Normal range (includes psyiological degeneration)	7	9	14	30
II Suspected rear labyrinth type	9	21	12	42
III Inner ear type, and others where the cause is known	2 4	3 7	2 6	24
	22	40	34	96

We intend to continue our studies on disorders of group II by referring to ophthalmological and neurological studies, since such disorders have not yet ruled out the effects of organic mercury as the cause of hearing difficulty.

2) On impairment of vestibular functions

Of the 72 individuals examined, 11 had eye tremors (+), and 3 (+),
mostly of the horizontal type. Three were of the perpendicular type, suggest-
ing impairment of the central nervous system. A careful study is under way,
since even in these cases one cannot say that there is a link with organic
mercury.

3) On olfactory impairment

A new method applied in our latest study was the use of an Arinamin
solution (50%, 20%) of a close density to determine the relationship, if any,
with the central nervous system. Determination could not be made in 12 out
of 72 cases, and some were in error. We wish to conduct a further study on
this matter.

7. 1) The Amount of Mercury in the Inner Ear Fluid in Experimental Organic
Mercury Poisoning

Otolaryngological Seminar, Department of Medicine,
Kumamoto University

Team member: Yasuji Nozaka
Research assistants: Yoshimi Mioki, Kazuki Umeda

Since the 1940 report of Hunter-Russell on organic mercury poisoning, the latter has been identified as a strange malady affecting the masses of Minamata district in 1953, followed by another similar outbreak in Niigata in 1964. As a result, remarkable progress has been and is being made in its study. Yet, there still remain many unknown matters, research on which is currently under way. In the area of ear and nose, impairment of hearing, equilibrium, speech, and taste functions are recognized, but the real nature of these impairments has not yet been established. With respect to hearing impairment, rear labyrinth type disorders are held primarily responsible, but inner ear type disorders also cannot be dismissed [2, 3, 4]. Thus, we measured the total volume of mercury in the inner ear fluid as a means to determine whether or not inner ear cells are directly impaired because of the contamination of the inner ear by organic mercury.

II. EXPERIMENTAL METHOD

1. Experimental animal

Mature cats, 2.0 - 4.0 kg in weight.

2. Materials administered

Into 1 ml of a puropyrene compound $[CH_3Hg]$ was dissolved methyl mercury iodide $[CH_3CH \cdot OH)CH_2OH]$, so that its proportion would be Hg 2.0 mg.

215

3. Method and period of administration

The foregoing solution was injected daily into the abdominal cavity of
the animal at a dosage of 0-1 mg - 2.0 mg per kg of weight until symptoms of
poisoning appeared. Before any loss of control of movement occurred, animals
Nos. 8, 10, 12, 17 suffered immensely from diarrhea, frothing, and runny nose,
and were in weak condition. Then, the injections were suspended and the ani-
mals were killed (Table 1).

TABLE 1

Cat	Daily dose/ kg	Period admin.	Total amount admin.	Inner ear	Spinal fluid	Blood	Brain	Remarks
.,	2 ung	13 days	101.0mg	0.54ppm			1⁻.15ppm	.
)	1.5	20	72 0	0.11	ppm	ppm	16 96	
1	1.0	23	57.5	0.50			21 45	
0	1.0	19	47.5	0.49			17.74	.
12	1.0	16	32.0	0 32			13 19	Debilitative death
16	1.0	22	44.0	0.31		12.22	16.88	
17	1 0	15	30 0	0.53		20.25	7 87	Debilitative death
	Average	19	42 5	0.46		16.24	17 32	
5	0.5	30	51.0	0.63		6.25	22.92	
9	0.5	32	40.0	0.57		2.44	12.80	
13	0.5	39	48.8	0.52		5.90	15.23	
	Average	34	47.6	0.57		4.86	16.68	
8	0.1	143	35.75	0.37	0.08	6.07	5.96	Slaughtered because of debilitation
10	0 1	137	34.25	0 38	0.17	6.97	6 89	Debilitative death
11	Control			0.39			0.64	
15	⁄⁄			0.49		0.05	0.08	
18	⁄⁄			0.37		0.06	0.07	
19	⁄⁄			0.49	0.35	0 08	0.15	
20	⁄⁄			0.49	0.10	0 02	0.05	
	Average			0.45		0.06	0 06	

4. Collection of material examined.

After anesthetization through abdominal injection of ketimine, bone
cells past the rear part of the pinna were removed, and a 1/4 ml hypodermic
syringe with a 26G 1/2 needle was inserted into the labyrinth through the
round window, drawing the inner ear fluid, while carefully avoiding a mixture
of blood. Since the amount of fluid from one ear was too small for examina-
tion, the fluid from both ears was combined.

216

For reference and comparison purposes, segments of other organs were also examined as follows.

(1) Brain: after incision on the head, the brain was severed at the mid-sagittal surface, and half of the portion was examined.

(2) Arterial blood: the carotid was severed, and the arterial blood drawn and examined.

(3) Spinal fluid: extended from the nape of the neck to the vertebrae in animals no. 8, 10, 19, 20; spinal fluid was drawn after opening the area between the vertebrae.

5. Quantitative determination of total
    amount of mercury

The procedure employed was the method devised by Winkler [5] and Sandell [6], as well as by Kai [8], which is patterned after Snell's [7] method.

(1) Conversion of objects of study into ashes: objects of study were converted into ashes by adding strong acids (nitric acid, sulfuric acid) and permanganate, and applying heat.

(2) Extraction of mercury: hydrochloric acid and hydroxylamine were added to nullify surplus permanganate; urea solution was then added to inactivate nitrous acid formed during ashing, after which dithizone was added. Mercury was then extracted for examination.

(3) Quantitative determination of total amount of mercury.

    (i) In solutions with a large mercury content, a QB50 type spectrophotometer was employed to conduct colorimetry at a wavelength of 500 mμ.

217

(ii) In the case of low mercury content, the solution was dried in a test tube, and the mercury therein was subjected to high temperature and vaporized. A Beckman mercury vapor meter (Model K24) was then used to determine the volume according to the ultraviolet, atomic absorption method [9].

III. RESULTS OF THE EXPERIMENT

1. Inner ear fluid (Tables 1 and 2)

(1) With respect to the group of 5 mice each administered 1 mg/kg per day, injection was continued for a 15 - 23 day period, for a total dosage of 30 mg - 57 mg. Of these, Nos. 12 and 17 died of disease during the same period. The total volume of mercury in the inner ear fluid was from 0.32 ppm to 0.53 ppm, or an average of 0.46 ppm, which was close to the 0.45 ppm average for the control group.

(2) With respect to the group of 3 mice administered 0.5 mg/kg daily, injection was continued from 30 - 39 days, for a total dosage of 40 - 54 mg. The total amount of mercury in the inner ear was 0.52 ppm - 0.63 ppm, with an average of 0.57 ppm, which was slightly higher than in the control group. The difference was not considered significant.

(3) In the case of the group of 2 mice administered a daily dose of 1 mg/kg for a prolonged period of approximately 140 days, the total volume was 34 - 36 mg, with an average 0.38 ppm in the inner ear fluid, and somewhat lower than in the control group. The difference was also considered insignificant.

(4) In the case of two mice administered a daily dose of 2.0 mg/kg and 1.5 mg/kg, respectively, the aggregate dosage for each was greater, with 104 mg and 72 mg. Yet, the total volume in the inner ear fluid was 0.54 and 0.44 ppm, with no significant differences from the control group.

Thus, no significant differences could be found between the entire groups and the control group with respect to the inner ear fluid.

2. Spinal fluid (Table 1)

Test specimens were obtained only from two cats administered mercury (Nos. 8 and 10) and an equal number of control cats (Nos. 19 and 20). Of those cats administered mercury, the average amount was 0.13 ppm, which was slightly lower than the 0.23 ppm registered by the control group. The difference was insignificant. Strictly speaking, while that value is lower than the value for the inner ear fluid, it would be safer to state that there was no significant difference between them.

3. Brain (Tables 1 and 3)

Cats affected by organic mercury poisoning registered over 16 ppm, while even that group administered 0.1 mg — although they did not develop mercury poisoning — showed 6 ppm, or more than a hundred-fold of the control group's 0.06 ppm. These values are almost identical to the amount of mercury in the brain of experimental Minamata diseased cats and those of the control groups as reported by Ujioka [19].

TABLE 2. COMPARISON OF TOTAL VOLUME OF MERCURY IN
INNER EAR FLUID

Administered groups (amount per day/kg)	Average value of total volume of mercury
2.0 mg Group (1)	0.54 ppm
1.5 mg " (1)	0.44
1.0 mg " (5)	0.46
0.5 mg " (3)	0.57
0.1 mg " (2)	0.38
Control group (5)	0.45

TABLE 3.   COMPARISON OF TOTAL MERCURY CONTENT
IN BRAIN

Administered group (daily dose/kg)	Average value of total mercury content
2.0 mg Group (1)	17.15 ppm
1.5 mg " (1)	16 06
1.0 mg " (4)	17 32
0.5 mg " (3)	16.08
0.1 mg " (2)	6.17
Control group (5)	0.06
Spontaneously afflicted cat* " " "	8.08 10.4
Experimentally afflicted cat* " " "	18 6 18.1 10.0
Control area cat*	0 05 0.04 0.02

*Findings by Ujioka [19] (1960)

4.   Blood (Tables 1 and 4)

The total amount of mercury in the blood of the group administered 1.0
mg mercury was especially high, with more than 12 ppm. In the case of groups
administered 0.5 mg and 0.1 mg, the lowest was 2.5 ppm, or an average of
5 - 6 ppm. This was approximately more than 40 times that of the control
group average 0.06 ppm.

TABLE 4. COMPARISON OF TOTAL VOLUME OF MERCURY
IN BLOOD

Administered group (daily dose/kg)	Average value of total amount of mercury
1.0 mg Group (2) 0.5 mg " (3) 0.1 mg " (2)	10.24 ppm 1.86 6.52
Control groups (4)	0.46
Spontaneously afflicted cat* " " "	10.6 15.8
Control area cat " " " " " "	0.13 0.08 0.06

*Findings by Ujioka (1960) [19].

IV. SUMMARY AND COMMENTS

It was reported in 1940 by Hunter [1] that hearing impairment was a serious clinical symptom of acute organic poisoning. In 1958, Nestrugina [4], after having conducted a hearing test of 60 patients with chronic organic poisoning, reported that the impairment could be of both peripheral and central nervous system types. Nozaka and his associates [2, 3] held that the disorder could be of the rear labyrinth, but stated that inner ear disorder could not be dismissed because of positive cases of recruitment. The reason for his assertion was that, under the present hearing test method relying on subjective examinations, there were limits in attempts to determine the impaired region. According to Matsumoto [10], changes occurred in the cerebrum frontal and parietal lobes, as a result of which mental and intellectual disorders and voluntary movement impairment developed. Prominent changes also occurred in the cerebellum, resulting in an advanced degree of loss of movement. Thus, reactions against tests have decreased in many cases. Even if there

were 100% changes and fallout of the cortical cells in the lateral, temporal side of the cerebrum — which is the hearing center as claimed by Matsumoto [10] and Takeuchi [11], it would still be necessary to be especially careful in the determination of results of the hearing test. As Matsuzaki [12] says, there are occasions where determination cannot be made.

Miyakawa [24] and his associates used rats to study changes in the peripheral nervous system. Based on their findings, they claimed that even before changes appear in the cerebellum due to organic mercury poisoning, the peripheral sensory nerve has already been affected, along with trifurcated and vestibular nerves. However, when the results of hearing tests are considered, along with the fact that there are almost no disorders of the retina or optic nerve, even in cases of prominent constriction of the visual field, the belief is that hearing impairment is due to rear labyrinth type disorders [10, 11]. In the hearing tests, there were cases of advanced degree of hearing difficulty or even deafness, although biopsy showed relatively slight changes in the central nervous system, making one suspect that inner ear disorders [10,2,11] were also involved. Histological studies of the inner ear are being attempted, but no reports have yet appeared because of various difficulties.

We measured the total amount of mercury in the inner ear fluid for the purpose of determining whether the contamination of the inner ear by organic mercury caused direct destruction of cells therein.

In a study on fetal Minamata disease, Fujita [13] reported that through use of radioactive materials, he had discovered that mercury in the mother's body was transferred to the fetus via the placenta, and that mercury was transferred to the child by the mother's milk. However, the blood of both the infant and the mother do not combine, and metabolism takes place through osmic action of the placenta and the peculiar permeatic nature of epithelium cells [14]. Thus, it was very interesting that organic mercury migrates from the mother's body under such circumstances. It also suggests the possibility

of organic mercury being transferred to the inner ear fluid or spinal fluid because of the peculiar permeability of epithelium cells or through blood vessels.

With respect to impairment of nerve cells by organic mercury, Kuwahara [15], Yoshino [16], Miyagawa [17], and Brown[18] contend that the reason may be due to obstruction of cell metabolism owing to interference of mercury alkylide with enzymes involved in protein formation by cells. Thus, if mercury contamination and histologic sedimentation in the inner ear can be established, there exists a possibility that a similar metabolic obstruction could develop in inner ear cells.

However, according to our findings relative to the total amount of mercury in the inner ear fluid, there were not significant differences as indicated in Tables 1 and 2. There also was almost no difference even in the case of spinal fluid (Table 1). Nevertheless, a remarkable difference of mercury level was noted in other internal organs (brain, blood) as compared to the control group (Tables 3 and 4). What is the reason that accumulation and transfer of mercury occurs in mother's milk and fetus, when there is virtually no such transfer in the inner ear fluid or spinal fluid?

It is said that endothelium cells of the vascular system engage in the production of endolymph fluid, whereas endothelium cells of cerebral capillary vessels take part in the production of spinal fluid, and that they — by means of differences of osmic pressure or ion density and unusual permeability — screen out the materials that pass through [20, 21]. Thus, it could be that in these endothelium cells, placenta, and mammary gland, physical and basic differences exist with respect to permeability.

It is further stated that mercury alkylide in the blood is found in very small quantity in blood serum, being found mostly in red blood corpuscles [19, 23]; that it circulates in the body by a weak combination with globin, a protein [13], enabling it to travel freely among proteins in internal

organs [8]. Thus, it is able to move easily from the mother's red blood corpuscles to those of the fetus, as well as to milk containing such proteins. However, it is believed difficult for it to travel from the blood serum to the inner ear fluid or spinal fluid which contain only a small number of proteins.

Thus, it is apparent that there was no such thing as mercury contamination of the inner ear fluid, and the possibility of organic mercury — a type of mercury — damaging inner ear cells was ruled out. However, it cannot be denied that there are other causes of hearing difficulty in the inner ear.

According to Takeuchi [22] and Matsumoto [10], vitreous, uniform changes of micro blood vessel walls were noted in the brain of patients suffering from organic mercury poisoning, as well as slight, unusual enlargement of wall endothelium cells and edema of the surrounding area of blood vessels. It is therefore conceivable that similar changes have occurred also in the vascular system in the inner ear. Deficiencies in the production of the inner ear fluid due to changes in the vascular system or in its formation could possibly lead to disorder of inner ear cells. It is also possible that because of changes in the vascular system, inner ear cells may be confronted with a chronic lack of oxygen and become irreversible.

According to results obtained by Ochiai, who made a histological study of the inner ear of poisoned cats used in our experiment, there were no differences in Corti's organ, spiral ganglion cells, and the vascular system when compared to those of the control cats. Thus, it can be stated on the basis of histological studies of the inner ear fluid and the inner ear that, in our method of experiment, we found no evidence of impairment of the inner ear brought on by organic mercury poisoning.

V. CONCLUSIONS

We conducted a study to determine whether or not mercury contamination developed in the inner ear fluid of cats suffering from organic mercury

poisoning. We injected methyl mercury iodide into the abdominal cavity of fully grown cats to develop poisoning symptoms. We then extracted all the mercury and measured its amount through colorimetry and the ultraviolet, atomic absorption method. For comparison with other internal organs, the total mercury content in the spinal fluid, brain, and blood was also measured according to the same method. As a result, it was found that the total amount of mercury in the inner ear fluid was not linked to the dosage or period of administration of mercury, for there were no differences from the control group. No differences were noted in the spinal fluid, but prominent differences were noted in the brain and blood between poisoned and control groups. Thus, we assumed that there was no contamination of inner fluid by organic mercury, or impairment of inner ear cells.

REFERENCES

1. H Hunter, D., et al. Poisoning by Methylmercury Compounds. Quart. J. Med., Vol. 9, 1940, p. 193.

2. Nozaka, Yasuji. Minamata Disease — Research on Organic Mercury Poisoning. Minamata Disease Research Team, Department of Medicine, Kumamoto University, 1966, p. 193.

3. Nozaka, Yasuji, et al. Effect of Impairment of Hearing, Vestibular Functions, Taste and Speech in Minamata Disease. Otorhinolarngy, Vol. 73, 1970, p. 1006.

4. Nestrugina, Z. F. Disturbance of Auditory and Vestibular Functions in Chronic Intoxication with Mercury. Vestn. Otorhinolarygn., Vol. 20, 1958, pp. 4, 32.

5. Winkler, W. O. Report on Mercury. Association of Official Agricultural Chemist., Vol. 21 (2), 1955, p. 220.

6. Sandell, E. B. Colorimetric Determination of Traces of Metals. Third Edition, 1959, p. 621.

7. Snell, F. D. and C. T. Snell. Colorimetric Methods of Analysis. Third Edition, 1958, p. 63.

8. Kai, Fumio. Trend in Animal Bodies of Organic Compounds and Methyl
    Mercury Compounds Found in Minamata Bay Fishes and Shellfishes.
    Kumamoto Medical Association Journal, Vol. 37 (12), 1963, p. 673.

9. Yamaguchi, S. Ultra Microdetermination of Mercury in Biological Mate-
    rials by Atomic Absorption Photometry. Industrial Medicine, Vol. 10
    (3), 1968, p. 125.

10. Matsumoto, Hiedyo. Neuropathological Study of Minamata Disease in
    Humans (Third Edition). Kumamoto Medical Association Journal,
    Vol. 35 (11), 1961, p. 1133.

11. Takeuchi, Tadao. Minamata Disease: Research on Organic Mercury Poison-
    ing. Minamata Disease Research Team, Department of Medicine,
    Kumamoto University, 1966, p. 264.

12. Matsuzaki, Chikara. Literary Reminiscences Concerning Hearing Sense
    Cortical Center and Cortical Hearing Difficulty. Autiology Japan,
    Vol. 12 (1), 1969, p. 43.

13. Fujita, Eisuke. Experimental Research on Organic Mercury Poisoning
    — Movement of Substances Producing Minamata Disease from the Body
    of Mother Rats to Their Offspring via the Placenta or Mother's Milk,
    and Trend of Movement in the Mother's Body. Kumamoto Medical
    Association Journal, Vol. 43 (1), 1969, p. 47.

14. Kaki, Michitaka. Obstetrics. Regular Edition. Nanzando, 1962, p. 34.

15. Kuwahara, Shunsuke. Effects of Mercury Compounds on Cerebral Functions
    — Especially Inhibitive Effect of Methyl Mercury Compounds on
    Transaminase, Aromatic Amino Acid Found in Brain of Rats. Kumamoto
    Medical Association Journal, Vol. 44 (3), 1970, p. 214.

16. Yoshino, Y., et al. Biochemical Changes in the Brain in Rats Poisoned
    with an Alkyl Mercury Compound, with Special Reference to the
    Inhibition of Protein Synthesis in Brain Cortex Slices. J. Neuro-
    chem., Vol. 13, 1966, p. 1223.

17. Miyagawa, Tahei, et al. Electron-Microscopic Study of Experimental
    Minamata Disease — Chronology of Development of Pathological Changes.
    Mental Nerve Journal, Vol. 69 (12), 1967, p. 1352.

18. Brown, W. J., et al. Organic Mercurial Encephalopathy — An Experi-
    mental Electron Microscope Study. Mental Progress, Vol. 9 (1),
    1965, p. 34.

19. Ujioka, Irei. Chemical And Analytical Study of Mercury Found in Food-
    stuffs and Animal Organs. Kumamoto Medical Association Journal,
    Vol. 34 (Supplement 2), 1960, p. 383.

20.   Nakamura, Fumio. Outline of Physiology — Physiology of Sensation.
      Igakushoin, 1967, p. 757.

21.   Broman, T., et al. An Experimental Study of Disorders in the Permeabil-
      ity of the Cerebral Vessels (the Blood-Brain Barrier) Produced by
      Chemical and Physiological Agents. Acta Physiol. Scandinav., Vol. 10,
      1945, p. 102.

22.   Takeuchi, Tadao. Pathology of Central Nervous Disorders (First Report).
      Kumamoto Medical Association, Vol. 31 (Supplement), 1957, p. 37.

23.   Katsunuma, Harue, et al. Study on Distribution of Various Mercury
      Compounds in the Body. Nisshin Medicine, Vol. 48 (6), 1961, p. 373.

24.   Miyakawa, T., et al. Experimental Organic Mercury Poisoning — Patho-
      logical Changes in Peripheral Nerves. Acta Neuropath. (Berl.), Vol.
      15, 1970, p. 45.

7. 2) Cochlear Reaction in Organic Mercury Poisoning and Changes in Hearing
Hearing Nerve Activity Potential

Otolaryngology Seminar, Department of Medicine, Kumamoto
University

Team member: Yasuji Nozaka
Research assistants: Kosei Shimoyama

Second Seminar, Pathology, Department of Medicine
Kumamoto Univeristy

I. PREFACE

In Minamata disease, hearing difficult is one of the major symptoms,
along with centripetal constriction of the visual field, loss of movement,
and articulation difficulty. As to the area affected in the case of hearing
difficulty, Nozaka [1, 2], as the result of hearing tests on Minamata disease
patients, concluded that the rear labyrinth was mostly affected, but also
suspected afflictions of the inner ear, based on the DL test and other findings.
On the other hand, Cho [3, 4] and his associates claimed, based on hearing
tests of pure tones on 50 ears in 25 cases and subsequent use of Békésy
audiometry, that hearing disorders in organic mercury poisoning begin with
cortical hearing difficulty, and that the rear labyrinth disorders which ex-
tend, as the condition worsens, to the base of No. 8 cranial nerve were the
chief area of concern. From the pathological viewpoint, Takeuchi [5] stated,
based on biopsy of Minamata patients, that the side region of the head, which
is the cortical center of the hearing sense, the falx cerebri, and the central
area of the occipital lobe were susceptible to damage with fallout of cortical
nerves being noted. As noted above, the nature of hearing impairment was
gradually being discovered. In order to study whether disorders occurred
in the inner ear and auditory nerve ends, using chronic electrodes we

administered Minamata disease-producing organic mercury to cats, and observed changes in the cochlear reaction [6, 7] (hereinafter referred to as CM) and auditory nerve activity potential [8] (hereinafter referred to as AP). Chronic electrode methods have been used on cats by Galambos [9] and Simmons [10, 11], and on domestic rabbits by Ouchi [12], Shiraiwa [13], Ogawa [14], and Otani [15] [15] to detect potential changes from poisons of the ear organ, such as di-hydrostreptomycin and kanamycin.

II. EXPERIMENTAL METHOD

1) Experimental animal

Cats used were those which had been established as having Minamata disease, naturally or experimentally, and on which pathological studies had been conducted [16, 17]. Of four fully grown mice weighing between 2.7 - 3.0 kg, two were tested on both ears, and the other two on one ear.

2) Electrodes

A silver ball tip 500 µ in diameter affixed to the end of a 200 µ-diameter steel wire was insulated with polyethylene tube and used.

3) Insertion of electrode

Insertion of terminal on top of head: Following anesthetization by muscular injection of about 40 mg/kg of hydrochloric ketimine, a skin incision was made on the top of the head under sterile conditions, the periosteum removed, four small holes made in the skull, and the terminal affixed with the 300 µ-diameter steel wire and dental cement (Repairsin F).

Electrode insertion surgery: After incision of the rear part of the ear, the bone cell of the inner ear was exposed, a hole approximately 4 mm was opened, using a bar, in the upper rear part of the bone cell. Using a

surgical binocular microscope, the silver ball tip of the electrode was affixed, so that it would touch the membrane above the round window. The electrode was affixed to the hole with dental cement, and the hole was closed. Next, the other end of the electrode was guided to the area of the top of the head through the periosteum, and affixed to the terminal on the top of the head. For a fukan [sic] electrode, a portion of the steel wire used to affix the terminal to the skull was utilized. Following the operation, penicillin and tetracycline were administered for about a week to prevent infections or tympanitis.

4) Experimental devices

Pure tones were applied for measurement (Audiometer Rion A-1002k). Sounds were directed to the cats' ears by the closed method from the 20 cm receiver, using a 4 mm-diameter vinyl tube. The vinyl tube was affixed with an ear-mold made of plaster to fit the cats' ears. In the AP measurement, a constant 0.05 msec impulse from an electronic stimulator (Nihon Koden MSE-3) was converted into a click sound by a specially-manufactured crystal receiver and guided to the cats' ears by the closed method. The level of click sounds was measured with a precise noise meter, NA-51, manufactured by Rion. CM and AP directed from the round window electrode was carried to the Braun tube oscilloscope (Nihon Koden VC-7) through the preamplifier, and then photographed.
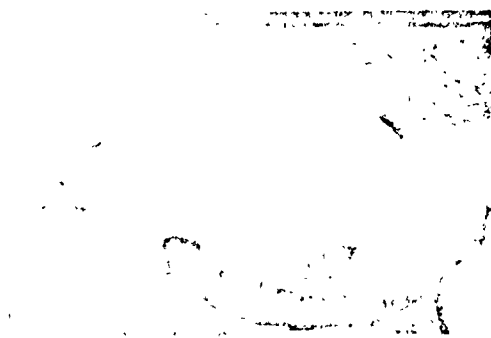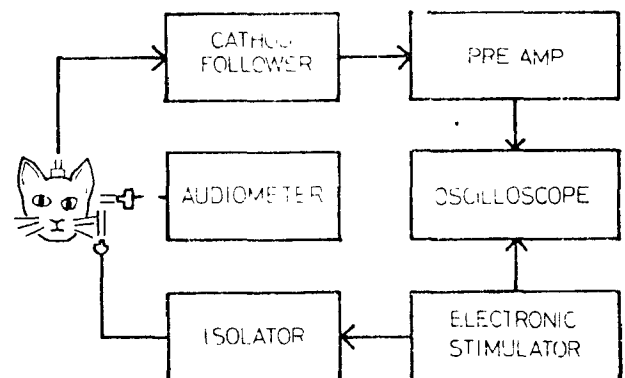
Photo 1   Figure 1. Experimental devices

5) Measurement

As a rule, measurements were taken every other day, when the animals were anesthetized with hydrochloric ketimine. In CM, the potential hearing level (hereinafter abbreviated as HL) of 90 dB stimulating sounds and the visual detection level (hereinafter abbreviated as VDL) or the minimum sound required to recognize CM were measured. In AP, the potential for 50 dB (SPL) of stimulating sounds, as well as the latent period, were determined. There were two negative peaks in AP, at N_1 and N_2. The potential (amplitude) extended from the base line to the peaks of N_1 and N_2, and the latent period was from the commencement of sound stimulation until the peaks of N_1 and N_2. A soundproof room was not used. The noise level at the time of measurement was 35 phons.

6) Method of administration of methyl mercury

Allowing for a three-week recovery time after positioning of the electrodes, 0.5 - 1.0 mg/kg mercury was injected daily into the abdominal cavity of the animals until poisoning symptoms appeared. For this purpose, methyl mercury iodide (CH_3HgI) was dissolved in $(CH_3CH_2OH)CH_2OH$ to reach a mercury content of 2 mg/ml (see Table 1).

7) Observation of entire body conditions

In addition to such three major characteristics as some loss of control in walking, spasmodic convulsions, and torpidity, as noted in cats afflicted with Minamata disease, there were other disorders such as loss of appetite, vomiting, diarrhea, a general weakening, loss of weight, eye lipid, and flow of saliva. These disorders were divided into four phases [18], and observed (see Table 1).

231

TABLE 1. AMOUNT OF MERCURY ADMINISTERED AND
ITS EFFECTS

		No. days until symptom appearance	No. days until death	Amt. Hg. administered per day (mg/kg)	Aggregate amount Hg administered (mg)	Walking, but some loss of control	Spasmodic convulsion	Torpidity	Flow of saliva	Eye lipid	Diarrhea	Weight (kg)	Weight loss (kg)
No. 3	RW 5	41	46	0.7	73.0	₩	₩	₩	₩	₩	+	2.7	0.2
No. 1	RW 16, 18	30	38	0.8	58.8	₩	₩	₩	₩	-	₩	2.7	0.6
No. 2	RW 17	35	42	0.8	74.8	₩	₩	₩	₩	₩	₩	3.0	0.4
No. 4	RW 20, 21	25	31	1.0	61.8	₩	—	₩	—	₩	₩	2.9	0.6

8) Pathological studies

The brain and auditory nerve of cats which had died, including those slaughtered because of imminent death, were fixed in formalin, and pathological studies were conducted thereof.

III. FINDINGS

1) Appearance of symptoms of organic mercury poisoning

Twenty-five to 41 days after commencement of administration of methyl mercury, initial symptoms of loss of movement in hind legs were noted. The aggregate amount of mercury administered was 58.8 - 74.8 mg. Until the initial symptoms appeared, the cats were generally in good condition; however, with the onset of the symptoms, they suddenly began to stagger to one side or collapse and were unable to walk. Spasmodic convulsions were observed in three cats. Toward the end, their condition deteriorated due to loss of appetite, vomiting and diarrhea. They appeared to be in a state of lethargy

and died 5 - 8 days following the outbreak of initial symptoms. Their weight loss ranged from 200 - 600 g.

2) Changes in CM and AP

Figure 2 reflects the changes in the average values of CM and AP in the case of 6 ears, following commencement of administration of methyl mercury. In CM, the pure tone stimulation of 90 dB (HL) did not cause any distinct lowering of either 1 KHz or 4 KHz, but in both ears of one cat, there was a loss of control of movement and a simultaneous decrease of the potential. Figure 3 shows this CM decline as a change of reaction intensity curve. The decrease of potential was slight, but was accompanied by a movement to the right. VDL was somewhat high at an average value of 60 - 70 dB (HL), but the effects of administration of methyl mercury were virtually unseen. A rise in the level was observed in only one ear.
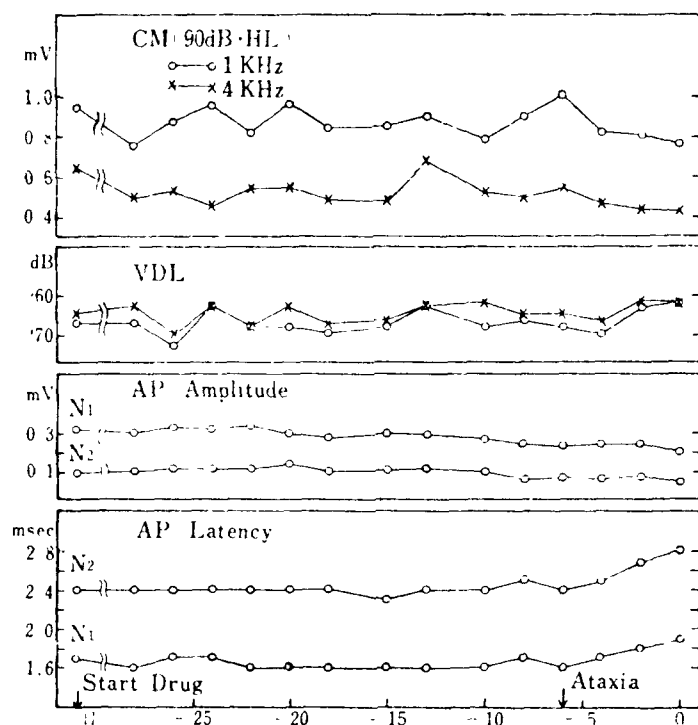
Figure 2. Changes in CM and AP following administration of methyl mercury (average values of 6 ears)
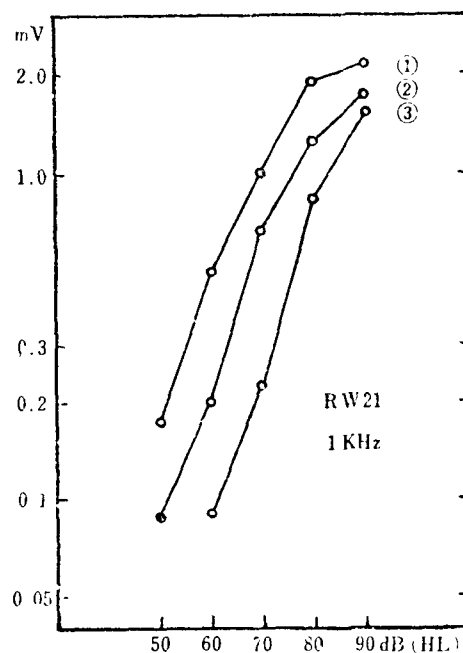
Figure 3. CM reaction intensity curve:

1 — Appearance of loss of control of movement; 2 — after 2 days; 3 — after 6 days

233

The average value of AP amplitude reflected a slight decrease as time progressed. Of the 5 ears which induced AP, 2 showed a decline of AP, but these were considered to be secondary changes because they were accompanied by a CM decline. The average value of AP latency was 1.6 msec for N_1, and 2.4 msec for N_2, but began to extend as loss of control of movement became noticeable. At the time of final determination, it was 1.9 msec for N_1, and 2.8 msec for N_2, or an extension of 0.3 msec for N_1 and 0.4 msec for N_2. This variation was common in all cases.

IV. COMMENTS

The plan was to determine the potential without putting the cat under anesthesia to avoid any changes in muscular reflex suppression in the inner ear. however, we were forced to resort to anesthesia because of the difficulty of securing the cat. The effects of anesthesia cannot be dismissed in light of Simmon's [20] experiment. He found that in a chronic experiment [11] using Nembutal as an anesthetic, the degree of potential variation did not differ from that when no anesthetic was used. Thus, it was believed that a variation of the potential could be avoided by controlling the strength of the anesthetics.

The lowering of CM in the two ears began as disease symptoms appeared, giving rise to the suspicion of inner ear disorders. However, since the other four ears were unaffected, it was necessary to examine more cases. In the case of two ears, variations of the reaction intensity curve were accompanied from the outset by a decline of the potential as the curve moved to the right. Ogawa considered that such a change in DHSM was due to variations of endocochlear potential as a result of changes in the endolymph structure.

The extension of AP latency was slight, but an important finding that was noted in all cases from the time of outbreak of symptoms. It is generally stated that AP latency [21, 22] is prolonged by diminished sound pressure,

masking, cooling, and anoxia. Otani, who has reported on KM with respect to drugs, contends that this may be due to the keen sensitivity of nerve endings of capillary cells, as in the case of synapses, to various attacks, when considering that variations in AP latency occurred prior to changes in the CM and AP amplitudes. Because of such an occurrence, even in cases without a lowering of CM and AP, it could mean the beginning of disorders of capillary cell nerve endings or of auditory nerve endings. Thus, a long term study would be required.

Pathological and histological studies

Pathological studies were conducted to verify the onset of symptoms, their extent, and auditory nerve afflictions of 4 cats used in this experiment.

1) Method of study

Visceral organs of the nervous system of 4 cases of slaughtered or dead animals were fixed in formalin and stained under the haematoxylin-eosin, Klüver and Barrera method. Studies were made chiefly of the posterior slice of the central area of the cerebrum, the posterior slice of the occipital lobe, the appendage of the cerebellum, lobe area, cervical spine, spinal nerve, and auditory nerve.

2) Findings

① Cerebrum: In all cases, an acute enlargement of small nerve cells was seen, not only in the optic region, but also in the cortex. Acute changes such as chromatolysis were observed, with numerous cells becoming large or being obliterated. An increase of glia was also noted in the area of cell obliteration. Some conical cells were also noted to have undergone some acute growth, with some showing a slight degree of hardening and atrophy. These pathological changes were observed most keenly in No. 3, and only to a slight degree in the other 3 cases (see Photo 2 and 3).
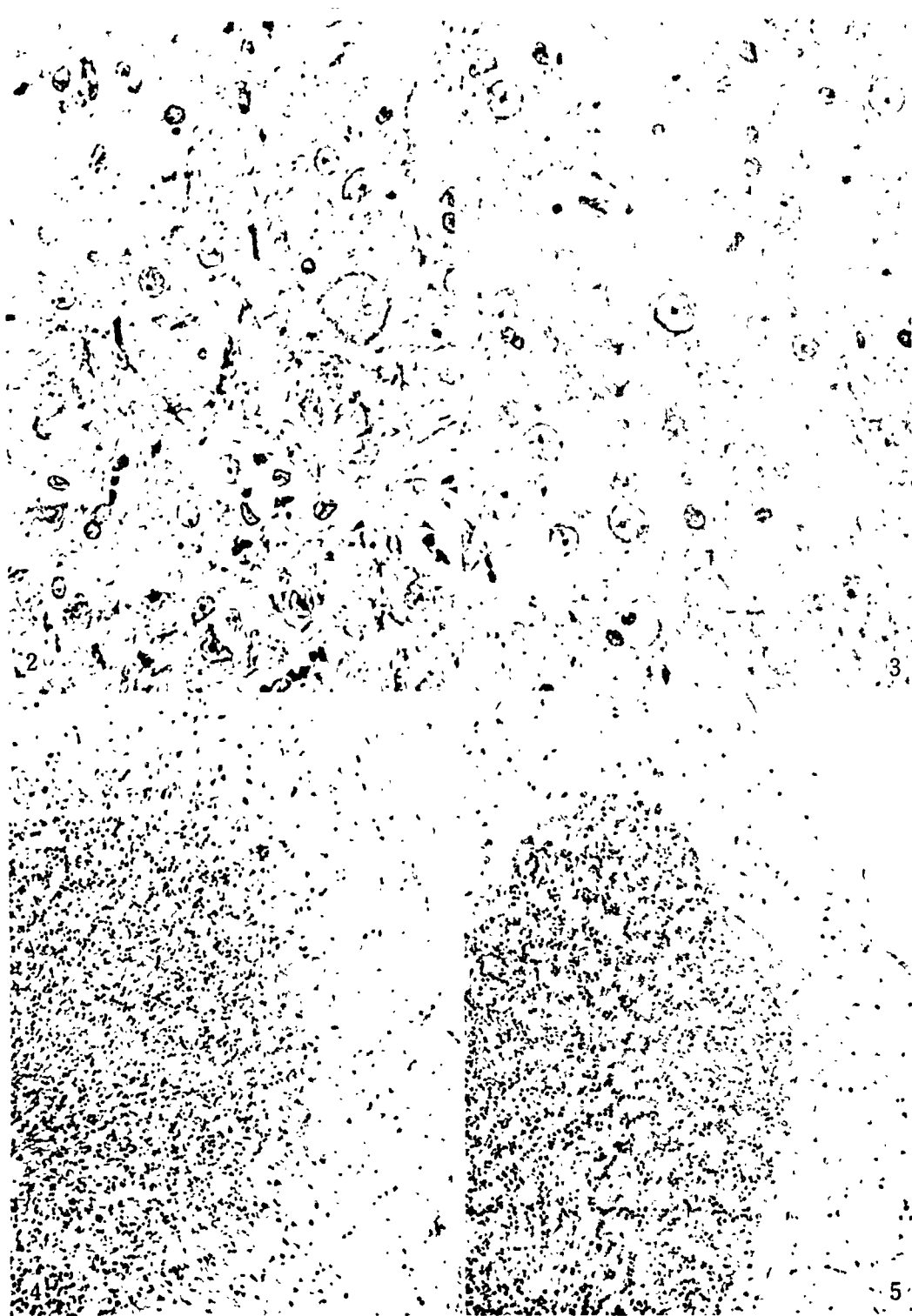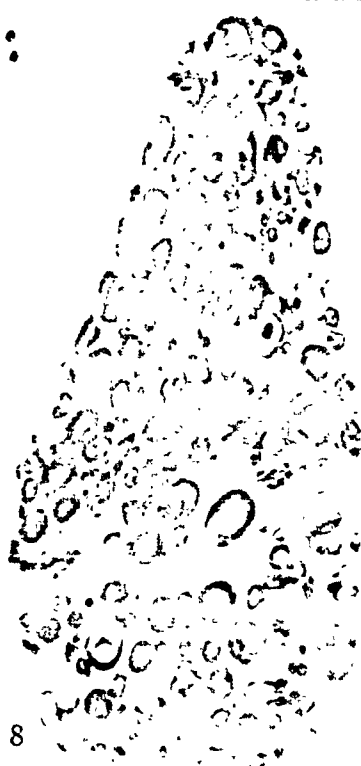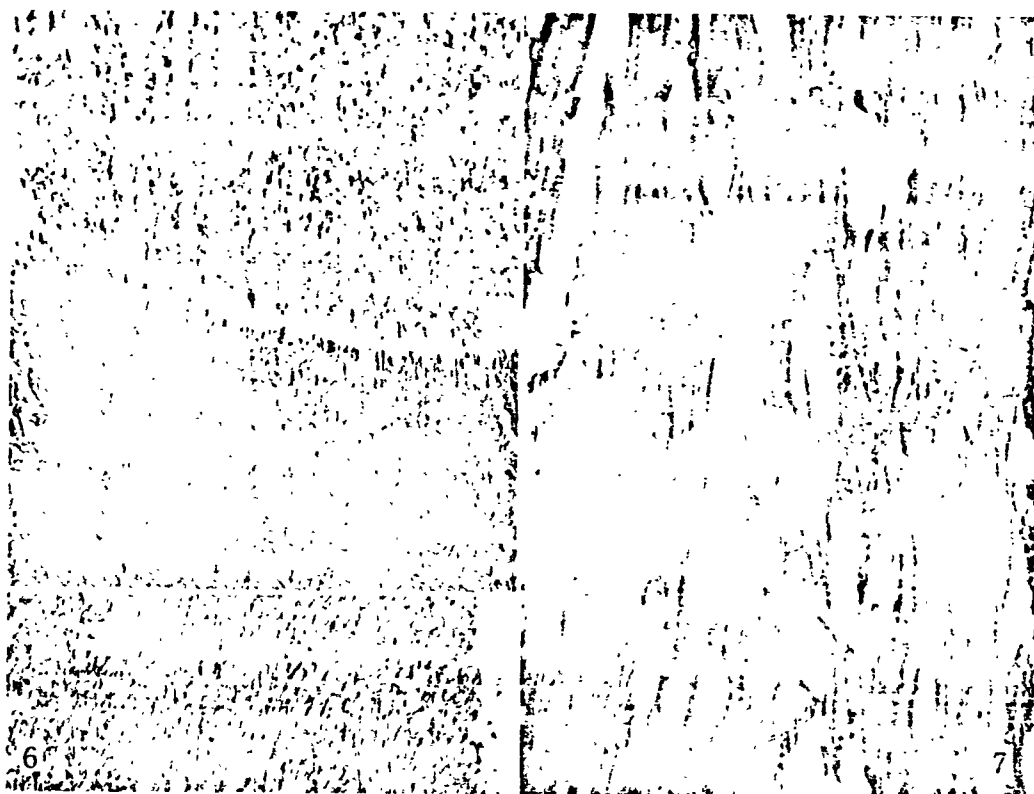
Description of Photos

Photo 2. Acute enlargement of conical cells in the cerebral occipital lobe cortex of No. 1, along with an acute enlargement of small nerve cells. Obliteration of cells can be observed.

Photo 3. Nuclear enlargement of small nerve cells in the cerebral temporal lobe cortex of No. 4; obliteration of cells can also be seen.

Photo 4. In the appendage of the cerebellum of No. 1, fallout of Purkinje cells is notable; an increase of Bergmann's glia is seen in the area of the fallout. Granular cells are falling off to some extent directly below the Purkinje cell layer.

Photo 5. Around and deep in the cerebellum of No. 4, there is a fallout of Purkinje cells and an increase of Bergmann's glia. A very few granular cells have decreased at the apical area.

Photo 6. Auditory nerve of No. 2, with the upper half being the center and the lower half being the peripheral nerve fibers. From the border area of the brain to the peripheral region can be seen a slight damage (upper side of photo), becoming more conspicuous in the peripheral region (lower side of photo).

Photo 7. Enlarged view of peripheral side of auditory nerve of No.1, showing ganglionic degeneration.

Photo 8. In the lateral cross section of the posterior root of the spine of No. 3, the disintegration of the medullary sheath and enlargement and/or contraction of the axons can be seen on a segment of the nerve fiber. A trend toward increase of Schwann cells is also noted.

Photo 9. In the anterior root nerve of the spine of No. 3, the nerve fibers are in normal position; disintegration of the medullary sheath is

238

seen only in a part of the nerve fibers. There is no increase of
Schwann cells (see Photos 2 and 3).

② Cerebellum: Acute enlargement or fallout of Purkinje cells in the
appendage was prominent in all cases, with some showing poor stainability and
disintegration or obliteration. A multiplication of Bergmann's glia was found
in the area of fallout. In Nos. 1 and 3, granular cells were also noted
undergoing fallout from directly below the Purkinje cell layer (see Photo 4).
In No. 4. a minute quantity of granular cells has decreased in the apical
region deep in the cerebellum (see Photo 5), but almost no fallout of granular
cells can be seen in No. 2. Thus, the fallout of granular cells was slight
in either case. Although there were some differences, it constituted only
a patchy fallout.

③ Nucleus: Even in nuclei in the brain stem, no prominent change was
noted in their nerve cells, there being only a slight growth of nuclei in a
part of the nerve cells.

④ Auditory nerve: Localized or glandular changes were seen in various
areas of the medullated nerve in all cases, and achromatism in the medullary
sheath, which showed an enlargement. Changes were more prominent towards the
periphery. Schwann cells showed a slight reaction, some reflecting a trend
toward increase. These changes were relatively great in the order of Nos. 3
and 1, but relatively slight in Nos. 2 and 4. As seen in No. 2, these
changes were slight in the region having nerve fibers in the center, but
suddenly became greater toward the periphery (see Photos 6 and 7).

⑤ Cervical spinal cord: In No. 3, changes were seen in the lateral
fiber, achromatism and irregular shapes in the medullary sheath, as well as
changes in the axons, accompanied by an increase of glia. In the cerebellum
of this case, localized, inflammatory cell infiltration was noted in one
region, but this was not believed to be due to poisoning.

239

⑥ Posterior root of spine: Here, changes have appeared in a part
(1/4 - 1/5) of nerve fibers, with disintegration of the medullary sheath and
enlargement of axons being noted. An increase of Schwann cells is also noted
(see Photo 8). The anterior root nerve is systematic; a slight destruction
of the medullary sheath due to changes in the nerve fiber was noted, but there
was no increase of Schwann cells (see Photo 9). This change was common in
all cases.

3) Comment

The pathological changes in the nervous system in our experimental
cases, such as disorders of the cerebral cortical cells, partial fallout of
granular cells in the cerebellum, Purkinje cell disorders, and peripheral
nerve changes, are all in accord with the findings of Takeuchi [5] and Takaya
[6], and attest to their causing methyl mercury poisoning.

Nerve cell disorders of the cerebral cortex have extended to the entire
cerebral cortex, and to the auditory center cortex. However, no noticeable
pathological changes were noted in the nerve fibers of the cerebrum and the
brain stem. Furthermore, almost no changes were noted in auditory nerve cells
in the central nervous system. However, in the peripheral nerve, there were
ganglionic or localized changes extending to nerve endings, accompanied by an
enlargement of the area. This change distinctly became greater toward the
periphery.

In our experiment, ganglion spirale cochleae and Corti's organ in the
inner ear were not studied. In a separate study, however, Ochiai studied
acute period pathological changes in methyl mercury poisoning cases, and dis-
covered that during the acute period, no great changes were induced as re-
gards ganglion cells of the ganglion spirale, and that no great changes were
equally induced in such Corti's organs as Zona pectinata, Zona arcuata,
Deiter cells, capillary cells, and Membrana tectoria. Thus, he found that
they maintained their own mutual structure and did not accept changes within
the cells. He claims, however, that chronic cases will require further study.

Thus, from the foregoing it can be seen that, in methyl mercury poisoning, cerebral, cortical cells sustain relatively greater damage than the nucleus or nerve cells, with respect to the central nervous system, and that changes in the nerve fibers tend to become greater toward the periphery, and that no pathological changes occur in the inner ear at the time of acute affliction. It may be more appropriate to consider electro-physical findings from the standpoint of changes in the peripheral region of the auditory nerve, rather than primary obstructions of nerve endings of capillary cells. No definite conclusion is possible, however, because of the small number of cases studied in our experiment and inadequacy of study of the inner ear. Further studies are required.

Conclusion

1) Changes of CM and AP were observed, using 6 ears of four cats on which chronic electrodes were attached and methyl mercury administered.

2) Under 90 dB (HL) of pure tone stimulation, the lowering of CM potential, suspected to be due to the effects of methyl mercury, was seen in two ears of one cat.

3) Elevation of VDL was seen in only one ear.

4) AP amplitude was not noted in cases with no CM decline.

5) The prolongation of AP latency was observed in all cases. It was assumed to be the beginning of disorders in the capillary cell nerve endings or in the periphery of the auditory nerve.

6) Pathologically speaking, unusual changes brought about by Minamata disease in the cerebrum, cerebellum, and peripheral nerve were confirmed. In the field of otorhinology, acute changes and patchy fallout were seen in cerebral, cortical nerve cells, and, in the auditory nerve, partial or ganglionic changes were seen in nerve fibers.

241

7. 3) <u>Pathological and Histological Changes in the Hearing Organ of Experimental Cats Poisoned by Organic Mercury</u>

Otolaryngology Seminar, Department of Medicine,
Kumamoto University

Team member: Yasuji Nozaka
Research assistant: Yoichiro Ochiai

In Minamata disease-related ear impairments, disorders are considered to be primarily of the rear labyrinth type, but on the basis of hearing tests, inner ear obstructions cannot be completely ruled out. Therefore, methyl mercury was injected into the abdominal cavity of fully grown cats to produce experimental organic mercury poisoning. Biological fixation was then performed and studies conducted on histological changes in the hearing organ. The results of the experiment are discussed below.

Experimental Method

Fully grown cats were used for the experiment. Methyl mercury iodide CH_3Hg I was added to 1 ml of $CH_3CH(OH)CH_2OH$ so as to attain a mercury content Hg 2.0 mg. The solution was administered daily into the abdominal cavity of the animal until there was a great loss of control of movement, at which time it was biologically fixed. The Hg administration rate in this experiment aggregated 30 - 72 mg. The period from the time of administration to the outbreak of the disease was from about 20 to 40 days, with apparent symptoms of acute poisoning. Tests were conducted on 12 ears of 6 cats. For control, 4 ears of 2 cats were utilized to effect comparison.

Biological_fixation_method

An incision was made on the cervical region of the cat, secured on its back, and its cervical veins and the carotid on either side exposed. Two

243

hypodermic needles attached to the end of a branched, rubber washing tube connected to an irrigator filled with biological saline solution were stuck into each side of the carotid, and the artery and the hypodermic needles were ligated firmly. Cervical veins on either side were severed to make an opening for the flow of the washing solution. Washing was continued until the washing solution flowing from the cervical vein was completely clear, without any reddish color (about 1000 ml of biological saline solution). Next, the fixing solution was administered. Initially, the fixing solution was added over the remaining biological saline solution, in an amount equal to the latter, and gradually replenished. The Wittmaack fixing solution was used.

Wittmack fixing solution:

5% heavy chromic acid, potash solution	83 ml
Japan Pharmaceutical Standard formalin	10 ml
Japan Pharmaceutical Standard glacial acetic acid	3 ml.

Following fixation, the auditory organ was removed, taking care to obtain a small specimen to include the auditory organ. Then, the middle ear cavity was opened, followed by post-fixation, ash removal, washing and drying. Soft tissues and unneeded bone fragments were removed as much as possible to obtain a small specimen. Tsueroijin [sic] covering was performed. The fragment was stained with haematoxylin-eosin, and pathological, histological studies were conducted with an optical microscope.

Findings

The Corti's organ, vascular system, and spiral nerve ganglia cells were studied and compared with those of normal cats.

1. Corti's organ

Both outer and inner capillary cells retained their shapes fairly well, with no signs of obliteration of the nucleus, or enlargement, swelling, or

244

fusion of cytoplasm. Almost no changes were noted in supporting cells, such as Deiter's cells or Claudius' cells (Photos 2 and 2').

2. Vascular system

Cell alignment in the surface layer was almost normal, with no indication of attrition of cytoplasm, obliteration of nucleus, or change (Photos 3 and 3').

3. Spiral nerve ganglia cells

Spiral nerve ganglia cells assisted in maintaining the normal pattern of the cells' internal structure, and virtually no changes, such as nucleus contraction, enlargement, or disintegration, were seen (Photos 4, 4').

Conclusion and comments

In this experiment, we produced experimental organic mercury poisoning in fully grown cats, or so-called acute poisoning, by administering 30 - 70 mg mercury for a period of from 20 - 40 days. We then performed biological fixation and observed changes in Corti's organs, vascular system, and spiral nerve ganglia cells. As anticipated, our examination under the optical micro-scope did not reveal any great change, and did not differ from those of con-trol cats. Such drugs as quinine, salicylic acid, alcohol, nicotine, arsenic agents, lead, carbon monoxide, and dihydrostreptomycin are known to cause toxic inner ear disorders. These, which all have an affinity with the audi-tory organ, caused changes of various degrees in Corti's organ, especially in the outer and inner capillary cells, spiral nerve ganglia, and the vascular system. However, Mioki of our classroom claimed that in the case of organic mercury, he found virtually no transfer of the aggregate mercury to the inner ear. Even though mercury may not directly impair the inner ear as in Minamata disease attributed to chronic organic mercury poisoning, it can be assumed that some secondary changes occur in the inner ear. Based on our experiment, we can say that in the case of acute poisoning from organic

245

Normal cat   Cat administered organic mercury
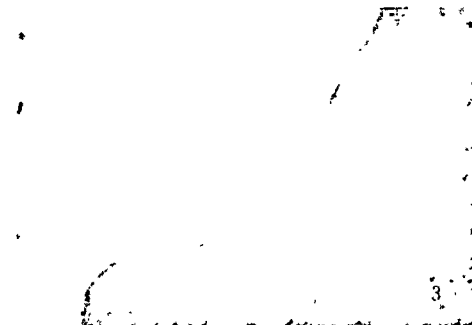
mercury, no pathological or histological changes (under an optical microscope) were noted in the inner ear as a result of organic mercury.

REFERENCES

1.   Nozaka, Yasuji. Minamata Disease — Study on Organic Mercury Poisoning. Kumamoto, 1966, pp. 139-148.

2.   Nozaka, Yasuji, et al. Effects of Auditory Power, Vestibular Functions, Sense of Taste and Speech Impairments in Minamata Disease. Japan Otorhinology, Vol. 73, 1970, pp. 1006-1007.

3.   Cho, Hatsuo. Hearing Power and Vestibular Equilibrium Function Impairment in Organic Mercury Poisoning. Otolaryngology, Vol. 42, 1970, pp. 165-170.

4.   Fujisaki, Ryuzo, et al. Auditory Impairment in Organic Mercury Poisoning. Audiology, Vol. 14, 1971, p. 484.

5.   Takeuchi, Tadao. Pathology of Minamata Disease — Study on Organic Mercury Poisoning. Kumamoto, 1966, pp. 264-265.

6.   Sakurai, Tokio. Study of Auditory Nerve Activity Potential Induced by Round Window and Summating Potential. Japan Otorhinology, Vol. 64, 1959, pp. 1766-1784.

7.   Wever, E. G., et al. Physiological Acoustics. 1954, p. 305.

8.   Murai, Kazuo. Study of Auditory Nerve Activity Potential of Domestic Rabbits. Japan Otorhinology, Vol. 72, 1969, pp. 1129-1139.

9.   Galambos, R. and A. Rupert. Action of the Middle Ear Muscles in Normal Cats. J. Acoust. Soc. Amer, Vol. 31, 1959, pp. 349-355.

10.   Simmons, F. B. and D. L. Beatty. The Significance of Roundwindow-Recorded Cochlear Potentials in Hearing. Ann. Otol., Vol. 71, 1962, pp. 767-800.

11.   Simmons, F. B., R. Galambos, et al. Serial Studies of the Onset and Progressions of Drug-Induced Cochlear Damage in Cats. Archives of Otolaryngology, Vol. 72, 1969, pp. 233-239.

12.   Ouchi, Hitoshi, et al. Study of Permanent Electrode Method in Cochlear Reaction. Japan Otorhinology, Vol. 67, 1964, pp. 426-427.

13. Shiraiwa, Hosei. Study of Permanent Electrode Method in Domestic Rabbits. Japan Otorhinology, Vol, 69, 1966, pp. 631-660.

14. Ogawa, Etsuji. Experimental Study of Attack of Dihydrostreptomyocin on Auditory Organ. Japan Otorhinology, Vol. 71, 1968, pp. 217-232.

15. Otani, Iwao. Acute Experimental Study on Auditory Organ Toxicity of Kanamycin. Japan Otorhinology, Vol. 71, 1968, pp. 688-707.

16. Takaya, Gochu. Experimental, Pathological Study on Genkai [sic] in Minamata Disease, Especially the Effect of Methyl Mercury Compounds on Cats. Kumamoto Medical Association Journal, Vol. 38, 1964, pp. 100-139.

17. Ushikusa, Sadao. Experimental Supplement on Oral Administration of Methyl Mercury Iodide to Cats. Kumamoto Medical Association Journal, Vol. 39, 1965, pp.33-39.

18. Grizzle, C. and W. C. Cutting. Vestibular Apparatus Intoxication of Experimental Animals with Streptomycin and Dihydrostreptomycin and Mixtures. Acta Oto-Laryng., Vol. 43, 1953, pp. 421-427.

19. Henriksson, N. G. et al. The Caloric Test in the Cat. Acta Otolaryng., Vol. 53, 1960, pp. 21-32.

20. Simmons, F. B. Middle Ear Muscle Activity at Moderate Sound Levels. Annals of Otology, Rhinology and Laryngology, Vol. 68, 1959, pp. 1126-1143.

21. Coats, A. S. Depression of the Click Action Potential by Attenuation, Cooling and Masking. Acta Otolaryng, Suppl. 284.

22. Deatherage, B. G. and D. H. Eldredge. Latency of Action Potentials in the Cochlea of the Guinea Pig. J. Acoust. Soc. Amer., Vol. 31, 1959, pp. 479-486.

248

8. STUDY ON PREVENTION AND TREATMENT OF MINAMATA DISEASE AND METHYL MERCURY POISONING

Biochemistry Faculty, Toxic Research Facility,
Department of Medicine, Kumamoto University

Team member: Hitoshi Takahashi
Research assistants: Kimiko Hirayama, Etsuko Hirata
Kenichi Enami

Purpose of Study

The purpose of this study, which will have a great effect on human health, is to seek biochemical and pharmacological supplemental methods of examination of socially-feared organic mercury, especially methyl mercury, with respect to its presence in the body, as well as its level; also, to discover safe and effective methods of treatment in the event of possible deposit in or sudden internal contamination of the body. Another purpose is to further the knowledge concerning metabolism of methyl mercury in the body, necessary to formulate treatment guidelines.

Summary of This Year's Report

As several items were clarified following publication of last year's report, we shall first follow up on our observations of metabolism of methyl mercury in the body, and on drugs for treatment. We shall then report on the results of experiment as an approach to clinical applications, utilizing drugs in examination and treatment.

Results of study and findings

1. Subsequent findings on metabolism of methyl
    mercury in the body

Many kinds of methyl mercury derivatives have been used in experiments,
and there exist equally various forms of methyl mercury in industrial
wastes. There are few or no research reports dealing with the possibility
of their being taken into the bodies of mammals, including humans. Speci-
fically, how and in what chemical form do they exist in blood, other body
fluids, tissue cells, bile, and urine, in a biological environment consisting
of a pool of numerous substances with pH of about 6 - 8? Reports are avail-
able concerning changes in distribution in animal bodies, based on utiliza-
tion of radioactive methyl mercury iodide $(CH_3-^{203}Hg-Cl)$, and many quantitative
experiments in which methyl mercury adhering to tissue protein is dissolved
and removed with such acids as hydrochloric acid are being performed. In
time, a change occurs in the distribution of this methyl mercury in the body,
with mercury undergoing a gradual decrease in the liver and kidney after
concentration in those areas. Conversely, methyl mercury content in the
central nervous system begins to rise to a peak several days later. Con-
sidering that the discovery of the form of methyl mercury moving in blood
plasma during this period would be necessary for prevention of its movement
in the brain, we studied the form of low molecular methyl mercury found in
small quantities in the blood serum. Also, it was necessary to learn
whether such mercury compounds can pass into urine and bile, from the
standpoint of treatment.

o On absorption

It was subsequently found that some methyl mercury contained in
fish flesh is dissolved by hydrochloric acid of stomach fluids and becomes
methyl mercury iodide, but, as in the case of ingestion of methyl mercury
iodide solution, it is not readily absorbed in that form, remaining for a
while on the epithelium of the digestive tract. But, as proteins are
digested and converted into amino acid, its cystine combines readily with
methyl mercury, and becomes methyl mercury cystine at a pH on the alkaline

250

side in the intestines; also, methyl mercury which had been in stagnation after merging with proteins in epithelial cells of the digestive tract is freed by cystine in the process of absorption as methyl mercury cystine, and by riding on the activity transport of amino acid is effectively and rapidly absorbed. As in the case of amino acid, the principal path of absorption to the liver is past the portal vein. Since methyl mercury iodide is rather insoluble in water, but soluble in fat, it was possible, when administered with fat, to gain entry together with chyle into the lymphatic stream and enter the entire blood stream by way of the thoracic duct. The following experiment was, therefore, conducted.

An operation was performed on a rat about 300 g in weight, at which time a small vinyl tube was attached to the thoracic duct. Following the operation, the rat was placed on a fixed platform for one day. The next day, at the moment when the chyle disappeared because of blood, an aqueous solution containing 1.25 mg (1.0 mg as Hg) of methyl mercury iodide — or the same solution but with a small quantity of salad oil added — was orally administered with a catheter. Subsequently, lymphs, up to a six-hour period, were collected and measured for their mercury content. It was found that the average content in the group of three mice administered an aqueous solution was about 1.1 µg, and in the group with an equal number of mice administered with a salad oil mixture, 0.9 µg, or only 0.1% of the amount of mercury administered, showing no difference between the two groups. At that time, the average amount of mercury in the liver was 48 µg and 55 µg, respectively, or an equivalent of 5%, a large amount of methyl mercury yet remaining in the digestive tract.

The above established that methyl mercury was absorbed by way of the portal vein system, and rejected the possibility of highly fat soluble methyl mercury iodide entering the lymphatic stream along with absorption of fat. Probably, when methyl mercury iodide was administered, it (methyl mercury iodide) became stagnated in epithelial cells of the digestive tract, and it was freed by low molecular SH compounds derived from subsequently absorbed cystine or cystine from the blood stream, entering the portal vein as a

251

component of such compounds. We previously reported about methyl mercury cystine passing through the digestive duct walls and being absorbed at about 10 times the speed of methyl mercury iodide. This type is not only absorbed, but can pass through the blood-brain barrier.

② Migration in the brain

Our experiment was conducted on the belief that the principal factor in cerebral migration of methyl mercury was in the form of methyl mercury cystine; that methyl mercury iodide used in the experiment was also ingested by some cell and combined with SH of its protein, and then freed later by cystine, by combining with the latter to enter the blood plasma and pass through the cerebral blood gateway, after which they were drawn into cerebral, parenchymal cells. It was necessary for us to repeat various indirect experiments because of the difficulty of establishing the foregoing, although it would have been simple if a perfusion test of the brain in the absence of blood corpuscles could have been performed.

We administered 2.5 mg (2.0 mg in Hg) of methyl mercury iodide dissolved in 1 ml of biological saline solution, or an equal amount of the former mixed with surplus cystine fluid and modified completely into a compound, at a specific speed into the tail vein of male Wistar strain rats weighing about 200 g. A comparison was then made of mercury compounds by studying the amount of methyl mercury in the brain over a given time (Figure 1) and in the liver (Figure 2). It was found that there was almost no rise in the mercury level in the brain after a lapse of 30 - 60 minutes following injection in the group administered methyl mercury iodide, but that a distinct rise occurred in the group administered cystine compounds. This was true also for the liver. Subsequently, however, both groups showed a similar rising trend of the methyl mercury level in the brain and a lowering trend in the liver. This was believed due to the fact that methyl mercury iodide which had infiltrated the liver and other organs was, because of the cystine

pool in the body, being guided by methyl mercury cystine and later behaved in a completely similar manner. When discussing the infiltration of these compounds in the brain through such an injection method, it would be best to rely on results obtained within 60 minutes after administration to preclude any chance of compounds undergoing changes.
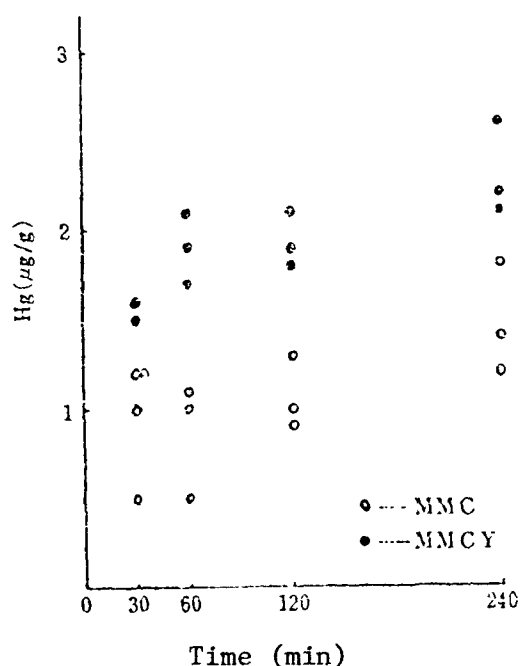
Figure 1. Changes over time of mercury level in brain following intravenous injection of methyl mercury compounds:

MMC (methyl mercury iodide) and MMCY (mixture of methyl mercury cystine and 9 times mole cystine) were intravenously administered (2.0 mg mercury). Subsequently, the cerebral mercury level, 30, 60, 120, and 240 minutes after injection was studied for each group of 3 mice at the respective time
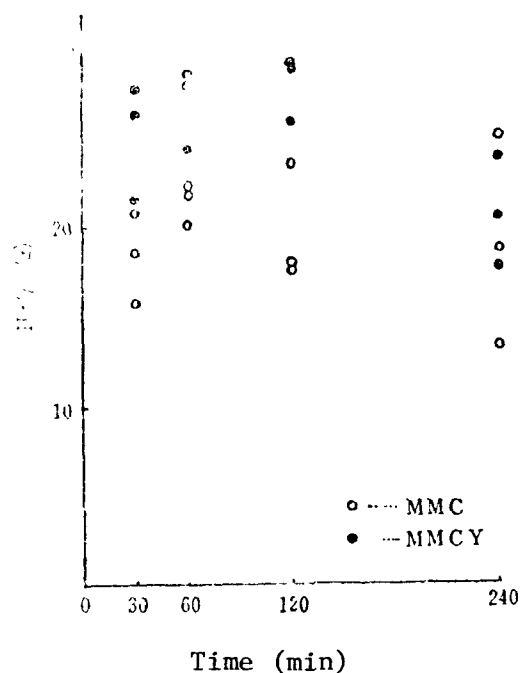
Figure 2. Changes over time in the mercury level in the liver after intravenous injection of methyl mercury compounds:

MMC (methyl mercury iodide) and MMCY (mixture of methyl mercury cystine and 9 times mole cystine) were intravenously injected (2.0 mg mercury). The mercury level in the liver at various times following administration is indicated. Three mice were examined at any one time

Figure 3 reflects the result of a similar experiment, the mercury level being studied after a 60-minute period following administration. In the brain, the methyl mercury iodide-administered group reflected a value close

253
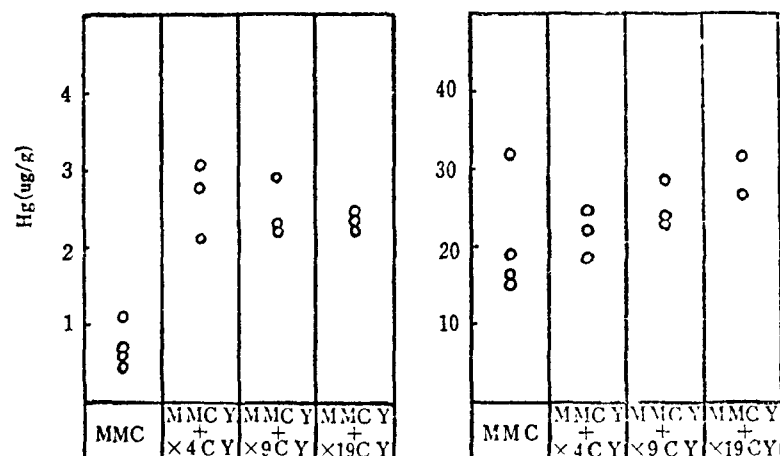
Figure 3. Mercury level in brain and liver at
time of density change of cystine:

MMC (methyl mercury iodide), MMCY (methyl mer-
cury cystine) and cystine of various density
(2.0 mg Hg) were administered intravenously
to rats. Mercury level in the brain and liver
one hour after injection is indicated

to that of the untreated brain; the cystine compound-administered group
showed a high intake level, irrespective of the amount of surplus cystine
administered. The cystine compound intake was also notable in the liver.

It was once considered that, because of solubility in fat, methyl mer-
cury iodide would more readily infiltrate into a brain which has fatty mate-
rial. However, water soluble methyl mercury cystine was able to infiltrate
more easily. This could not be explained in terms of fat solubility.

Figure 4 reflects the results of an experiment wherein three compounds
— i.e., synthesized, unadulterated methyl mercury iodide, methyl mercury
cystine, and methyl mercury acetylcystine — were added to blood, and their
distribution into blood corpuscles and blood plasma was determined over a

254
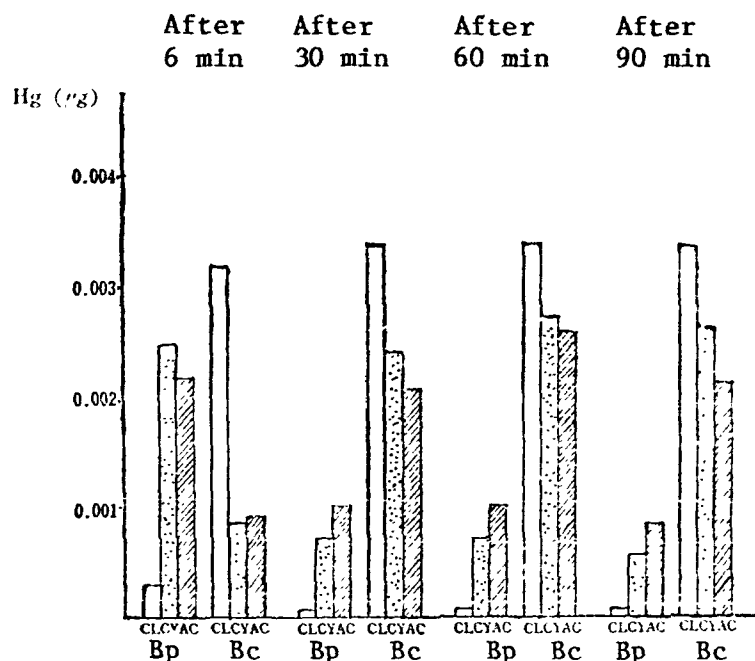
Figure 4.   Distribution of methyl mercury deriva-
tives in blood:

0.5 μ mole each of methyl mercury chloride, methyl
mercury cystine, and methyl mercury acetylcystine
was added to 3 ml human blood and mixed well. Its
distribution in blood corpuscles and blood plasma
at the end of 6, 30, 60, and 90 minutes is shown
(6N HCl was added to 200 μℓ of sample, extracted
with 1 ml of benzene and 4 μℓ subjected to gas
chromatography)

Bp —— in blood plasma; Bc ——— in blood corpuscles

given time. The latter two compounds reflected almost the same distribution
rate, whereas methyl mercury iodide immediately entered into the blood cor-
puscles. In order to enter the brain, it would be necessary for it to assume
another shape and be released from inside the corpuscles to blood plasma.
The cystine pool in the body is believed to become involved here.

As an experiment in support of our belief, 10 male mice of the Wistar
strain, weighing about 200 g, were divided into two groups. One group was
fed synthetic feed containing relatively little cystine, and the other group ——
the same feed but containing 0.5% cystine. From the sixth day onward for a
period of five days, 1 mg methyl mercury iodide was dissolved in biological

255

saline solution and administered daily by muscular injection to each group, for a total of 5 mg. On the twelfth day of the experiemnt, they were decapitated, blood drained, and the mercury level in the brain and liver was measured. It was determined that in the group which did not ingest cystine there was little migration of methyl mercury from the liver and, consequently, a low mercury level in the brain (Figure 5).
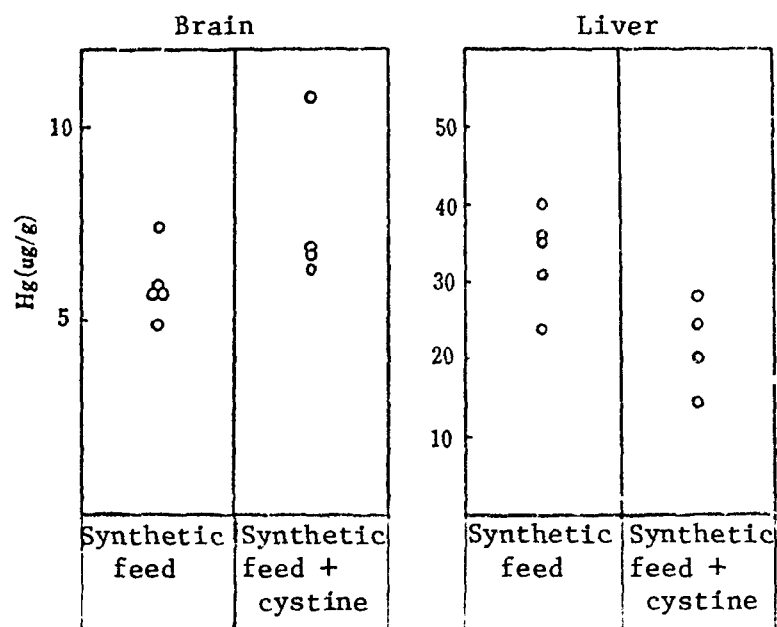
Figure 5. Effects of low cystine feed and cystine-supplemented feed on mercury level in visceral organs:

Mercury level in brain and liver of rats. Rats were administered synthetic feed containing a small amount of cystine and also synthetic feed containing a greater amount for a period of 10 days. During the last 5 days, they were also administered 1 mg methyl mercury iodide, or a total or 5 mg, by muscular injection, and decapitated and drained of blood 48 hours after the last injection

The intake difference will probably become larger if the animal can be made to survive even under the most severe conditions lacking cystine. The use of halogenized benzene to cause dissipation and deficiency of cystine as mercaptan acid weakens the animal excessively, and for that reason has not yet been successful.

256

⑤ Discharge into the bile

When methyl mercury was administered by muscular injection to animals, a considerable amount of methyl mercury appeared in the bile for a while thereafter. Figure 6 reflects the methyl mercury level in the bile of a rat during a two-hour period of time. The rat involved, about 250 g in weight, was administered through muscular injection 1 mg of methyl mercury iodide daily in biological saline solution, or a total of 5 mg in a five-day period, with methyl mercury being drawn from the rat on the 12th, 24th, 36th, and 48th days. Even at the end of 48 days, discharge continued. A considerable amount was discharged in the bile daily. Our calculations may appear to be off, due to such a constant discharge. However, as described in our next experiment, this is because this form of methyl mercury is mostly in the form of readily re-absorbable methyl mercury cystine, which circulates in the liver and intestines.

Figure 8 reveals the results of an analysis of a portion of bile derived on the 12th day under the improved analytical method (Figure 7) for methyl mercury compounds, integrating isolation by negative ion exchange, as explained in our previous report, and quantitative determination method by gas-chromatography. As can be seen, much of the methyl mercury is in the form of methyl mercury cystine, with some methyl mercury acetylcystine present. Under this improved method, it is possible to concentrate mercury compounds in the fluids without destruction. Therefore, thin layer chromatography was conducted on a specimen, and we were able to establish by comparison with synthetic products that they were the actual compounds. We were unable to discover at this point any trace of methyl mercury iodide used in injection in the bile. It is possible to conclude that this effective circulation in the liver and intestines of methyl mercury cystine is a key factor in continued mercury residues in the animal body. This knowledge should help when examining methyl mercury found in the bile, or in treatment by preventing circulation in the liver and intestines in a manner to be described later, and effecting elimination through excrement by connection to that part of the organ which does not absorb mercury.

257
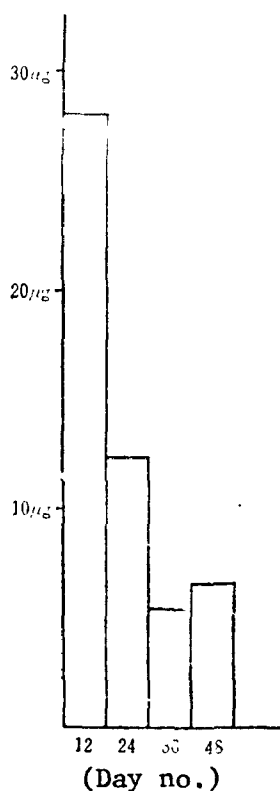
Figure 6. Quantitative changes over time of methyl mercury compounds in bile of rats administered methyl mercury:

Methyl mercury level excreted within 2 hours after collection of bile on 12th, 24th, 36th, and 48th days from rats administered, by muscular injection, 1 mg/day of methyl mercury iodide over a 5-day period (as methyl mercury iodide)

Figure 7. Chromatography showing separation of pure methyl mercury compounds:

Extraction using Dowex-1-Cl-resin (1.2 x 18 cm) and gradual increase of NaCl (0.05M phosphoric acid buffer solution pH 7.6) of methyl mercury iodide, methyl mercury cystine, and methyl mercury acetylcystine (50 μ moles each); hydrochloric acid added to each extracted solution with extraction by 2 ml benzene; 1 μℓ subjected to gas-chromatography

Ⓔ Discharge into urine

It was previously reported, and many people are aware, that excretion of methyl mercury in urine is extremely serious. It is possible that at the time of its excretion there are, aside from the importance of low molecular substances in blood plasma, a minute amount of methyl mercury in blood plasma, which probably is in the form of methyl mercury cystine carried to the brain. Methyl mercury on this amino acid is apparently highly re-absorbable, for intravenous injection of synthetic methyl mercury swiftly into a rat did not result in its elimination in the urine.
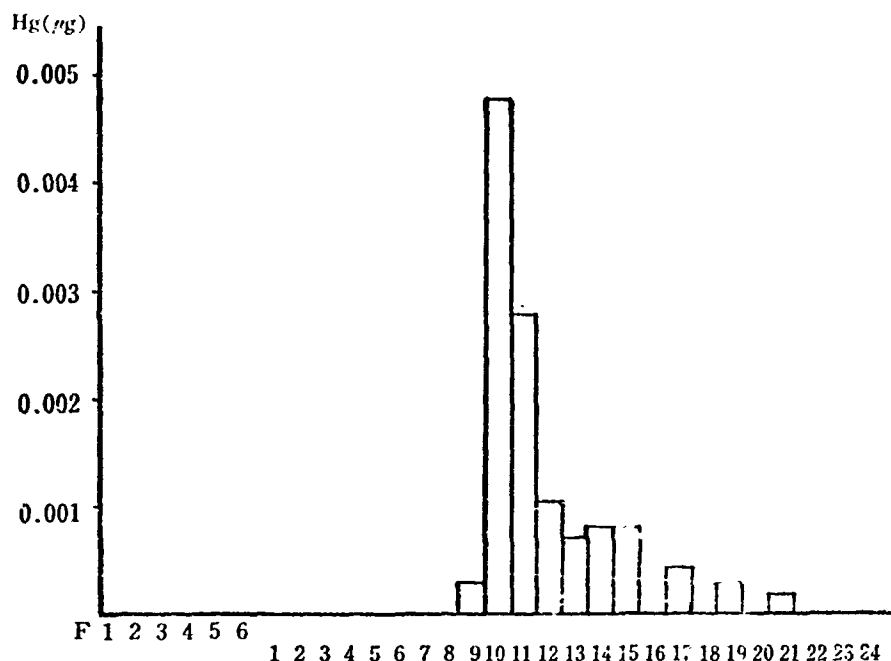
Figure 8. Chromatography of isolation of bile:

Bile of rat administered 1 mg methyl mercury idodide
for 5 days was isolated according to the method
outline in Figure 7

Figure 9 reflects the findings obtained as a result of surgical placement
of a catheter in the peripheral ureter of a male rat about 400 g in weight
for taking urine specimens at a specified time. Five μ moles methyl mercury
iodide, methyl mercury cystine, or methyl mercury acetylcystine, together
with phenolsulfophthalein (PSP), were injected into tail veins to observe
the secretion of chromosomes and methyl mercury. No excretion was observed
in the form of the first two, but a considerable amount of methyl mercury
acetylcystine was excreted during a short 20-minute period. In methyl mercury
adhered to amino acid, the amino group clogged with acetyl group showed poor
re-absorption capability in the kidney, accounting for the good "clearance"
into urine.

Compounds mixed with this acetylcystine are generally known as mercaptan
acid. It is a well known fact that when animals are administered halogenized
benzene, the mercaptan acid-containing body purified in the liver is secreted
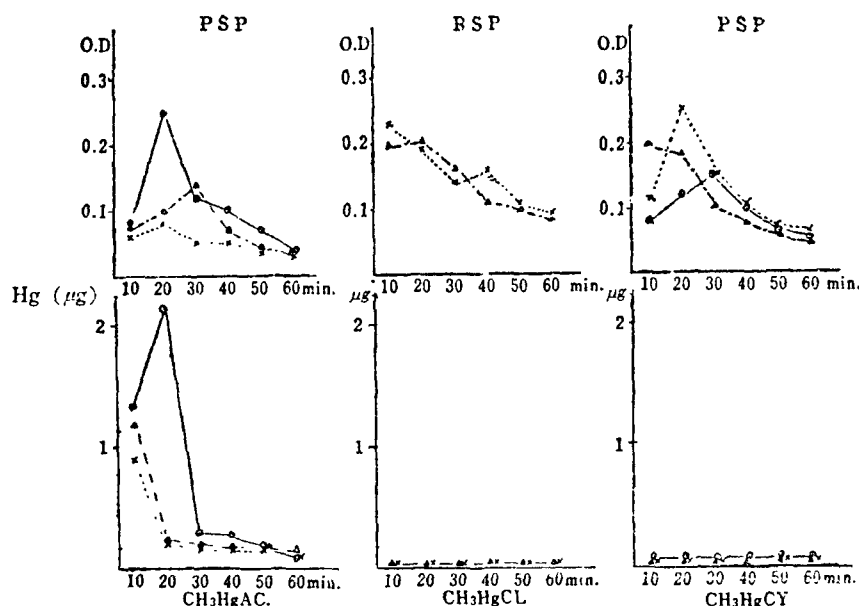
Figure 9. Excretion from kidney:

Amount of methyl mercury compounds excreted from peripheral ureter of a rat about 400 g in weight was observed when intravenously administered with 5 μ moles methyl mercury iodide, methyl mercury cystine, or methyl acetylcystine. PSP had been added to methyl mercury compounds

in the urine. On the belief that such a purification function may occur even with respect to methyl mercury, we tested for methyl mercury acetylcystine as a metabolic product, and finally succeeded in discovering it, even though in a small quantity, in the urine of methyl mercury poisoned rats. Figure 10 shows the results of an experiment in which rats which had been administered by muscular injection 1 mg per day of methyl mercury iodide solution for five days were also administered, every other day, 12.1 mg (100 μ moles)/2 ml biological saline solution, 16.3 g (100 μ moles)/2 ml acetylcystine. Urine excreted during the subsequent 24-hour period was then examined according to the method outlined in Figure 7. In all cases, peaks denoting methyl mercury acetylcystine (can also be termed methyl mercury mercaptan acid) were observed, which was not unusual in the case of acetylcystine-administered animals. As such peaks were found also in other animals, we prepared other similarly

260

poisoned rats and collected
and condensed their urine
by chromatogic separation,
and established the pre-
sence of methyl mercury ace-
tylcystine when it matched
with the synthetic sample
under the secondary devel-
opment process of thin layer
chromatography. It was
possible to find such meta-
bolic products, although in
a small quantity because of
their characteristics
readily appearing in urine.

In the results of the
experiment reflected in
Figure 11, the aim was to
cause, through administra-
tion of acetylcystine, ex-
cretion of methyl mercury
in the form of mercaptan
acid, which is readily dis-
charged by urine. We ad-
ministered 16.3 mg/2 ml
acetylcystine mixed with PSP

Figure 10. Analysis of urine following
the administration of table salt (top),
cystine (middle) and acetylcystine
(bottom) to methyl mercury poisoned
rats   rats

intravenously to two groups of rats: one group —— 10 days after muscular in-
jection of a total of 5 mg of methyl mercury iodide, and the other group ——
one hour after having been injected 2.5 mg/2 ml of methyl mercury iodide. A
rise in the excretion of chromosomes and methyl mercury was noted. Additional
efforts are thus being exerted, so that the characteristics of this substance
can benefit diagnosis and treatment.

Figure 11. Clearance from the kidney:

Left chart is an analysis of urine extracted from the bladder of a 250 g rat on the tenth day following muscular injection of 1 mg methyl mercury iodide for 5 days

Right chart reflects the results of intravenous injection of 16.3 mg acetyl cystine with PSP, an hour after injection of 10 μ moles of methyl mercury + PSP

2. Therapeutic agents

Excluding therapeutic agents now under experimentation, we turn our attention to Pyridoxine-4-thiol and Pyridoxine-5-thiol, which we believe are the closest to practical application. These two substances have a structure resembling vitamin B_6, and a SH radical easily bondable with methyl mercury.

We previously reported on their effect in lowering the mercury level in visceral organs (brain and liver) of methyl mercury poisoned rats through subcutaneous injection.

Figure 12 shows the results of oral administration of these two substances, and also the oxidized form of Pyridoxine-4-thiol, on a specific day
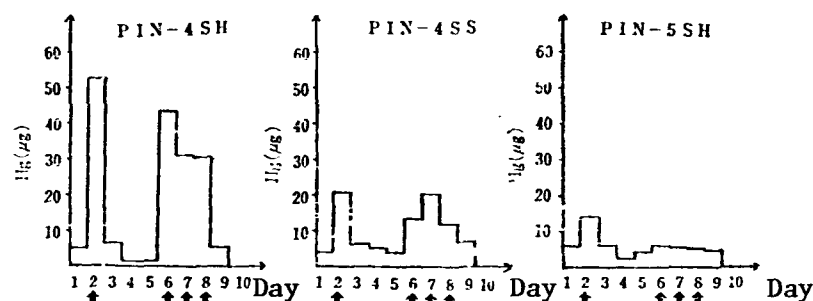
Figure 12. Level of methyl mercury excreted in urine after oral administration of various drugs:

Methyl mercury excreted in urine after oral administration of 22 mg Pyridoxine-4-thiol, oxidized form of Pyridoxine-4-thiol, or Pyridoxine-5-thiol on the 2nd, 6th, 7th, and 8th day following intravenous injection of 2.5 mg methyl mercury iodide (shown as mercury level)

during a 9-day period, when about 200 g rats were intravenously injected with 2.5 mg methyl mercury iodide and 22 mg Pyridoxine-4-thiol. The methyl mercury level in the urine was determined by gas-chromatography. Even in oral administration, there was sufficient inducement of methyl mercury toward urine, the greatest effectiveness being observed in the order of Pyridoxine-4-thiol > oxidized form of Pyridoxine-4-thiol > Pyridoxine-5-thiol. The effectiveness of this oxidized form suggests that it becomes a monomer, upon reduction in the body.

Figure 13 reflects the mercury induced in urine and excrement when, as in the case above, equal moles (22 mg) of Pyridoxine-4-thiol and -5-thiol were orally administered continuously for a period of 7 days to approximately 200 g rats which had been intravenously injected with 2.5 mg of methyl mercury iodide. Both caused inducement of mercury toward urine and excrement, with Pyridoxine-4-thiol showing the greatest effectiveness. As Pyridoxine-5-thiol becomes an energizer of cerebral metabolism known as Pyrithioxine (Figure 14) when oxidized in the body, its ability to remove mercury may be somewhat weaker, but may be highly effective from the symptomatic and functional aspects. We wish to utilize this if we can correctly determine the type of treatment required for poisoned animals, as explained later.

263
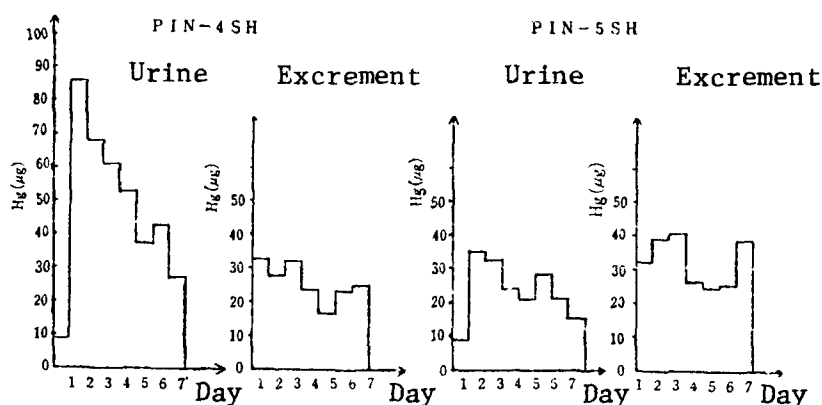
Figure 13. Mercury level in urine and excrement after continuous administration of drugs for 7 days:

Aggregate mercury level in urine and excrement upon continuous oral administration for 7 days of 22 mg Pyridoxine-4-thiol and Pyridoxine-5-thiol subsequent to intravenous injection of 2.5 mg methyl mercury iodide

② <u>Agents to capture methyl mercury of the type discharged by bile</u>
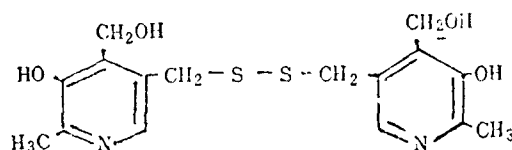
Figure 14. Structure of Pyrithioxine

It was previously stated that the reason for prolonged excretion of methyl mercury into the bile was circulation between the intestines and the liver. As a means to prevent re-absorption, substances with a capability to bond with methyl mercury and not prone to digestion and absorption are effective. With this in mind, the use of reduced pulverized human hair was beneficial. We were able to utilize SH synthetic resin, developed by Kokoku Jinken Co. Of the polyacrylic acid type substance, 1 gram of the product indicated an absorption capability of 277 mg methyl mercury iodide at pH 7.4.

Eleven male Wistar strain rats weighing about 220 g were intravenously injected in the tail veins with 2.5 mg methyl mercury iodide dissolved in 1 ml of biological saline solution, and put into cages. Four rats of the

264

control group were fed commer-
cially available pulverized
feed, and another group of 7
rats were given the same feed,
but with 1% of SH resin added,
for a period of 12 days. Dur-
ing this period, excrement and
urine were separately collected
daily, and their mercury level
determined. On the thirteenth
day, they were decapitated and
drained of blood, and the mer-
cury levels in the brain,
liver, and kidney measured.
Figure 15 is a graph of the mer-
cury level in the excrement of
the animals. The value of the
group administered SH resin
was 2 - 3 times more than in
the control animals. Figure 16
is a comparison of the mercury
level in visceral organs. In
the treated group, mercury in
the liver was significantly low
(P<0.005) ; no significant dif-
ference was noted between the
brain and the kidney.

Figure 15. Effect of SH resin in causing
excretion of mercury in excrement:

Level of mercury excreted in excrement of
group of rats administered feed contain-
ing 1% SH resin and by control group, sub-
sequent to intravenous injection of 2.5
mg methyl mercury iodide

In addition, 0.2 ml biologi-
cal saline solution in which methyl mercury iodide had been dissolved to make
a density of 1 mg/ml was administered in both thighs (total amount, 400 μg)
of each of 48 DD-strain mice weighing about 17 grams. Divided into two groups
of 24 mice each, one group was fed commercially available pulverized feed,

Liver   Kidney   Brain

Hg (μg/g)

10

5

40

20

4

2

Mean±Sd | 11.08±0.61 | 7.24±1.65 | 25.53±1.34 | 24 30±5 1 | 2 03·0.23 | 1.84±0 3‹

Treat. Contr.   Treat. Contr.   Treat. Contr.

Figure 16. Changes in mercury level in visceral organs
due to oral administration of SH resin:

Level of mercury in visceral organs of group of rats
provided with feed containing 1% SH resin for a period
of 12 days, and of the group supplied with ordinary
feed, subsequent to intravenous injection of 2.5 mg
methyl mercury iodide

and the other group was fed the same but containing 1% SH resin, for 20 days.
On the 21st day, they were decapitated and their blood drained. Aggregate
mercury level in the body was then determined. The average value and the
plus or minus difference was 94 ± 25 μg for the experimental group, and 108.±
27 μg for the control group. The significant difference was 0.1 > P > 0.05.
Prolonged administration of SH resin inhibits weight increase. Since a
greater effect can be anticipated by use of reduced, pulverized hair, we are
currently considering the use of another animal protein for the therapeutic
method.

3. Experimental administration of drug inducing
methyl mercury in urine, as a supplemental
examination

As Pyridoxine-4-thiol can strongly induce methyl mercury in the body
toward urine and also lower mercury level in visceral organs, we conducted

an experiment to determine whether it can be utilized as a supplemental examination method for detecting residual methyl mercury in the body.

We administered 2.5 mg methyl mercury dissolved in biological saline solution intravenously to rats weighing about 200 g, and two days later, varying amounts of Pyridoxine-4-thiol were administered orally or through abdominal cavity injection. When the mercury level induced toward urine was measured at the time of the above-described administration, the increase of mercury excretion was commensurate with the amount administered; also, a considerable amount of mercury was found even where only 2 mg/200 g had been administered. Where a large dosage was involved, oral administration was more beneficial in inducing mercury toward the urine, probably due to high blood density over a longer period. After one month, when the mercury level in the body greatly decreased (half-life is said to be 7 days), the animals were once again treated in the same way as before. It was found that a small amount cf mercury , commensurate with the dosage administered, was being excreted (Figure 17). Because of extreme sensitivity to methyl mercury in
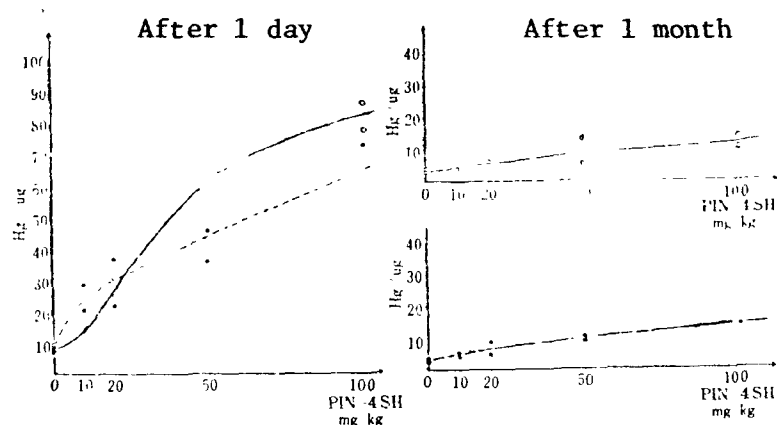
Figure 17. Relationship of amount of PIN-4-SH administered to amount of mercury excreted in urine:

Aggregate level of mercury excreted in urine upon oral administration and abdominal cavity administration ☉ of various Pyridoxine-4-thiol, one day after and one month after intravenous injection of 2.5 mg methyl mercury iodide

267

gas-chromatography, 1 μg of methyl mercury chloride was added to 100 ml urine; the benzene was extracted and transferred to glutathione solution. Finally, when extraction was achieved with 2 ml benzene, an approximately 70% recovery rate upon injection of 5 μl was sufficient to conduct measurement.

Hoping that this method might disclose any residual methyl mercury, if any, in cats inhabiting the Minamata district, we administered Pyridoxine-4-thiol (20 mg/kg) to three cats, and attempted to measure the mercury level in their urine, but not enough was excreted to permit a measurement. We are studying the toxicity of this drug in the hope that the drug can be used to treat those with possible methyl mercury contamination, such as tuna fishermen and others with Minamata disease symptoms. No strong toxicity, however, has been noted so far.

4. Effectiveness of therapeutic agents on methyl
    mercury poisoned animals

In order to determine the effectiveness of therapeutic agents in the elimination of mercury, animals having a specific amount of mercury in their bodies are required. We administered drugs to them, measured the amount of mercury excreted or in their visceral organs to determine the effectiveness. When an increased amount of mercury was administered, the animals exhibited loss of control of movement, particularly of their hind legs, loss of appetite, loss of weight, and went into a stationary state, with extreme irregularity in the mercury level in visceral organs. Determination of effectiveness of any therapeutic agent was thus made difficult. Yet there was great interest in whether any improvement could actually be effected in their ill conditions. Treatment was rendered to diseased rats, but most died during the early stages of treatment, without any indication of the effectiveness of treatment.

As a new method of treatment, we recently acquired an electronic scale to monitor and record the animals' actions. Thus, before the animals' conditions could deteriorate to the point where further treatment would be futile, unusual symptoms were detected and therapeutic drugs were administered.

268
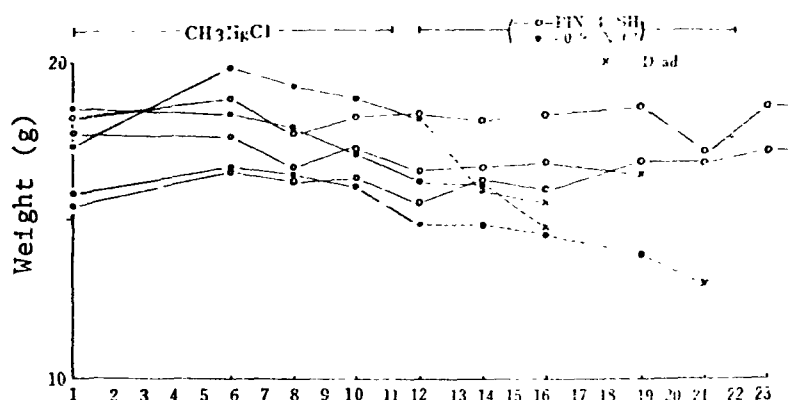
Figure 18. Changes in body weight of stricken mice
and day of death:

Methyl mercury iodide was administered for 10 days;
of the 7 mice which showed an abnormal condition when
observed with the electron scale, one group of 3
mice was treated with Pyridoxine-4-thiol, and the
other group of 3, with 0.9% saline solution, all
from the 12th day and in the abdominal cavity.

PIN-4-SH treated group — no great change in weight,
one death; 0.9% saline solution treated group —
great change in weight; all died

Each of the 10 male (DD strain) mice weighing about 20 g was supplied
with feed consisting of a mixture of methyl murcury and fish protein, remov-
ing any bitter taste. Pulverized feed was then added and solidified, and
300 µg methyl mercury iodide was put into 5 g feed, the daily portion for a
mouse. Body weight was taken every two or three days, and the animals'
actions monitored and recorded every 10 minutes during the morning with the
electronic scale. About seven mice lost some weight, and their actions slowed
down during the 10-day period. Thus, three mice each were selected from the
two groups of mice formed. One group was administered biological saline
solution, and the other group — the same, but with 3 mg of Pyridoxine-4-
thiol added. Injections were made into the animals' abdominal cavities for
a period of 10 days. Regular solid feed was supplied. Figure 18 shows the
impact of treatment on body weight and their lives; Figures 19 and 20 give a
record of findings using the electronic scale of mice which recovered after
treatment, and of mice which died without receiving any treatment. We

269
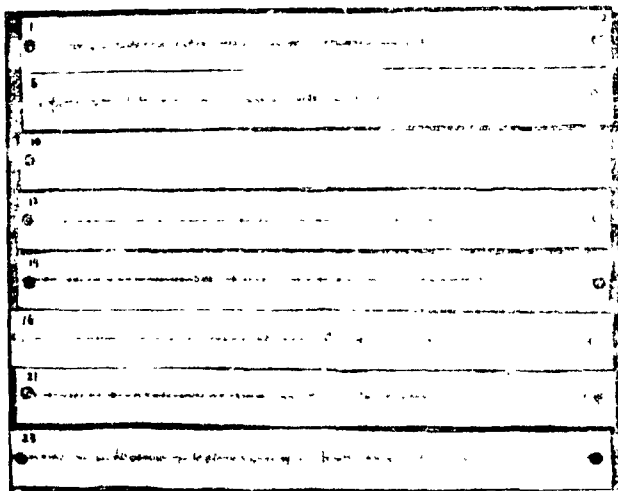
Figure 19. Activity of stricken mice as determined by electronic scale (PIN-4-SH treated cases):

1st day — normal movement (before administration of Hg) 6th day — normal movement (during administration of HG); 10th day — statical movement (Hg administration suspended the following day); 12th day — statical movement (administration of PIN-4-SH commenced); 21st day — recovery of movement (during PIN-4-SH administration); 23rd day — normal movement (PIN-4-SH administration suspended)
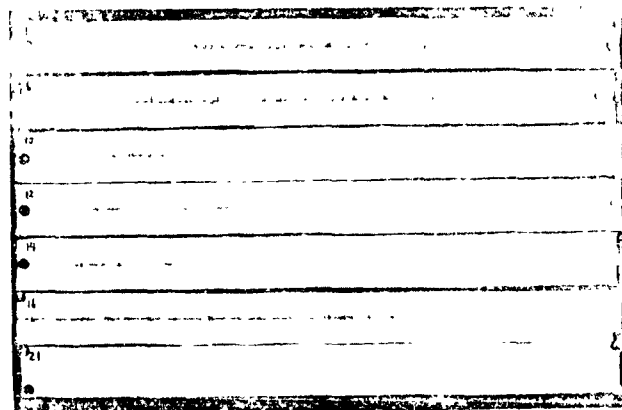
Figure 20. Activity of stricken mice as determined by electronic scale (0.9% saline solution administered cases):

1st day — normal movement (before Hg administration); 6th day (some statical movement (during administration of Hg); 10th day — statical movement (Hg administration suspended the following day); 12th day — statical movement (0.9% saline solution administration commenced) 16th day — some recovery of movement (during administration of saline solution); 21st day — statical movement (death on following day

realize the need for repeated experiments hereafter in order to come up with an effective method of treatment for methyl mercury poisoning.

Conclusion

As a result of this study, we were able to enhance our knowledge of biological metabolism of methyl mercury and apply it in formulating guidelines for treatment. We wish to continue our study on effective treatment, examination and preventive medicine to arrive at a general method of treatment. We wish lastly to acknowledge the receipt of scientific research assistance funds from Kumamoto Prefecture for this study.

APPENDIX

TRIAL APPLICATION OF COMPUTER CARDS FOR USE IN
EXAMINATIONS FOR MINAMATA DISEASE

Minamata Disease Research Team, Department of
Medicine, Kumamoto University

In order to promote efficiency in examination of Minamata disease, a trial card was completed after study by all departments concerned to provide selection of patients through the Tanac-type computer. By means of this card, it will be possible to automatically categorize cases into confirmed Minamata disease, possible Minamata disease, suspected Minamata disease, or doubtful Minamata disease. The card shown in Figure 1 was prepared on the basis of important elements of information furnished by various departments.

The upper six columns are for neuro-internal medicine affairs. Main elements of information are printed in a block, with space on its right being blank. This space is to be darkened in the event that positive findings are indicated. The Tanac-type computer will automatically read the shaded area and make a selection of cards containing the appropriate data. The lower columns are reserved for ophthalmological, otorhinological, and neurological matters.

About 2/5 of the space on the right is not linked to the computer selection, being used to enter such information as name, sex, date of birth, findings, etc.

Figure 2 is a sample card with entries on Minamata disease
(Tsutsui, Ophthalmology)

The assistance of Professors Araki of Kawasaki Medical College and Igata of Kagoshima University and Assistant Professor Okashima of Kumamoto University in programming this card is gratefully acknowledged.

271

Minamata disease examination card

Dept. of Medicine, Kumamota University

a b c d e f ** g ** h ** ** i i j k ** **
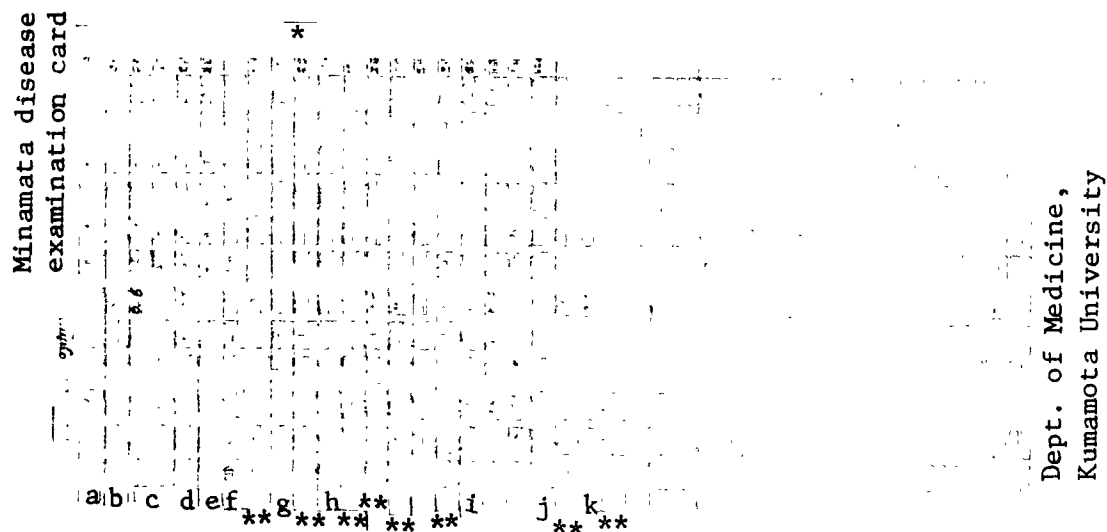
Figure 1. Minamata disease examination card:

a — visual constriction; b — visual sedimenta-
tion; c — eye movement; d — hearing difficulty;
(left); e — hearing difficulty (right); f — eye
disease; g — strabismus; h — bone; i — sense
of smell; j — intellect; k — mercury

*Translator's note. Column headings at the top
of the card are all illegible in the original
foreign text.

**Translator's note. Illegible in the original
foreign text.

Minamata disease examination card

Yamaotao

Minamata Disease

Dept. of Medicine, Kumamota Univ.
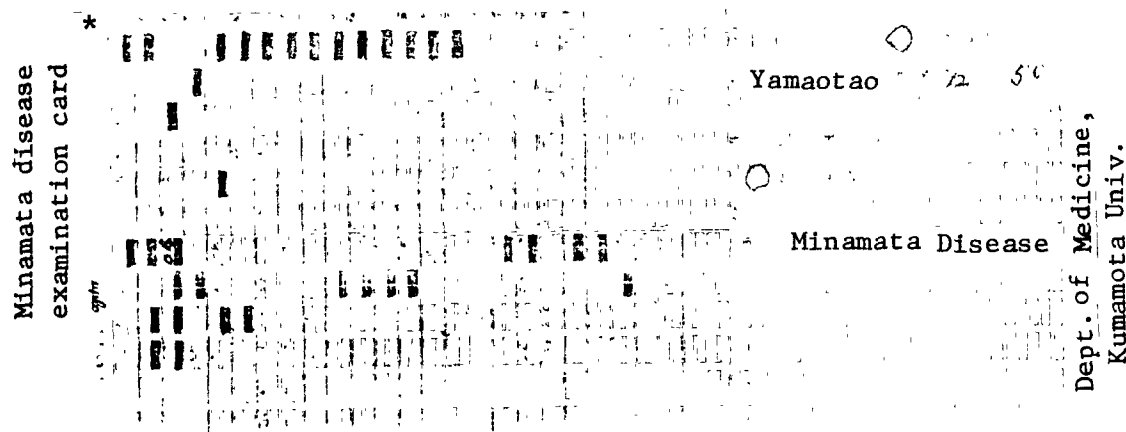
Figure 2. Minamata disease examination card
*Translators note. Illegible in the original
foreign text.

IV. GENERAL FINDINGS AS RESULT OF EXAMINATION

All members of Minamata Disease Research Team
Department of Medicine, Kumamoto University

Our first year's report covered the results of the epidemiological survey of all residents of our research area, particularly on how we collected information on disorders through questionnaire surveys or general examinations and selected those individuals with neurological disorders who may possibly have contracted Minamata disease. Specifically, out of 304 households or 1119 individuals (520 male, 599 female) of Minamata district (Yudo, Dezuki, Tsukiura), 459 households or 1871 individuals (902 male, 969 female) of Goshonoura district (Ariashiguchi), and 278 households or 1180 individuals (570 male, 610 female) of Ariake district (Akazaki, Zushi, Oura), the latter as control area (although this was inappropriate), those with possible neurological disorders numbered 315 (33.7%), 135 (8.5%), and 29 (3.1%) in Minamata, Goshonoura, and Ariake districts, respectively.

This year, we classified and examined the foregoing individuals into those with Minamata disease, possible Minamata disease, undetermined and requiring further observation, other disorders, miscellaneous. We wish at this time to report on the results of general studies, based on detailed examinations by the Neuropsychiatric Departments and epidemiological and clinical observations (ophthalmological, otorhinological, pediatrics, and minute examinations).

Findings

1. The following cases were diagnosed this fiscal year (until the end of March, 1963) by the Neuropsychiatric Department:

273

A total of 245 patients (113 male, 132 female) were examined from Minamata district. Of these, those confirmed as probable cases of Minamata disease numbered 191 (91 male, 100 female) or 78.0%; those with possible or doubtful affliction — 20 individuals (9 male, 11 female) or 8.2%; undetermined — 24 (9 male, 15 female) or 9.8%; other ailments, 12 (5 male, 7 female) or 4.8%; and healthy individuals and unknown cases — 1 each.

From the Goshonoura district, a total of 134 individuals (61 male, 73 female) were examined. Of these, probable cases numbered 25 individuals (13 male, 12 female) or 19.4%; possible or doubtful cases — 34 individuals (21 male, 13 female) or 25.4%; undetermined — 30 individuals (12 male, 18 female or 22.4%; other ailments, 34 individuals (11 male, 23 female) or 25.4%; unknown — 1, and virtually no disorder in 10 individuals.

From Ariake district, a total of 23 patients (12 male, 11 female) were examined. Of these, those having all of the major symptoms and confirmed as Minamata cases numbered 5 individuals (4 male, 1 female) of 21.7%; those with all of the Minamata disease symptoms except for articulation difficulty or other ailments — 3 (0 male 3 female) or 13.0%; those with symptoms similar to those of Minamata disease — 2 (2 male, 0 female) or 8.7%; undetermined — 9 (5 male, 4 female) or 39.2%; other ailments — 2 (1 male, 1 female), and others — 2.

2. With the participation of members of the Epidemiological and Clinical Departments, there was sufficient basis for the findings.

During the examination, all clinical symptoms related to Minamata disease were probed. Major symptoms included unique sensory disorders (such as those on the area of the mouth, extremities of limbs, or throughout the body; senses of touch, pain, warmth, coldness, position, motion, etc.), loss of coordination (impairment in walking straight, turning right), standing on one leg, bending backwards, squatting, difficulty in following instructions, foot ailment, difficulty in getting up or lying down, resilience, penmanship, line stretching, imitating, etc.), articulation difficulty, field of vision

274

constriction, abnormality of eyeball movement, and hearing difficulty —
especially involving the rear labyrinth. Others included such motor disorders
as difficulty in walking, tremor, loss of energy, increase or decrease of
muscular tension, reflex disorder, limping in one leg, muscular atrophy, etc.
In the psychiatric field, intellectual and emotional disorders were observed,
as well as mental diseases in special cases. Observations were conducted from
all aspects, including senses of taste and smell and dysphagia.

The utmost efforts were made to avoid any erroneous findings, taking
into consideration proprioceptive symptoms and various other test results.

When there was a probability of mercury contamination as indicated by
epidemiological tests accompanied by the outbreak of complete major symptoms,
the case was diagnosed as a probable Minamata disease case. If the symptoms
were considered to be unique, encompassing the vast field of cerebral cortex
and cerebellum and peripheral nerves (sensory and articulation difficulty,
loss of coordination, constriction of visual field, hearing difficulty, and
intellectual-emotional obstructions), the case was treated as involving Mina-
mata disease even in the absence of major symptoms. An example of this would
be abnormality of eyeball movement, rather than constriction of the visual
field. In the case of other symptoms where they could not be distinguished
from other ailments, such matters as positive findings of mercury tests,
serious contamination of family members, and uniqueness of sensory disorders
were taken into consideration before the case was judged to be Minamata
disease related. Cases having all the required symptoms but without the
presence of mercury or absence of any seriously contaminated family member
were treated as possible or doubtful cases. This means that while many Mina-
mata disease patients come under this category, a definitive medical decision
was difficult. By undetermined is meant cases in which further monitoring is
required, such as where individuals reside in a contaminated district and
consume fish and shellfish, but display few symptoms of the disease. This
category is likely to include those with a slight degree of disease.

Based on overall observations, medical determinations were made as follows (see table).

TABLE 1

District Determination	Minamata		Goshonoura		Ariake	
	N.D.	G.d.	N.D.	G.d.	N.D.	G.d.
Minamata disease	191 {♂ 91, ♀ 99}	150 {♂ 71, ♀ 79}	25 {♂ 13, ♀ 12}	16 {♂ 5, ♀ 8}	5 {♂ 1, ♀ 4}	5 {♂ 4, ♀ 4}
Probable Minamata disease	20 {♂ 9, ♀ 11}	20 {♂ 7, ♀ 13}	34 {♂ 21, ♀ 13}	8 {♂ 7, ♀ 1}	2 {♂ 2, ♀ 0}	2 {♂ 2, ♀ 0}
Undetermined	20 {♂ 7, ♀ 13}	24 {♂ 9, ♀ 15}	30 {♂ 12, ♀ 18}	9 {♂ 4, ♀ 5}	9 {♂ 5, ♀ 4}	9 {♂ 5, ♀ 4}
Other ailments	12 {♂ 5, ♀ 7}	0	34 {♂ 11, ♀ 23}	5 {♂ 3, ♀ 2}	2 {♂ 1, ♀ 1}	2 {♂ 1, ♀ 1}
Unknown	1 {♂ 1, ♀ 0}	0	1 {♀ 0, ♂ 1}	0	0	0
Sound health	1 {♂ 0, ♀ 1}	1 {♂ 0, ♀ 1}	10 {♂ 4, ♀ 6}	1 {♂ 0, ♀ 1}	2 {♂ 0, ♀ 2}	2 {♂ 0, ♀ 2}
Aggregate total	245 {♂ 113, ♀ 132}	195 {♂ 88, ♀ 107}	134 {♂ 61, ♀ 73}	39 {♂ 22, ♀ 17}	23 {♂ 12, ♀ 11}	23 {♂ 12, ♀ 11}
Recognized by March 30, 1973	81 {♂ 36 (Death - 1), ♀ 45 (" - 1)}		0		0	

Remarks: Figures for the Neuropsychiatric Department shown in III - 4 are as of May,1950, while those in this table are as of the end of March.

N.D. — Neuropsychiatric Department; G.d. — general determination

Individuals from Minamata district who were subject to general examinations totalled 195 (88 male, 107 female). Of these, 150 (71 male, 79 female) or 77.0% were determined to have Minamata disease; 20 (7 male, 13 female) or 10.2% were diagnosed as probable cases; 24 (9 male, 15 female) or 12.3% were undetermined, and others — 1.

From the Goshonoura district, a total of 39 persons (22 male, 17 female) were given general examinations. Of these, 16 (8 male, 8 female) or 41.0% were diagnosed as having Minamata disease; 8 (7 male, 1 female) or 20.5%

were probable cases; 9 (4 male, 5 female) or 23.1% were undetermined; 5 had other ailments, and others — 1.

From the Ariake district, 23 were examined. Of these 8 (4 male, 4 female) had symptoms considered likely to be Minamata disease, of which 5 displayed all major symptoms of the disease, while the other 3 evidenced symptoms of visual field constriction, sensory disorder, loss of coordination, and hearing difficulty. Two (male) were diagnosed as probable cases, having such symptoms (3) as sensory disorder, loss of coordination and constriction of visual field. Nine cases (5 male, 4 female) were undetermined; 2 had other ailments, and another 2 were in fairly good health.

Incidentally, 81 (36 male, 45 female) out of 195 from Minamata district had previously been designated as having Minamata disease.

Note: Figures cited in III - 4 for the Neuopsychiatric Department
        are not in agreement with those cited in this section. This
        was because the department continued to collect data even
        after the end of March of this year, and then readjusted its
        system, resulting in a delay of submission of data. Hence,
        the general determinations reported herein cover the period
        up to the end of March. The differences must be reviewed in
        the future.

(Takeuchi)

V. PATHOLOGICAL STUDY OF MINAMATA DISEASE (Part 2)

9. PATHOLOGICAL RESEARCH OF MINAMATA DISEASE TEN YEARS LATER (Part 3)

1) Especially, Cases of Autopsy in Chronic Symptoms and Chronic Minamata Disease

Second Seminar, Pathology, Department of Medicine, Kumamoto
University

Team member: Tadao Takeuchi

Research assistants: Mitsuaki Eto, Hidetoshi Kojima, Ken Sakai,

Tohiko Miyayama, Shuji Suko; Nobuyoshi

Sakurama, Kazuko Sato, Koji Shigenaga,

Shinichi Tokumitsu, Teruo Iwamasa

I. PREFACE

In last year's report [1] dealing with results of autopsy in Minamata disease cases over a lengthy period of time, we pointed out that there were acute as well as chronic symptoms of Minamata disease, among which were those difficult to examine clinically, such as partial Minamata disease, masked Minamata disease, abnormal Minamata disease, diffusive cerebral disorder and non-manifest type Minamata disease. Occasionally, therefore, Minamata disease could not be diagnosed.

Subsequently, based upon 5 cases of autopsy and 10 cases mentioned in our previous report, we found there were certain developments until the outbreak of acute symptoms of Minamata disease and possible appearance of new symptoms because of an accumulation of attritional changes. These are reported herein.

II. STUDY MATERIALS AND METHOD

Autopsy was focused around 5 recent, unreported chronic cases, reference being made to cases discussed in last year's report. Of the 5 cases, one (No. 5946) had been diagnosed prior to death as having Minamata disease, and four (Nos. 5987, 5972, 6013, and 5952) had died from possible Minamata disease while they were petitioning for designation as Minamata disease carrier. The disease was confirmed as a result of biopsy in these cases.

The method of study is not described herein as it was mentioned in last year's report.

III. FINDINGS

General items are noted in Tables 1 and 2 (see Tables 1 and 2). Other matters will be described in detail with respect to each case.

TABLE 1. LIST OF AUTOPSY CASES INVOLVING CHRONIC SYMPTOMS OF MINAMATA DISEASE

Biopsy no.	Name	Sex	Age	Years trans-pired	Cerebral weight, g	Remarks
5987	Miya, Mu	M	64	(3)	1050	Infarction of myocardium*
5972	Yoshi, Go	F	81	10	1375	Cerebral hemorrhage*
6013	Hayashi, Fumi	F	75	12	1100	Bronchopneumonia*
5946	Funa, Iwa	F	79	14	1020	Minamata disease
5952	Taki, Ta	M	85	17.5	1150	Infarction of myocardium

*Determined to be Minamata disease as result of biopsy.

Symptom \\ Name	Visual field constriction	Hearing disorder	Speech disorder	Walk impairment	Loss of movement coordination	Sensory disorder	Tremor	Efferent pathway symptoms	Pathological reflex	Paralysis of movement	Mental disorder
Miya, Mu	−	+	+							−	
Yoshi, Go	+	+	+	++	+	+	+				+
Hayashi, Fumi		+++	+	+++	+	++	++	++		+++	+++
Funa, Iwa	++	++	++	+++	++	++	++			+++	++
Taki, Ta		+++	−	+++	NT*	NT*	−	+	−	−	+++

*NT — Unable to test.

Amount of Mercury Deposition

The amount of mercury deposition in 5 cases in which autopsy was per-
formed was measured by Professor Fujiki of the Hygiene Facility of this
University. Study was made of the liver, kidney, and brain, and, in addition
to the aggregate amount of mercury, some methyl mercury was also found in a
few cases (see Table 3).

Case No. I. Miya, Mu; female, Tsukinoura, Minamata-shi.

Date of birth: October 10, 1907
Date afflicted: 1969
Date of death: March 30, 1972 (64 years old)
Period transpired: 3 years
Family background: Mother (80 years old) died around 1960, after her
                    limbs became seriously affected.

TABLE 3. LIST OF MERCURY LEVELS IN VISCERAL ORGANS OF HUMANS
AFFECTED WITH CHRONIC MINAMATA DISEASE

Biopsy	Sex	Age	Years transpired	Liver ppm	Kidney ppm	Brain ppm	
						Cerebellum	Cerebrum
··	·	64	(3)	0.388 (0.024)	2.479 (0.011)	0.032 (0.010)	0.033 (0.013)
··	·	51	10	0.745	1.385	0.068	0.029
··	·	75	12	0.400	0.225	0.178	0.016
··	·	79	14	1.261 (0.037)	10.045 (0.016)	1.515 (0.036)	1.338 (0.017)
5952	·	85	17.5	0.280	0.263	0.083	0.211

( ) — Numbers in parentheses indicate the amount of mercury (measured by
Hygiene Facility).

History of employment. Engaged in agriculture, and resided in Tsukinoura
since around 1947; consumed fish, shellfish, oysters and shrimp in great quan-
tity; caught and consumed shellfish and oysters, in particular.

Current medical history. Paralysis in both hands, swelling sensation in
limbs since 1969, subsequently unable to move fingers, drops things, unable
to button up, hands quiver when carrying heavy objects, footgear comes off,
eyes hurt, hearing difficulty, stutters, headache, irritable, forgetful,
unable to think clearly, dislikes to do anything, depressive, and unable to
sleep. She was unaware that she had contracted Minamata disease all this
time. In September, 1971, she was examined at the Psychiatric Department,
with the following diagnosis. Unable to walk upright. Romberg (-). Unable
to stand on either foot with eyes closed; finger test: clumsiness on either
side, slow on the right, as well as on the left; foot test: clumsiness on
either side; Barré symptoms on the right (+) and left (+); motor coordination
disorder (++); articulation disorder (+); eyeball movement restricted in
either direction; pupils small on either side; auditory disorder (+); olfac-
tory and taste senses almost normal; muscular tension generally progressive,

spasmic contraction (++), solidus contraction (+); local reflex intensified in upper leg and knee on either side; Hoffmann, Wartenberg positivity on either side; sensory disorder on both hands, but none on lips; no centric field of vision constriction; no excessive perspiration or muscular atrophy as symptom of autonomous nerve affliction; deformity of left hand; adduction of I and V fingers, and bayonet-attached-gun type I, III, and IV fingers; mental disorders as sensory affliction (+), temperament (+), lack of aggressiveness and emotional insensitivity. Blood pressure, 118/86 mm Hg.

Because of the foregoing observations, the individual was treated as a probable case of Minamata disease. From May 27, 1969 to June 30 of the same year, she was hospitalized at Minamata Municipal Hospital for congestive heart disorder and a clot in the right leg. On May 8, 1970, she suffered edema of the whole body, and was readmitted; while in the hospital, she developed jaundice, but was released on June 23. In 1956, she had undergone cholecystectomy.

From mid-February, 1971, she felt fatigue throughout her body, had disorder of mood and emotion and lost her appetite, and was hospitalized on April 20. She was administered a liver protecting agent and a drug for urine condition, and was released on May 14. Subsequently, she was examined as an out patient, but on December 1, she complained of chest pain and respiratory difficulty, and was readmitted after she was found to be suffering from liver enlargement. An electrocardiogram indicated a clot in the right leg, and after failing to respond to treatment, she died on March 30, 1972.

The patient applied for designation as a Minamata disease patient on September 20, 1971, and died while her application was still pending. An autopsy established that she had Minamata disease.

Pathological Considerations

<u>Autopsy examination (autopsy no. 5987)</u>

1. Arteriosclerosis type changes

1) Hardening of arteries: i) Degeneration and hardening of middle membrane of arteries in the brain; softening of the brain: right diencephalon, right occipital lobe (macro), right lobe (macro), cerebellum (macro). ii) Hardening of coronary artery and scar of heart muscle, a growth on heart muscle and swelling, enlargement of the lumen. iii) Arteriosclerosis type atrophorous kidney. iv) Hardening of brachiocephalic trunk artery. v) Spleen, etc.

2) Atherosclerosis

2. Minamata disease: cerebral atrophy (1050 g)

1) Patchy fallout of cerebral cortical nerve cells; optic center, front center area, parietal lobe, temporal lobe, frontal lobe, etc.

2) Degeneration of cerebral medulla (especially, occipital lobe).

3) Fallout (moderate) and thinning of centric granular cells of cerebellum.

4) Degeneration of long fibers in medulla and atrophy and fallout of anterior horn cells (moderate).

5) Pathological changes of peripheral nerves (posterior root, lumbar ischium, gastrocnemius):

      i) loss, regeneration of posterior root and sensory nerve fibers;
      ii) increase of Schwann's cells.

3. Condition following cholecystectomy

1) Adhesion of portal area of liver with duodenum and transverse colon, and enlargement of chorion.

2) Liver: chronic inflammation of the bile duct and centric, fat accumulation in small lobe.

4. Congestion in various visceral organs

1) Congestion, dropsy of lungs as well as brownish coloration and hardening.

2) Congestion in other visceral organs.

5. Edema: water in lungs (left, 200 ml; right, 50 ml)

6. Chronic cystitis

7. Atrophy, scar formation in pancreas islets of Langerhans. Cause of death: heart disorder.

General External Appearance and Visual Observations of the Nervous System

Nutrition and skeletal growth were of moderate degree. Muscles of thenar eminence, thenar prominence on either side had a slight degree of atrophy, and some bending and contraction from the No. II joint of either hand to gliding joint. No muscular atrophy was observed in the femoral and gastrocnemius muscles. The heart was 460 g in weight, with enlargement of the cavity of the left atrium and left ventricle markedly visible. No clot was seen, but some crookedness and hardening of the coronary artery was found. Congestion of various visceral organs throughout the body was noted.

The weight of the brain was 1050 g, its size was generally smaller, and the fissure was wider. Atrophy was especially noted in the occipital lobes on either side. In one section, atrophy was found in the cortex, as well as atrophy in some portion of the optic center. A moderate degree of softening of the brain was noted in the right diencephalon, and a trace of softening in the right frontal lobe, right lobe, and cerebellum. No marked changes were found in pons, medulla oblongata, or spine. Some atheroma was noted in the brachiocephalic trunk artery.

Histological Observations on Cerebrospinal, Peripheral Nerves

Cerebrum: A moderate degree of cortical atrophy was noted in the occipital lobe, but was particularly deep in the cleft of falx cerebri. The layer structure was relatively preserved, but there were a disarray of nerve cells, patchy fallout, and an increase of glia. Atrophy and hardening was also noted in some nerve cells. A partial loss of the marrow line was seen, as well as diffusive spottiness deep in the medulla. In the forefront of the occipital lobe, there was a moderate degree of atrophy in the cortex, and the layer structure was relatively intact.

Spottiness is seen in Layer I; a moderate degree of fallout and decrease of conical cells deep in Layers II and IV, with localized glia multiplication in some areas, are also visible. Some spottiness is seen in the medulla, and a thickening of the walls of small veins. Infiltration of glia cells is also observed. At the optic chiasma, thickening and hardening of the inner membrane of small blood vessels is seen in the area of the aorta, including some softening over a relatively wide area. Softening is also visible in the right diencephalon. The softening involved the blood vessels, and this condition was similarly noted in the temporal lobe and parietal lobe. Generally, in the cerebral cortex, all layers showed patchy fallout of nerve cells (Photo 1) and some spottiness (Photo 2). Some increase of glia cells was found in the fallout area of nerve cells. Many of the remaining nerve cells were undergoing consumption, in addition to hardening and atrophy.
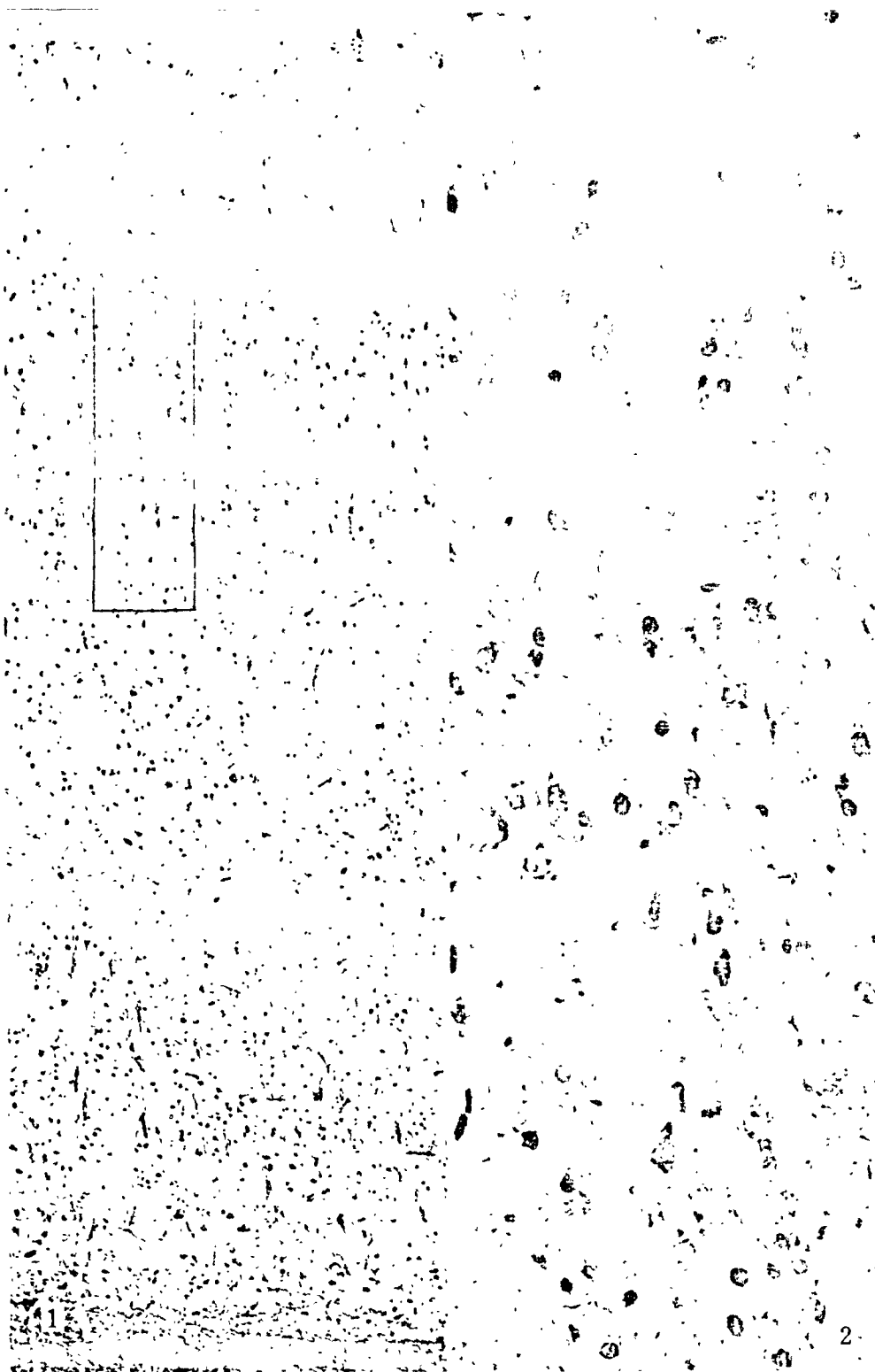
Photo 1. Case No. 5987 cerebral cortex (falx cerebri), some expansion (KB-stained) and cortical layer; also, extensive patchy fallout of nerve cells and increase of star-shaped glia

Photo 2. Enlarged view of cortical layers II - III, same area as above in same case. Early stage of microscopic, spongy condition and spottiness is seen in Layer II. Spottiness due to marked nerve cell fallout, diffusive multiplication of star-shaped glia cells can be noted. The remaining cells are also undergoing degenerative changes
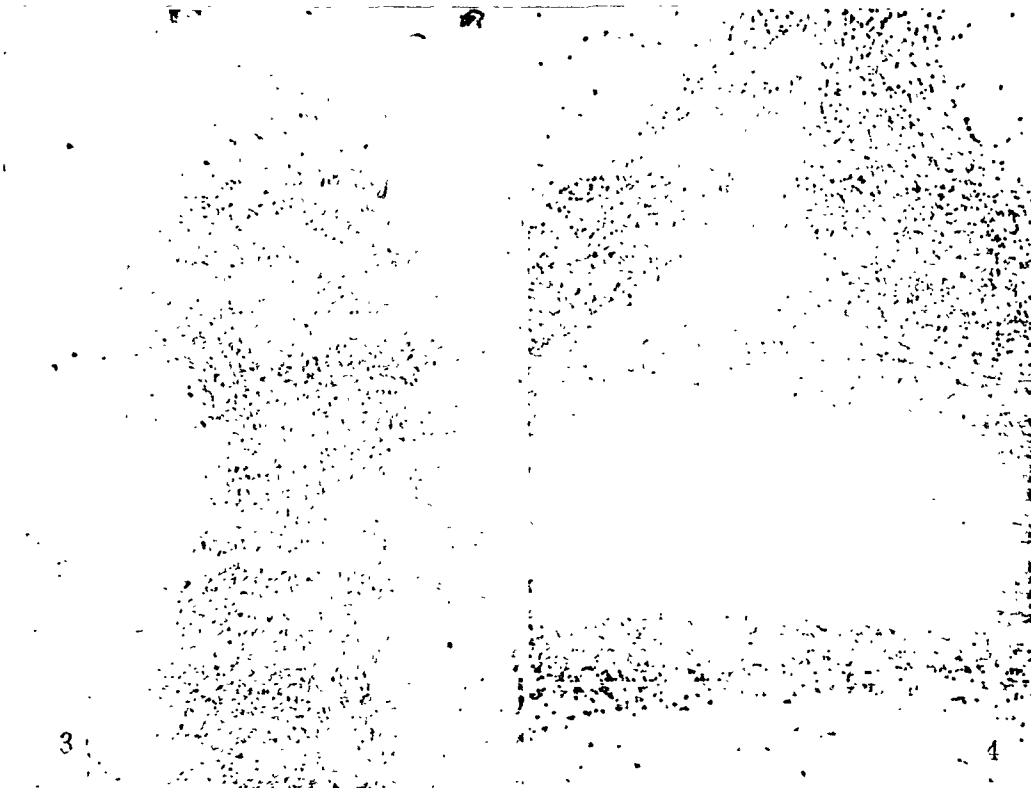
Photo 3. Noted near the center of Cerebellum (HE-stained) of Case No. 5987 was atrophy, extensive fallout of granular cells and apical scar formation; also partial fallout of Purkinje cells and Bermann's glia cells increase

Photo 4. Atrophy of cerebellum and spottiness of medulla in above case (NO. 5987)

Cerebellum: Slight atrophy was seen around the brain, but was rather distinct near the wall of No. 4 cavity. Partial fallout of Purkinje cells and an increase of Bergmann's glia cells was noted, with fallout of granular cells from directly beneath Purkinje cells (Photo 3). Spottiness was noted in the medullary sheath (Photo 4). Apical scar formation was also noted in some areas. A calcified cell bundle which developed subsequent to the softening was seen in a molecular layer of the lobe.

Mesencephalon: No marked changes were found.

Photo 5. Case No. 5987 irregular
axons in posterior root of spinal
nerves, irregular large fibers and
  multiplication of microfibers

Case No. 6. Through medullary sheath
staining of anterior root of spine
(No. 5987), new changes were found in
both sides of Ranvier membrane

Spine: Changing properties of axons attributed to achromatism of the
medullary sheath was systematically noted. Some decrease of anterior horn
and central duct of the horn cells was noted. Between the lumbar vertebrae
was a localized, softened area.

Peripheral nerves: In the posterior root, irregularity and nonuniformity
of the medullary sheath was seen; also, an increase of Schwann's neurons,
partial scar formation and cell infiltration. Also noted was an irregularity
of axons and an increase of small fibers (photo 5). New changes were also
noted in the medullary sheath (Photo 6). In the anterior root, the medullary
sheath was in relatively good shape. A trace of scar formation and micro-
fibers were found.

The case discussed here is that primarily of cerebrovascular disease, accompanied by Minamata disease. Three years had passed since the patient was stricken in 1969, but the mercury level in her visceral organs was normal. It is possible that she had been exposed to mercury contamination for a long time prior to that period. The nerve cells in the cerebral cortex showed regressive diffusive changes, in addition to fallout, indicating a phenomenon which occurred over many years.

Case No. II. Yoshi, Go; male; Idemizu-shi, Kagoshima-ken.

Date of birth: August 15, 1890.

Date afflicted: 1962.

Date of death: February 19, 1972 (81 years old).

Years transpired: 10 years.

Family history: Second son (47 years old) was treated at a mental hospital in Idemizu-shi about 7 - 8 years ago; received head injury (hospitalized 2 days) in a traffic accident in May, 1970; even now complains of headache and numbness in limbs. Third son (41 years old) also complains of numbness of hands.

Life history: Engaged in fishery since childhood; loves to eat fish; ate fish even during the days when fishing was prohibited. Unable to work for 14 years because of physical deterioration.

Current medical history: Hands tremble, numbness of fingers and partial blindness from about 10 years ago. Is unaware even when his footgear comes off; staggers, and from about 5 - 6 years ago, felt twitching sensation in feet; unable to speak clearly, and forgetful. Believed at first to be senile problems.

Symptoms at time of examination on December 12, 1971 (by Dr. Hirata): Senses of touch and pain impaired in both forearms and lower thighs, hearing difficulty, articulation difficulty, positive Lonberg's phenomenon, etc.

The field of vision in the right eye was: external 55°, internal 45°, top, 40°, bottom, 50°; the left eye could not be examined because of pterygoid fragment.

Noted were trembling of hand, involuntary movement, and changes in nails; also poor memory and difficulty in mental calculations.

Patient was diagnosed as a probable case of Minamata disease, and having high blood pressure.

He was treated by a certain doctor for respiratory condition in April, 1969 (allegedly, bronchitis since 1945); suffered paralysis on the right side of body on February 4, 1972, and temporarily lost consciousness; bed-ridden ever since; on liquid diet; again became unconscious on February 17, 1972, and died on February 19.

Patient died while his application for designation as Minamata disease, filed on January 18, 1972, was still pending. An autopsy confirmed that he had Minamata disease.

Pathological Matters

Autopsy examination (Case No. 5972)

1. Cerebral hemorrhage (left diencephalon); weight of brain: 1375 g (to include swollen area on left side).

2. Arteriosclerosis, especially sclerosis of arterioles

1) Atrophy of the kidney, of the arteriole-sclerotic type (accompanied by partial arteriosclerotic cicatrice); 2) papilloma of the heart muscle and hardening of the coronary artery; 3) hardening of cranial vessels and mortification of the middle membrane of arterioles.

3. Minamata disease

1) Extensive fallout of nerve cells of cerebral cortex (including optic center); 2) spottiness of cerebral medulla; 3) fallout of centric granular cells of cerebellum (including apical scar formation); 4) moderate spottiness of axons of spine; 5) relatively marked pathological changes in peripheral nerves (including sensory organ): i) loss of fibers, regeneration and scar formation in posterior root greater than in anterior root; ii) multiplication of Schwann's cells.

4. Moderate degree of bronchopneumonia and chronic bronchitis, and emphysema.

5. Scarred, pathological changes in upper lobe of the lung and tuberculosis of the lymph glands (moderate).

6. Atrophy of various visceral organs (spleen, liver, etc.).

Cause of death: cerebral hemorrhage.

General External Appearance and Visual Observation
of Nervous System

A small skeletal framework, malnutrition, muscular atrophy throughout body, and loss of weight in the liver, 715 g, spleen, 25 g, and kidney, 75 g. Brain weight, 1375 g, with moderate dispersion of soft membrane. Atrophy on right side of brain, being edematic in the opposite hemisphere, extending from the apex to the occipital lobe. As for the divided side, there was a sign of serious hemorrhage on the left diencephalon, which proved fatal. Relatively serious atrophy on right occipital lobe, with edematic condition on left occipital lobe and a wider medulla; narrower cortex, expecially in falx cerebri; moderate atheroma formation in cephalic trunk artery.

Histological Observations of the Nervous System

Cerebral hemisphere: Bleeding in left diencephalon region, extending from the capsula interna to putamen, with surrounding tissues of the edematic type; red blood corpuscles still present, with hemolysis in the central area; infiltration of undernourished cells and multiplication of star-shaped glia, with a trace of hemosiderin cells; slight hemorrhage in right diencephalon, with some thickening of vessel walls; no marked changes or loss of basal nucleus of cells.

In the occipital lobe, the region transforming into a cleft manifested a thinning of the cortex and indistinct layers, each layer showing a marked fallout of nerve cells even around the surface layer (Photos 7, 8). Great fallout of nerve cells was also seen in Layers II - IV, microscopically in a spongy condition (Photo 8), as well as in Layers IV - VI (Photo 7). Spottiness of the medullary sheath and partial loss of the marrow line was visible.

In the right lobe (non-hemorrhage region), some fallout was noted; also, a slight sign of bleeding in the medulla, and a slight spottiness. Even now, there were consumptive changes in the nerve cells, undergoing atrophy and obliteration. Hardening of cells and degeneration were also seen in the gyrus. In the left gyrus (hemorrhaged area) where there was a swelling caused by hemorrhage of putamen, cranial edema was noted, with bleeding in the vicinity of small vessels, extending to the medulla. A reactory multiplication of glia and spottiness of tissue were also observed.

Cerebellum: At the center of the appendage, fallout of granular cells from directly beneath the Purkinje cells was noted (Photo 12). A slight fallout of even Purkinje cells was noted, with an increase of glia in the molecular layer. The medulla was narrowed, with slight fallout. No vascular disease was seen in this area. Slight changes were seen in the hemisphere as well as a trace of apical scar formation near the ventricle. Signs of new bleeding were observed in the medulla opposite the No. 4 ventricle.

292
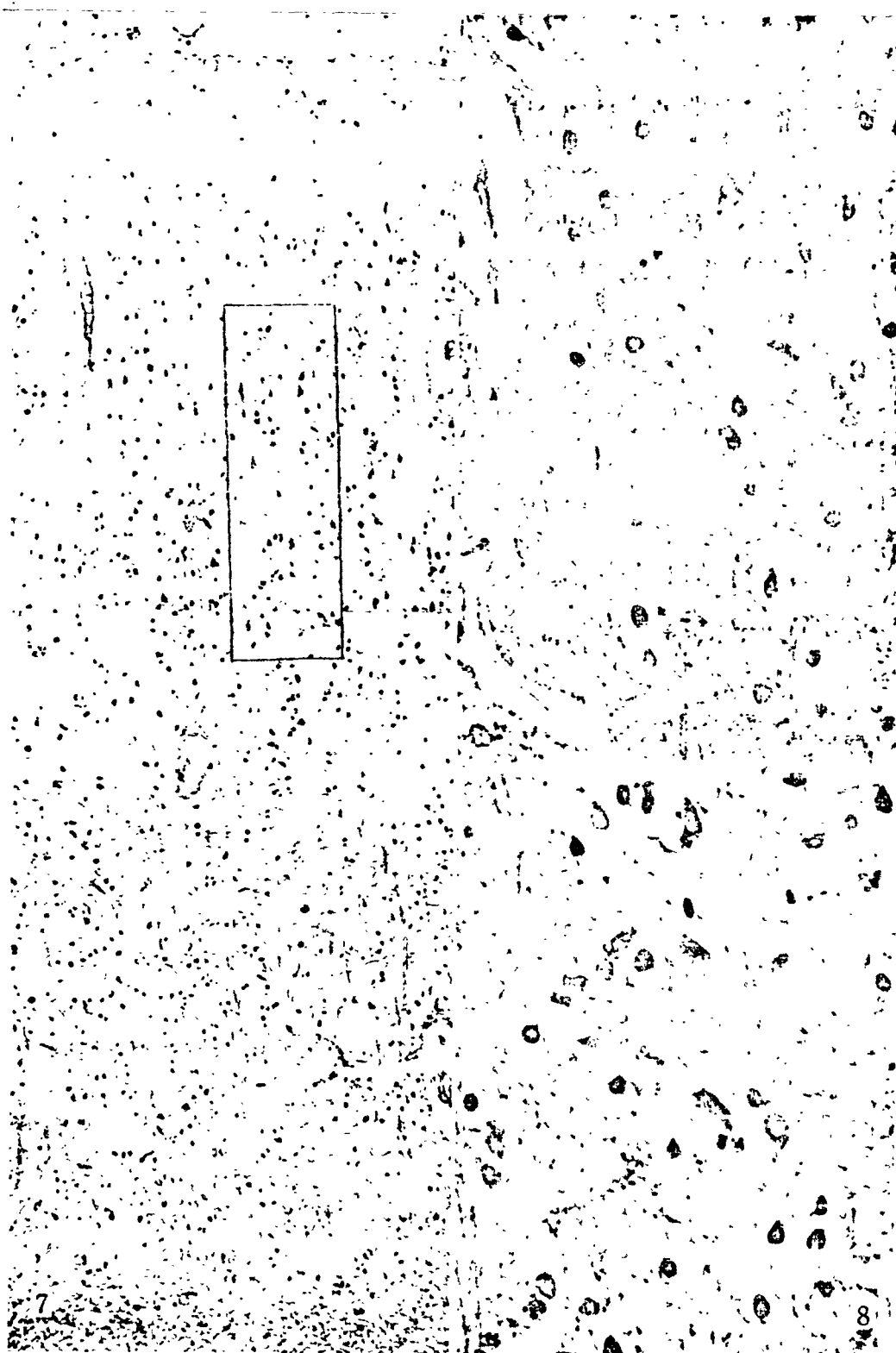
Photo 7. Slight expansion (KB-stained), patchy fallout of nerve cells throughout cerebral falx celebri (optic center) cortex of No. 5972; decrease and loss of marrow line

Photo 8. Enlarged view of cortex of same region as above (No. 5972) in Layers III - IV. Heavy fallout of nerve cells, with some thinning and multiplication of star-shaped glia; atrophy and hardening of remaining nerve cells, becoming consumptive
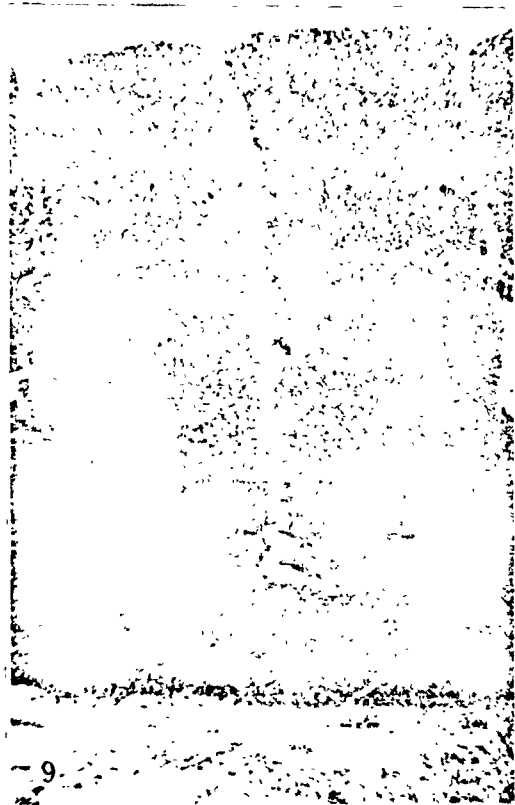
Photo 9. Spinal cord (cervical) of No. 5972; non-stainablity of posterior Goll fibers following degeneration can be observed
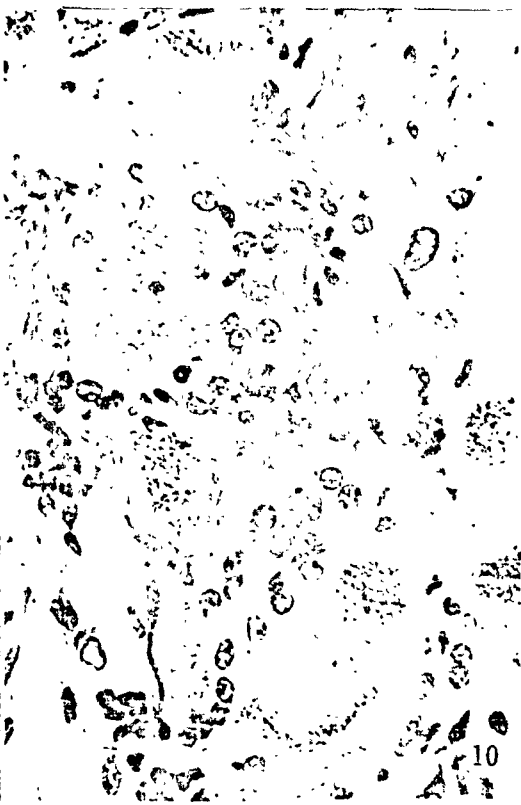
Photo 10. Ganglion of spinal cord in No. 5972; ganglion cells are generally intact, but some fallout are seen, with an increase of satellite cells; also marked increase of consumptive pigments in ganglion cells

Pons: A slight retrogressive degeneration was seen in olive nucleus, and a slight fallout and swelling of nerve cells. Degeneration and fallout of nerve cells of trigeminal nerve was noted; also spheriod bodies in the vicinity of medulla oblongata and the soft membrane. A trace of hemorrhage was seen in a part of nucleus dorsalis; also, localized, achromatic medullary sheath in the left conical groove region.

Spine: Achromatic medullary sheath due to slight changes in posterior fibers in the cervical spine; these changes lessened in the lower area, being found in the thoracic but not in the lumbar regions. Slight achromatism
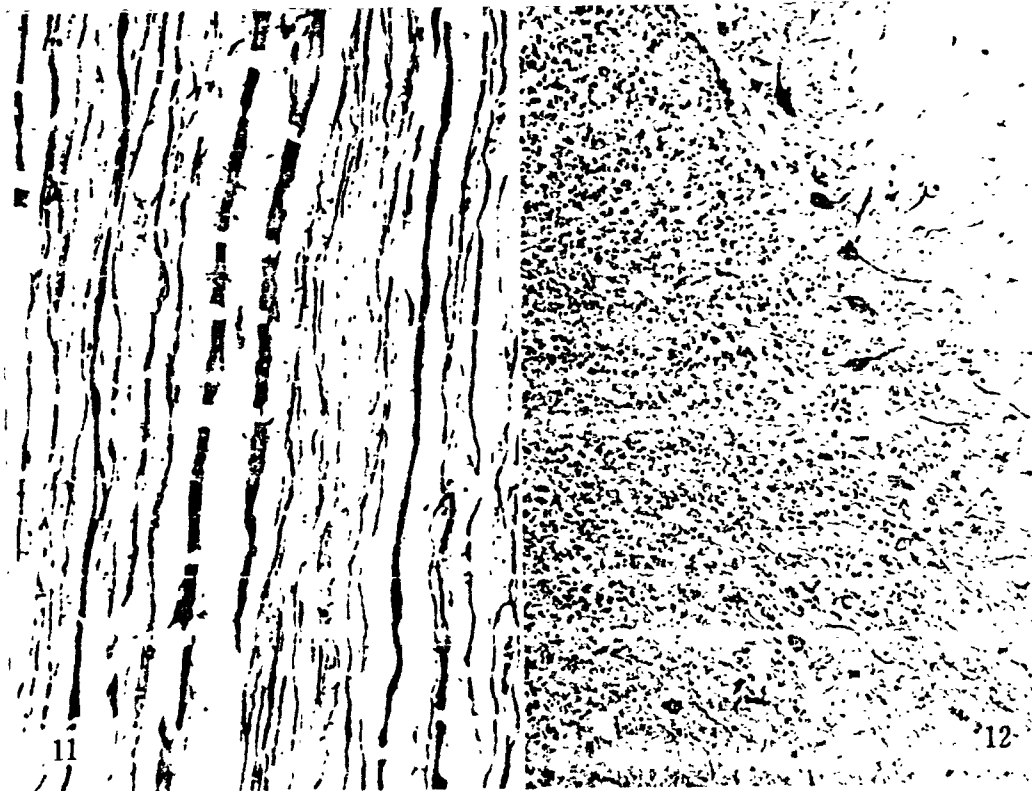
Photo 11. Posterior root of spinal cord of No. 9572; marked irregularity of axons and an irregular increase of micro-fibers; regenerative image is visible

Photo 12. Cerebellum of No. 5972; thinning and fallout from directly below the Purkinje cell layer

of medullary sheath was found in the anterior fibers in the lumbar region. Anterior horn cells were few, due to fallout, and were hardening, especially in the surrounding area; spheriod bodies were also detected.

Posterior root ganglion: Ganglion cells were relatively intact, but an increase of consumptive pigment in nerve cells was found. A slight increase of Schwann's cells was found in fibers (Photo 10).

Root nerves and cranial nerves: In the anterior root, there was a slight increase of Schwann's cells, loss of medullary sheath, increase of microfibers and restructure (Photo 11). The trigeminal nerve also showed changes as in the posterior root, though to a lesser extent.

295

Although this case focused primarily on senile changes, in discussing vascular, pathological changes, typical symptoms of Minamata disease were also found. It appeared that the senile changes have been compounded by symptoms of Minamata disease — representing one type of acute case among the elderly.

Case No. III.   Funa, Iwa; male; Tsunaki-mura, Ashikita-gun.

Date of birth: June 1, 1892.

Date afflicted: 1957.

Date of death: December 17, 1971 (79 years old).

No. of years transpired: 14.

Family history: Son succumbed December, 1957, from Minamata disease.

Current medical history.   Initially became aware of tremor of hands about 1957; neglected treatment on belief that he was an alcoholic, since tremor ceased when he drank; more violent tremor; unable to converse; paralysis of limbs; hearing and walking difficulty since late September, 1959.

Admitted to Minamata Municipal Hospital on October 14, 1959; unable to walk since November of same year; speech and hearing ability restored somewhat in mid-December; able to take five or six steps before collapsing; diagnosed as Minamata disease at time of admission; had been found to be a Minamata disease patient on October 14, 1959.

Began to complain about pain in limb joints in January, 1960; confined to bed and unable to walk since; muscular atrophy of hands since around April of the same year; stiffness of wrist and finger joints.

Transferred to Yunoji Hospital on March 10, 1965, for purpose of rehabilitation; tremor of both hands, difficulty in speaking, constriction of visual field, loss of coordination, hearing difficulty, drooling, limb deformity; somewhat undernourished; difficulty in changing body position in bed,

but able to sit up on own power with aid of device; unable to stand without help; wholly unable to walk; barely able to brush teeth; unable to wash face or comb hair; required toilet assistance and in changing clothes.

Partial results of tests were: PSP, 18.5% at 15 minutes, 61.4% at two hours; cholesterol, 248 mg/dl, CRP (++++), RA (+).

Treatment was administered on the basis of diagnosis of Minamata disease, acute articular rheumatism, and intestinal disorders.

Was able to walk using parallel bars from around September, 1965, until June of the following year.

Confined to bed because of difficulty in urinating since June 22, 1966; increasing stiffness of limbs; gradually started to mumble and drool due to glossitis and inflammation of inner mouth.

Fever developed in April 1969; urine volume decreased; blood precipitation increased in June of same year; coughed at night, with greater expectoration; blood pressure tended to increase from around July of the same year (180/86 mm Hg).

Strong sense of fatigue in entire body since around February, 1971; vomiting, fever, intensive expectoration, irregular heartbeat and edema of lower limbs since early November of same year.

Succumbed on December 16, 1971, due to heart disorder attributed to general deterioration (according to records of Doctor Misumi of Yunoji Hospital Annex).

Pathological Considerations

Autopsy examination (autopsy no. 5946)

1. Minamata disease: cerebral atrophy (1020 g)

1) Extensive thinning and fallout of nerve cells in cerebral cortex (extensive in optic center and others).

2) Spottiness in cerebral medulla.

3) Centric atrophy of cerebellum: i) fallout of granular cells; ii) fallout and part of Purkinje cells; iii) apical scar formation.

4) Medullary oblongata: changes in conical groove section.

5) Spine: changes in posterior fibers (cervical, thoracic), and in side fibers (cervical, thoracic, lumbar).

6) Peripheral nerves: i) obliteration, regeneration of posterior root and sensory nerve cells; scar formation; ii) slight similar changes also in anterior root; iii) increase of Schwann's cells.

2. Bronchopneumonia (primarily pneumonia of the infectious, effusive and bleeding type on the right side).

3. Condyloma-type pericarditis.

4. Arteriosclerosis: 1) sclerosis of arterioles (kidney, spleen); 2) sclerosis of the aorta; 3) papilloma on the heart muscle.

5. Tuberculosis (slight): 1) tuberculosis of right lung, of the small lobe, nodulous type; 2) empyemic pleura adhesion on the right side, calcification, scar formation.

298

6. Atrophy of various visceral organs: liver (580 g), spleen (40 g), and others.

7. Tumor in the prostrate and edema of scrotum.

8. Fibrous/round stomach tumor.

9. Atrophy of islets of Langerhans in pancreas, scar formation.

10. Marked deformation of fingers and of other joints.

Cause of death: bronchopneumonia.

General External Appearance and Visual Observation
of Nervous System

The skeleton was of moderate growth, and there was considerable mal-
nutrition. Muscular atrophy was noted in the arm, forearm, and fingers, with
contraction in fingers. In both hands, the III and IV fingers were crooked
in the direction of the palm, leaving only II and V fingers for grasping
(Photo 13). No swelling of PIP, MIP, GIP joints was noted; diffusive atrophy
of all fingers and deformed nails were seen. Muscular atrophy was also noted
in lower extremities, especially in gastrocnemius of the shank. Moderate
contraction was also seen in joints of both elbow and legs; the right elbow
joint was swollen, had luster and was slightly crooked; and a slight con-
traction at the pointed end of the foot. Moderate edema was noted in the
instep of the left foot and in the frontal tibia. In the small of the back
was a bedsore 10 x 10 cm in size.

The weight of the heart was 230 g, and rather small. A wart about the
size of a bean was found in the area of the semilunar valve of the aorta.
The weight of the right lung had increased to 890 g; it was tinged with red-
dish color, and had an increase of fluid and blood. Clumps of bood were
dispersed; also, hyperplasia of the plura and adhesion was noted on either

299
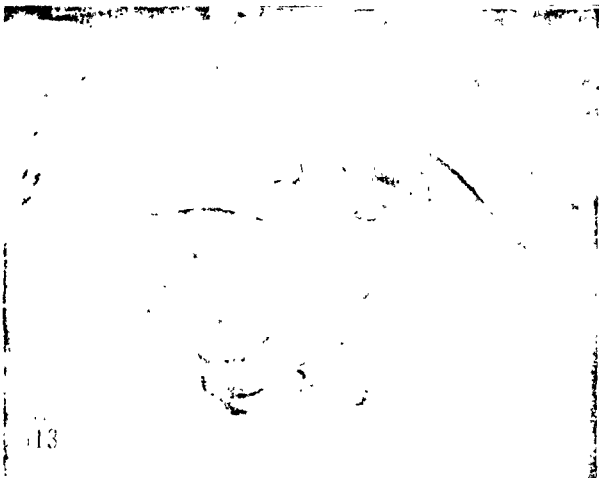
Photo 13. Deformed fingers of No. 5946, due to Minamata disease and articular rheumatism
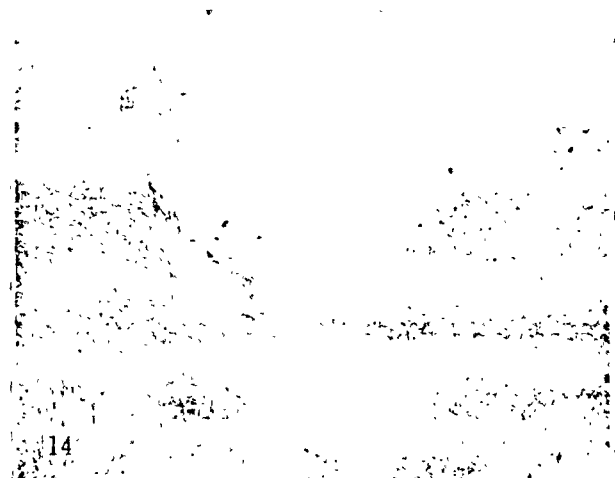
Photo 14. Atrophy of cerebellum of No. 5946; slight spottiness in medullary sheath

side. Atrophy of the liver (580 g), spleen (40 g), and kidney (60 g) was seen.

The brain weighed 1020 g, and the pia mater was generally turbid; atrophy was noted around the brain, especially on the occipital lobe. A slight atheroma was noted in the brachiocephalic trunk artery. No visual indication of softening was seen in the cerebral parenchyma.

Histological Observations of Nervous System

Photo 15. Atrophy of cerebellum of No. 5946; diffusive fallout of granular cells and also great, localized fallout of many Purkinje cells (due to Minamata disease and pathological changes involving lack of oxygen)

Cerebral hemisphere: Atrophy of the cortex was notable, expecially in the occipital lobe and falx cerebri groove, where even the layer structure was indistinct and thin (Photo 17). Fallout of nerve cells was great (Photo

18), and appeared to be microscopically spongy (Photos 19 - 20). Fallout was also noted in the cortical area, but there glia increase was moderate (Photos 19 - 20). An increase of brown pigment was seen in remaining nerve cells, as well as loss of the medullary line. Diffusive spottiness was also seen deep in the cortex.

In the temporal lobe, Layers II - IV appeared to be spongy, and fallout of nerve cells was notable. Atrophy and hardening was seen in remaining nerve cells, some on the verge of disintegration.

In the central fissure in the parietal lobe, the layer structure was intact, but fallout was seen, with a notable decrease of Betz cells.

Similar changes were found in other cerebral cortex.

Cerebellum: In general, there was atrophy with a thinning of the molecular layer. Relatively heavy fallout of Purkinje cells; granular cells were not clustered together in a normal pattern, and heavy fallout had caused their number to decrease, but no large scale fallout was seen
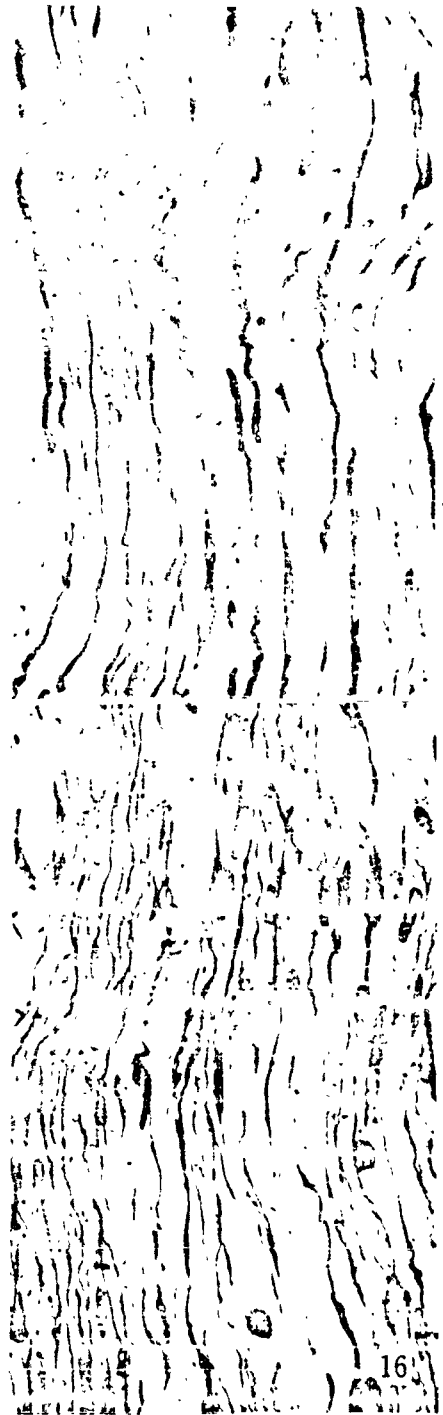
Photo 16. Anterior (upper) and posterior (lower half) root of No. 5946; axons highly irregular in both; unusual multiplication of microfibers seen in anterior root; greater changes noted in posterior root; increase of Schwann's nucleus
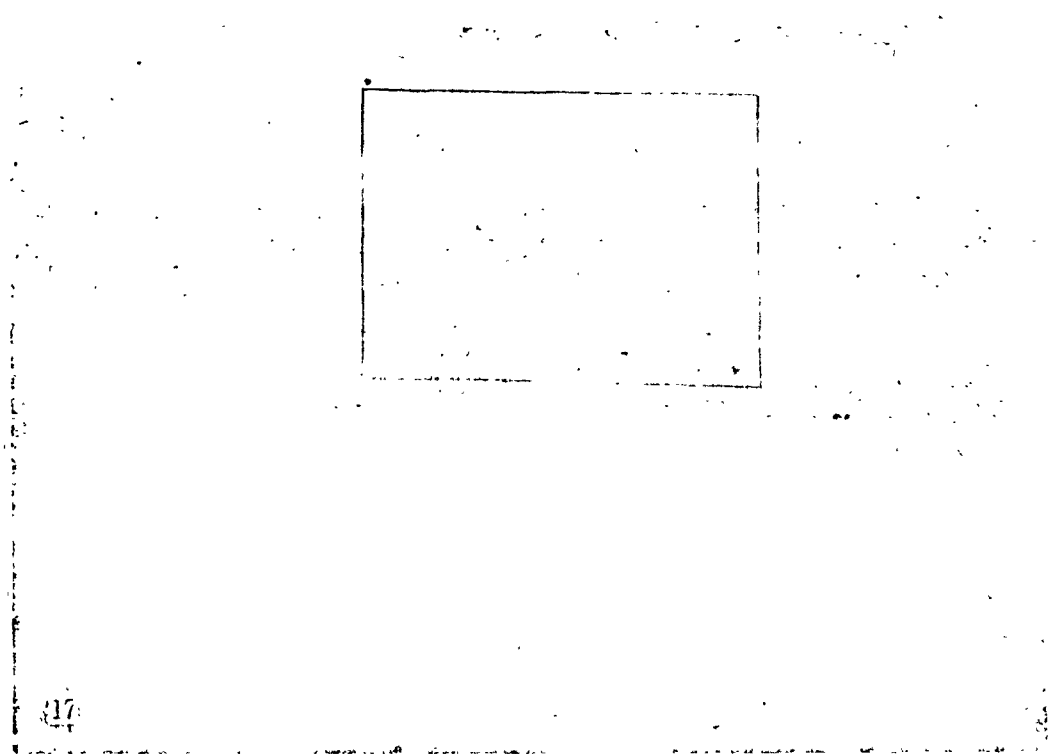
Figure 17.   Cerebral cortex (optic center) of No. 5946; marked
atrophy in cortex (KB-stained)

(Photos 14 - 15).   Apical scar formation was noted.   Increase of both glia
and Bergmann cells in the mocecular layer was observed; also, slight thicken-
ing of the middle and outer membranes of the meninges.

Pons: Various nuclei are intact, but achromatism of the medullary
sheath of Tr. mesencephalicus n. trigemini (V) was noted.

Medullary oblongata: Achromatism of the medullary sheath of Fasciculus
gracilis, Fibrae pyramidales was seen.

Spine: Achromatic medullary sheath, with general changes in posterior
fibers; secondary changes in the conical groove section, with some atrophy
and fallout of anterior horn cells of certical vertebrae and thoracic verte-
brae.   Some anterior horn cells in the lumbar vertibrae are in an atro-
phorous condition, but most are intact.

302
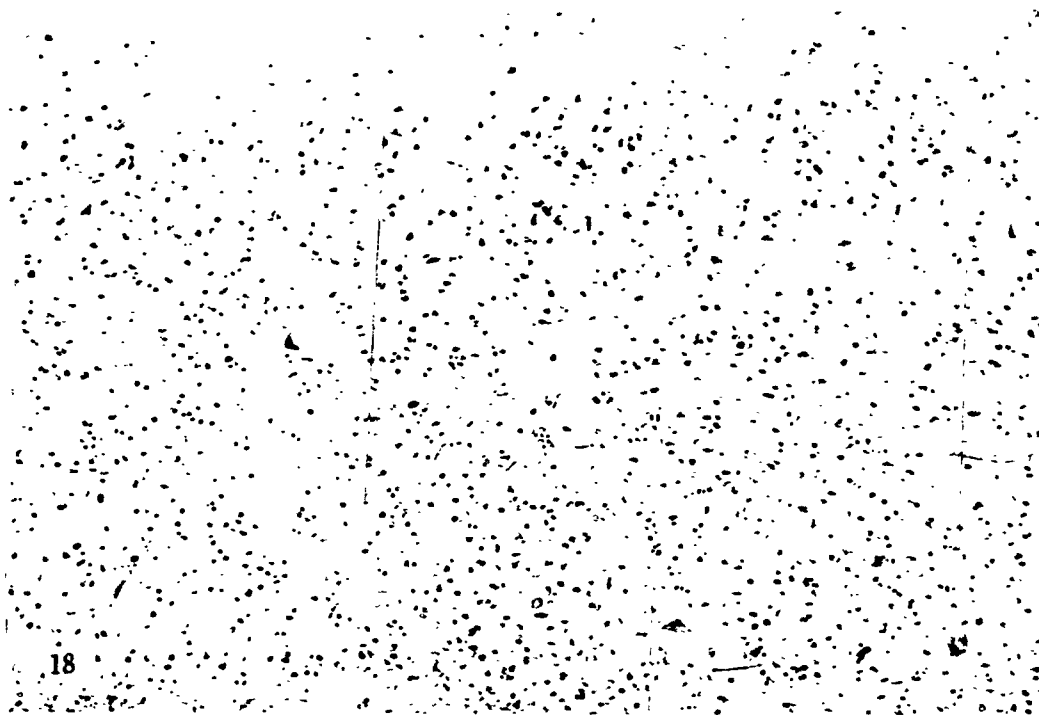
Figure 18. Enlarged view of of outlined area in Figure 17
(Case No. 5946); much thinning and fallout of nerve cells;
some increase of glia; modified form of remaining conical
cells is visible

Peripheral nerves: Increase of Schwann's cell nucleus in both anterior
and posterior roots; irregularity of an achromatic medullary sheath; irre-
gularity in color of axons; appearance of microfibers. A greater part of
the gastrocnemius nerve bore scars.

This case was of a fairly chronic type which became aggravated with the
lapse of time and was recognized as a typical Minamata disease. During the
ten years that have elapsed, the patient also developed articular rheumatism.
Abnormalities of the limbs and deformed fingers subsequently developed, due
to additional conical groove symptoms induced by motor nerve disorder as a
result of extensive cortical impairment of the cerebrum.
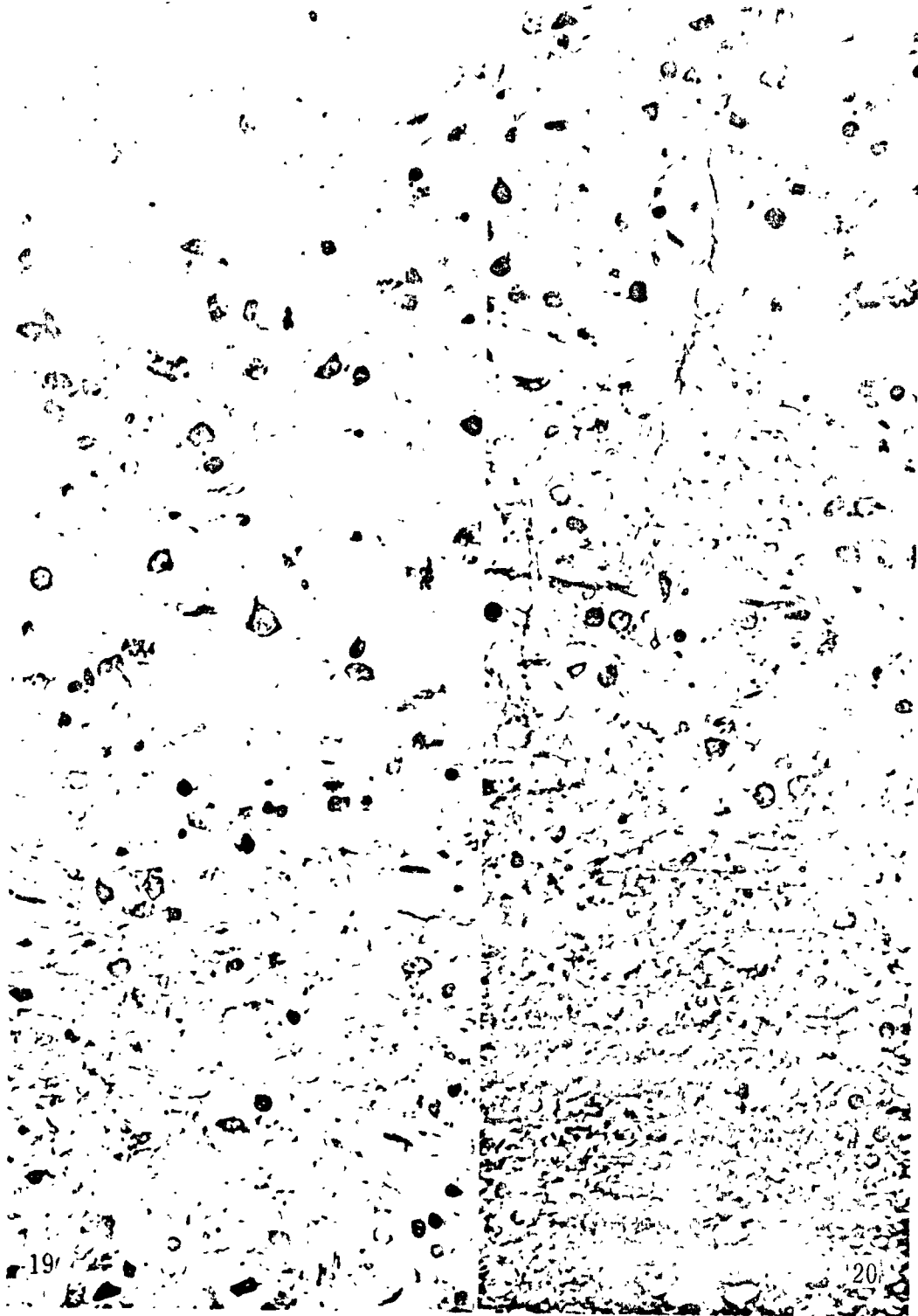
Figures 19 - 20. Enlarged view of cortical layers in same area in the same case (No. 5946) (KB-stained). Majority of nerve cells have fallen out in the layers and are dispersed. Microscopically, they appear to be spongy; no great increase of glia cells; nerve cells were greatly damaged, and many are disappearing. 19 — I, II, III, IV cortical layers; 20 — IV, V, VI cortical layers, medullary

304

Case No. IV. Hayashi, Fumi; male; Tsunaki-machi; Ashikita-gun.

Date of Birth: January 20, 1897.

Date afflicted: 1960.

Date of death: June 22, 1972 (75 years old).

Years transpired: 12 years.

Family history: Sensory disorders and lack of coordination noted in
                 wife (61 years old) (Tachizu).

Life history. Former marine carpenter; loved to eat fish; consumed fish
three times a day; ate fish until about March or April of 1971.

Current medical history. Unable to work well because of impairment of
feet, hands, and eye from around 1960 (63 years old); a large flow of sputum;
paralysis of hands and legs since 1962; difficulty in speaking from 1964;
difficulty in walking since 1965; unable to express himself due to indistinct
words; bedridden since 1969; since symptoms progressed slowly did not know
whether diesease was due to old age or Minamata disease.

Examined at the Psychiatric Department of this University on June 3,
1972. 1) Was unable to change body position on his own power when lying down;
both legs crooked at knee joint, with right leg extending outward, and left
leg upright. Peculiar deformity also noted in fingers: left fingers were
crooked at first and third joints; extended at second joint; in the right
hand, the first finger was crooked at the first joint and rested atop the
second finger.

2) In the upper extremities, muscular atrophy was particularly marked
around the middle finger, small finger, thumb, mid-palm, near the elbow of
the forearm, and from the thigh to the shank. 3) Constantly hollered
and occasionally motioned, as if brushing thing aside with hands. 4) Rigid-
ity: slight degree on head (+), moderate to advanced degree in upper extremi-
ties on either side; rigidity in lower extremities on either side, unable to
stretch beyond 30 degrees. 5) Slight paralysis on either side, moderate

305

loss of coordination in right hand; slight degree of voluntary tremor (+).
6) Evasive reaction against painful stimulation noted in upper arm and shoulders, even though absent in back of hands and sole of foot. 7) Great deterioration of hearing capacity and vision; no blinking reaction even when eye was poked; slight cataract in left eye. 8) Talks nonsense. 9) Furrows on tongue. 10) Grasping reflex on both hands, and partial resistance in upper right extremities. 11) High degree of intellectual and personality disorder lacking inhibition, lack of emotion. 12) Blood pressure: 185/100 mm Hg. 13) Constantly complains of headache and demands massage of legs by spouse. 14) Requires nursing care. The patient was diagnosed as having Minamata disease because of the foregoing symptoms.

The patient developed fever on May 30, 1972, lost appetite, and also developed bronchial pneumonia; succumbed on June 22, 1972.

The patient was subjected to autopsy examination, while his application of October 16, 1971, for designation as a Minamata disease patient was still pending.

Pathological Considerations

Autopsy examination (autopsy no. 6013)

1. Minamata disease: cerebral atrophy (1100 g)

1) Extensive fallout of nerve cells in cerebral cortex (optic center and other areas).

2) Spottiness in cerebral cortex.

3) Centric atrophy of cerebellum (slight): i) deciduation of granular cells; ii) apical scar formation; iii) spottiness in medulla of cerebellum.

4) Peripheral nerve changes: i) obliteration, especially of posterior root and sensory nerves, regeneration, and scar formation; ii) increase of Schwann's cells.

2. Senile changes

1) Arteriosclerosis: i) hardening of arterioles and atrophy of the kidney; ii) hardening of the coronary artery and a slight growth on heart muscle, changes in heart muscle; iii) changes in other arterioles, including arteriosclerosis.

2) Softening of the brain: left diencephalon — microsoftening of cerebrum, cerebellum and pons.

3) Atrophy of various visceral organs (liver, spleen).

3. Bronchopneumonia

4. Stones in kidney, bladder, and hydronephorsis

5. Rigidity of limbs

Cause of death: bronchopneumonia

General External Appearance and Visual Observation
of the Nervous System

The skeleton was of moderate development; general loss of weight, muscular atrophy throughout body, especially in the limbs; deformity and rigidity of small fingers on left and right hands in upper extremities; knee joint crooked and rigid to about 120 degrees, and rigid foot joints in lower extremities, bedsores under first toe of left foot, another 3 x 3 cm in size on heel, and another 8 x 8 cm in size on the small of the back; the heart weighed 210 g; coronary artery was hard, and followed a curved course;

diffusion of focus associated with bronchopneumonia and bleeding spots seen in divided side of the lungs, which was the cause of death; the kidney weighed 90 g, with a countless number of stones, ranging in size from pebbles to that of a soy bean, in the renal pelvis cavity on either side; as a result of the stones, the ureter was enlarged and hydronephrosis was present; also, many stones were evident in the bladder.

The brain weighed 1100 g, with indications of general atrophy, slight hemorrhage under the arachnoid membrane at the tip of the frontal lobe, and turbidity of the cerebral membrane in the same area; relatively large soft spots on left diencephalon on the divided side, also micro-soft spots in the cerebrum, cerebellum, and pons; relatively stronger atrophy in the cortex of the occipital lobe on either side, compared to the other areas; slight atheroma in brachiocephalic trunk artery.

Histological Observations of Cerebrospinal Peripheral Nerves

Cerebral hemisphere: Relatively strong atrophy of the cortex in the optic area of the occipital lobe; greater cleavage in falx cerebri groove; thinning of layer structure, with reduction of conical cells (Photo 21); microscopically, spongy condition noted (Photo 22); diffusive multiplication of glia cells (Photo 22); diffusive spottiness in medulla, and reduction of medullary line.

Similar changes, although to a different degree, in other areas of the cerebrum, with fallout in front center and reduction of Betz cells (Photo 23); considerable fallout of nerve cells from Layer IV to Layer V; increase of pigment, and consumptive changes in remaining nerve cells (Photo 24); also, similar changes in the frontal lobe (Photos 25 - 26).

Diencephalon: Slightly soft spot in the white matter on the left side, and many spheroid bodies in the surrounding area; spottiness following softness visible by staining the medullary sheath.

308

Photo 21.   Falx cerebri on cerebrum of No. 6013 (KB-stained); thinning of the cortex, accompanied by marked atrophy; coarseness of meninges; marked fallout of nerve cells.

Photo 22.   Enlarged view of cortical III-IV layers in optic center of No. 6013; spongy condition accompanied by strong fallout of nerve cells and sporadic increase of glia   cells; remaining nerve cells vanishing as consumptive changes occur.
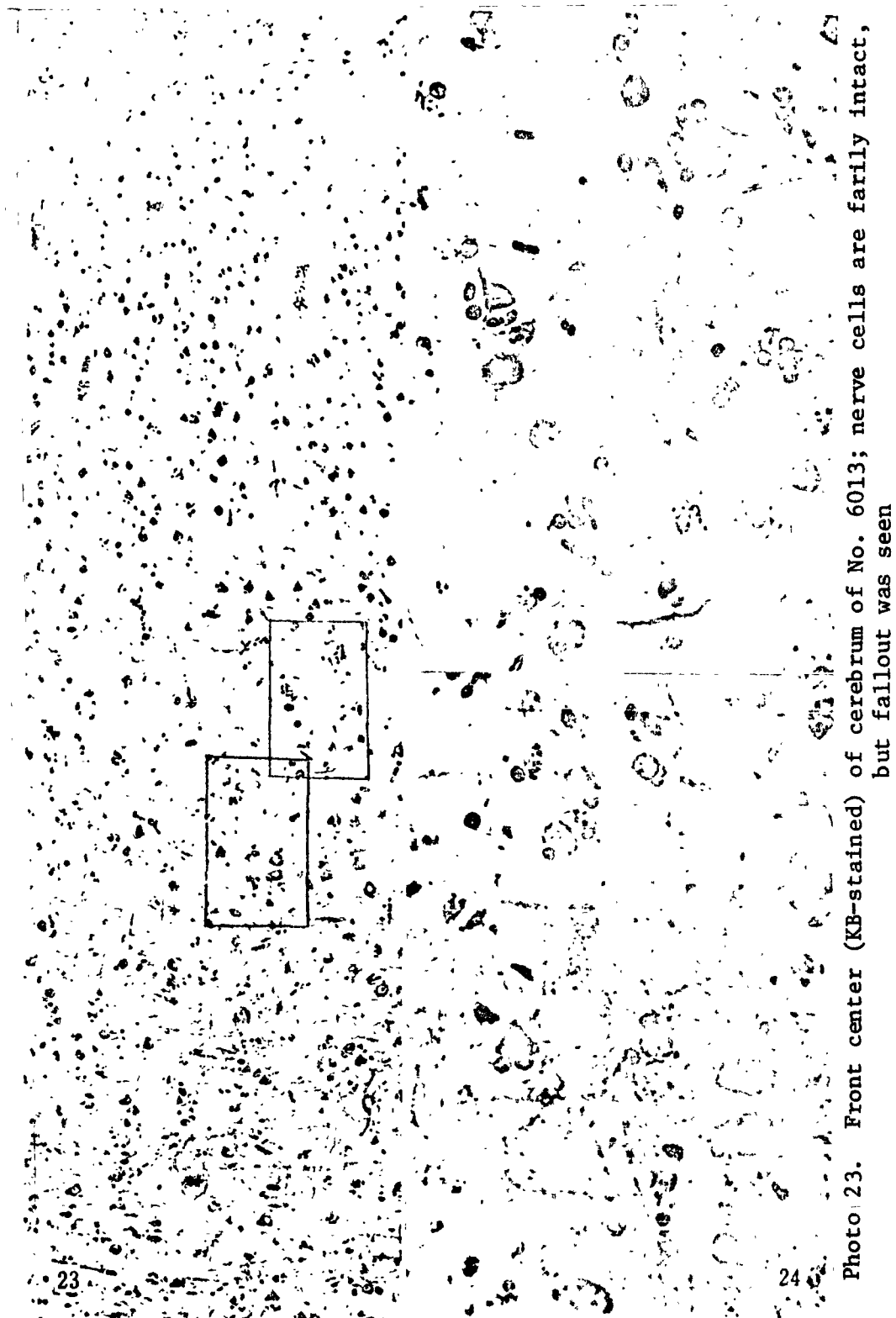
Photo 23. Front center (KB-stained) of cerebrum of No. 6013; nerve cells are farily intact, but fallout was seen

Photo 24. Enlarged view of part of Layer III and Layers IV - V of the same area in the same case (No. 6013); distinct fallout of nerve cells, consumptive changes in remaining nerve cells, increase of pigment, atrophy and hardening, consumption, and glia increase

310

Photo 25. Fallout and increase of star-shaped glia in frontal lobe cortex and occipital lobe of cerebrum of No. 6013; many remaining nerve cells undergoing consumptive changes as hardening, atrophy, and increase of pigment

311

Photo 26. Enlarged view of Layer III of same region in same case (No. 6013), showing similar observations

Photo 27. Cerebellum of No. 6013. Diffusive fallout of granular cells due to Minamata disease, accompanied by localized, vascular scars

Pons: Micro-soft spot in the middle neurons intact.

Medulla oblongata: Spheriod bodies in the surrounding area; spottiness in the surrounding area of 4th ventricle; increase of glia; olive nucleus intact.

Cerebellum: Atrophy around the middle; partial fallout of Purkinje cells; slight fallout of granular cells; apical scar formation; scars following development of vascular micro-spots in some areas (Photo 27).

Spine: Slight spottiness in posterior fibers of cervical vertebrae; many spheroid cells; reduction of anterior horn cells; absence of spottiness in thoracic vertibrae, but spheroid bodies present in the medulla; slight

312

spottiness in posterior fibers of lumbar vertibrae and spheriod boides in the medulla; anterior horn cells intact.

Peripheral nerves: Increase of Schwann's cells and scar formation in posterior root nerves; countless microfibers found by staining of axons and regeneration in process; medullary sheath intact and of normal size in anterior root nerves, partial increase of Schwann's cells and few microfibers seen.

This case involved diffusive, cerebral pathological changes associated with chronic Minamata disease, and localized vascular and senile, consumptive changes.

Case No. V.   Taki, Ta; female; Taimodo, Minamata-shi.

Date of Birth: December 24, 1886.
Date afflicted: Summer of 1954.
Date of death: January 4, 1972 (85 years old).
Years transpired: 17.5 years.
Family history: Grandson had fetal Minamata disease; mercury level in his mother's hair was 72.9 ppm in December, 1968.

Life history. Fished with fishing pole or net until 1954; ate all the fish and shellfish caught.

Current medical history. Numbness below the hip, sore foot, tremor of hands and inability to hold plates or food since summer of 1954; inability to walk without collapsing or hurting herself; such conditions temporarily alleviated around 1965; then stupefaction and inability to do anything by herself. As of July, 1971, suffered from numbness of hands, tremor, hearing difficulty, acataphasia, flow of sputum, and virtual inability to walk.

Was examined by Dr. Mishima of the Internal Medicine Department, Minamata Municipal Hospital on July 6, 1971; found to have poor judgment, loss of

memory; occasional emotional incontinence, uncooperative during examination; lacks facial expression; virtually deaf; slow tongue movement; some muscular strength in upper extremities; absence of tremor or speech disorders; coordination, sensory disorders and vision undetermined because of inability to perform test, due to imbecility of patient.

On December 29, 1971, the patient appeared to have less strength than usual and could not even hold an apple to her mouth, although there was no change in the facial color. On the next day, on December 30, the patient was able to pick pieces of fish with her own fingers, but often dropped them around her. On January 1, 1972, the same conditions persisted; on the evening of January 3, was unable to sit on her own power and could not talk; could not swallow food, and eyeballs drooped and were still. She succumbed the following day on January 4.

The patient was diagnosed as having softening of the brain; she had applied for designation as a Minamata disease patient on June 16, 1971.

Pathological Considerations

Autopsy examination (autopsy no. 5952)

1. Minamata disease: cerebroatrophy (1,150 g)

1) Extensive fallout of nerve cells in the cerebral cortex (optic center and other cortex).

2) Spottiness in medulla of the cerebrum.

3) Centric atrophy of cerebellum (slight): i) fallout of granular cells; ii) apical scar formation.

4) Fallout of anterior horn cells in the spine; slight spottiness of posterior fibers.

314

5) Pathological changes of peripheral nerves: i) obliteration and regeneration of posterior root, sensory nerve fibers and scar formation; ii) increase of Schwann's cells.

2. Senile changes

1) Arteriosclerosis: i) hardening of arterioles and atrophy of the kidney of the arteriosclerosis type; ii) papilloma on heart muscle; iii) other pathological changes of the arteriole-arteriosclerosis type.

2) Softening of the brain (diencephalon; other small, localized softening of the cerebrum and cerebellum).

3) Atrophy of various visceral organs (liver, spleen).

3. Chronic bronchitis.

Cause of death: softening of the brain and Minamata disease.

General External Appearance and Visual Observations
of the Nervous System

The skeleton was of normal development; slightly undernourished; no muscular atrophy; heart weighed 450 g, with hypertrophy in left ventricle (3 cm thick); no marked visual changes except atrophy of the liver and spleen; general atrophy around the brain, especially in the occipital lobe; atrophy of falx cerebri in the occipital lobe on the divided side, with deeper cerebral fissure; small softened area on left diencephalon.

Histological Observations of Cerebrospinal
Peripheral Nerves

Cerebral hemisphere: Layer stricture intact in the optic area of the occipital lobe, but thinning and fallout in certain areas, yielding a microscopically spongy appearance (Photo 28); increase of consumptive pigment and consumptive atrophy and loss; reduction of conical cells; diffusive
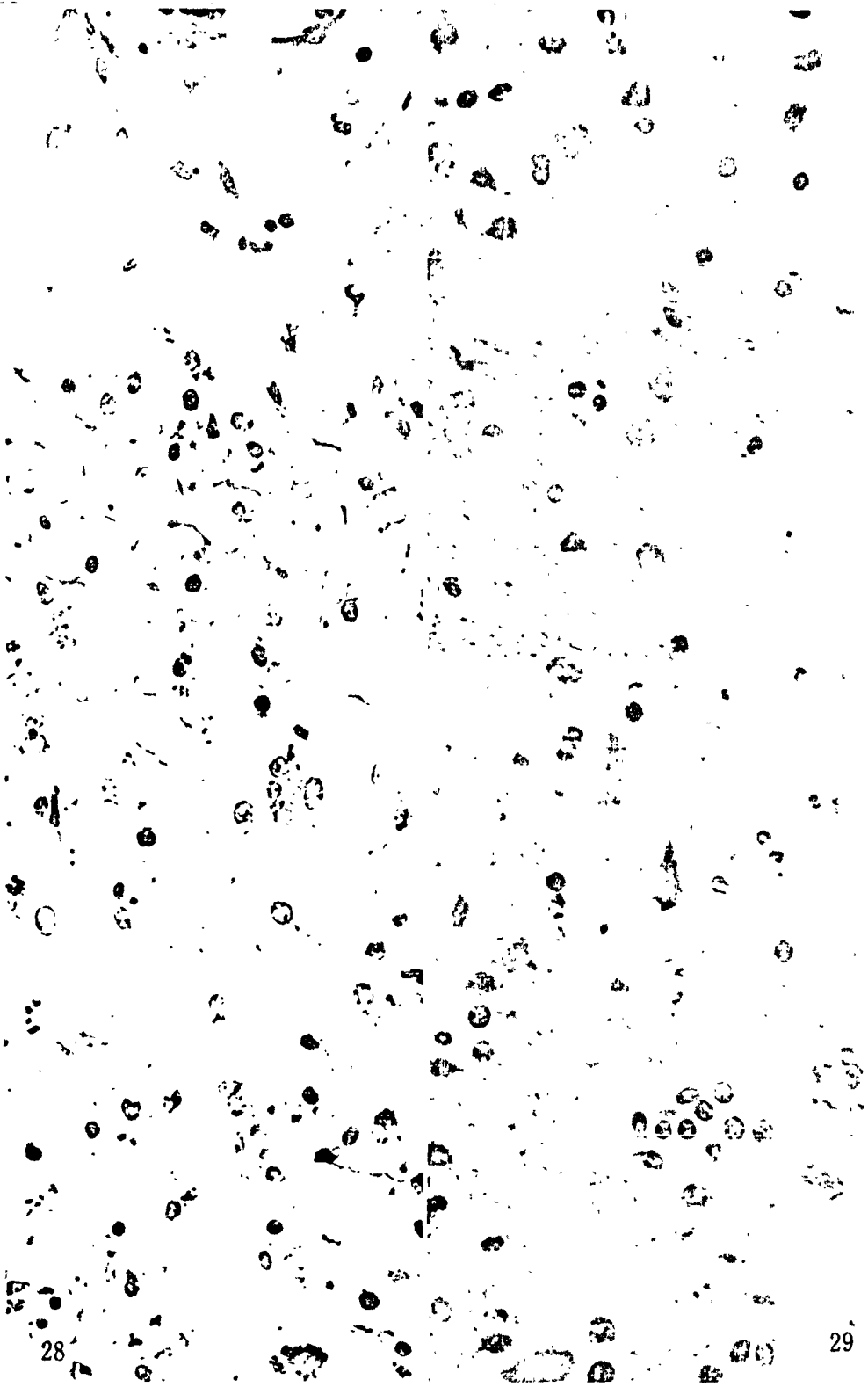
Photo 28. Optic center of cerebral cortex of No. 5952. Marked fallout of nerve cells, and spongy appearance

Photo 29. Part of cortex of cerebral temporal lobe of No. 5952, showing fallout of nerve cells and consumptive changes in remaining nerve cells

increase of glia cells; diffusive spheroid bodies, especially in the ridges, as spottiness progressed in Layer I; diffusive spottiness deep in the medulla, with spheroid bodies in the vascular area; coarse meninges, with thickening of the outer membrane of blood vessels; signs of calcification, subsequent to softening, in a part of the cortex of the occipital lobe (mid-position) and scar formation, the boundary relatively clear, but with spottiness in the medulla underneath; spottiness in Layer I in the occipital lobe, and diffused spheroid bodies; slight fallout trend in Layer 5, but relatively intact in Layer III, although hardening of nerve cells noted; few cells in Layer IV; considerable Betz cells in Layer V, with precipitation of brownish pigment and reduction of small cells; diffusive spottiness in the medulla; sign of localized softening in the medulla of the gyrus, as well as vascular, patho-logical changes.

Similar cortical, pathological changes in the frontal lobe and temporal lobe, accompanied by diffusive fallout and cells undergoing consumptive changes, atrophy and obliteration (Photos 28 - 29); glia cells localized and increasing in the fallout area of nerve cells (Photo 29), but generally limited in number, with star-shaped glia being notable (Photos 30 -31).

Signs of empyemic softening in the left diencephalon, extending from the inner covering to lens neurons, with many fat cells and spottiness in the surrounding area, together with numerous spheroid bodies; spottiness in part of the thalamus; hyperplasia of the inner membrane of blood vessels, morti-fication of middle membrane and partial calcium precipitation.

Cerebellum: Marked apical scar formation around cerebellum; slight fallout of Purkinje cells; atrophy in mid-cerebellum notable, but changes were not great (Photo 32); in the area of glia increase in the molecular layer and fallout of granular cells, reduction of the medullary sheath noted through KB-staining, but not extensive, diffusive spottiness; nerve cells having odontoid nuclei were intact; as for the walls of blood vessels in the meninges, hyperplasia of the outer membrane was noted, but not so much in the inner membrane.

Figure 30. Cerebral occipital lobe
of No. 5952. Diffusive increase of
star-shaped glia associated with
fallout of nerve cells

Figure 31. Partial expanded view of
same case (No. 5952). Increase of
star-shaped glia

Mesencephalon: Various nuclei
intact.

Medulla oblongata: Spheroid
bodies in the surrounding area; olive
nuclei intact; spottiness in part
of the 4th ventricle, and an increase
of star-shaped glia cells; calcium
precipitation directly below upper
covering of cerebral cavity; no
changes in various nuclei.

318
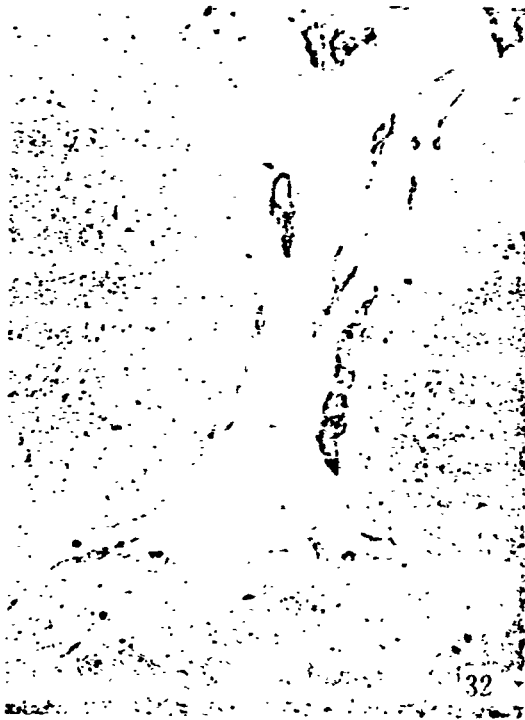
Figure 32. Area near the 4th
ventricle of cerebellum of No. 2592.
A peculiar apical scar formation is
seen in modest pathological changes
and partial fallout of cells
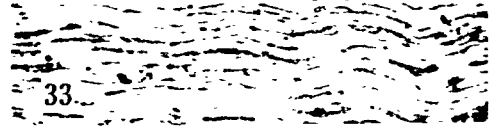
Figure 33. Regeneration of posterior
root nerve fibers

Spine: Few anterior horn cells
in spine; spottiness in posterior
fibers; loss of posterior horn nerve
cells and brownish pigment precipi-
tation; fallout of anterior horn
cells of the thoracic vertebrae, and
changes in transverse fibers; an-
terior horn cells intact in lumbar
vertebrae, but slight changes in
posterior fibers.

Peripheral nerves: Achromatism
of the medullary sheath, increase of
microfibers, and permeation of few
lymphocytes in the posterior root; some increase of Schwann's cells, partial
scar formation, and relatively uniform fibers in the anterior root.

This case involved pathological changes brought on by chronic Minamata
disease, accompanied by localized, vascular and senile changes.

IV. SUMMARY AND COMMENTS

The five cases of autopsy are unique in that the early symptoms of Mina-
mata disease, such as sensory, cerebellum and cerebrum cortex disorders,
gradually developed and with the passage of time induced the typical symptoms
of Minamata disease. As the symptoms accompanying the phenomenon of growing
old were diagnosed and clarified, the emergence of four cases of chronic

319

Minamata disease among the elderly involving senile, pathological changes, and one case (No. 5987) involving the emergence of symptoms as one grew older was interesting.

1. Acute symptoms induced by advancement in age
    and their pathological changes

No. 5987 is a typical case. This patient was afflicted with a sensory disorder, beginning with numbness of limbs in 1969 (61 years old), followed by a loss of coordination, articular and intellectual distrubances. As symptoms were relative light, she neglected medical care and, finally, in September, 1971 (63 years old), visited the Neuropsychiatric Department of this University, when she was diagnosed as a probable case of Minamata disease. The patient had previously been hospitalized for heart and liver ailments, but Minamata disease could not be detected at the time. Biopsy revealed localized, vascular changes in the brain, with pathological changes [2] of Minamata disease apparent over an extensive area. A look at the behavior of nerve cells revealed fallout of nerve cells, a manifestation of the peculiar distribution existing at the time of methyl mercury poisoning, and also increase of pigment precipitation in remaining nerve cells that occurs in advanced age, sclerosis, atrophy, and consumptive changes. The mercury level in the visceral organs was already normal, even in the brain where mercury can easily settle.

Judging from our previous examination [3] of the mercury level in visceral organs, it was our belief that such a level could not have developed during the three-year period since the patient's exposure to methyl mercury contamination. Rather than initially being afflicted in 1969, the patient probably had hidden or incomplete symptoms from before; as the patient advanced in age beyond 60, fallout of nerve cells in Minamata disease became aggravated by consumption associated with senile, consumptive changes, thereby exposing Minamata disease symptoms. Symptoms such as these which appear with advancing age take on a different meaning from that of late symptoms of mercury level as expounded by Tsubaki and others [4], in that the latter

320

even strikes the young, whereas the former strikes about the time when one advances into the senile period. We would like to name such symptoms as age-accumulative, late symptoms, a type of chronic Minamata disease.

Cases 5972, 6013, and 5952 can be considered as age-accumulative late Minamata disease, symptoms of which appeared 10 to 17 years later. At the onset, the symptoms were slight, and the patient believed it to be due to old age, neglecting any medical care. As time progressed, sclerosis type vascular changes took place, masking the typical Minamata disease symptoms until much later. Diffusive, peculiar pathological changes of Minamata disease were found to be linked with localized vascular changes and senile consumptive changes.

2. Chronic symptoms of Minamata disease

The chronic symptoms of Minamata disease consist of the aforementioned age-accumulative, Tsubaki's, and others' late symptoms and the common chronic symptoms. Clinically, it is sometimes possible to distinguish between them, and at other times it is difficult to do so.

No. 5946 is a typical common case of chronic symptoms. This patient was aware of hand tremors since 1957, but felt that it was due to drinking sake. The tremors gradually became worse, and during the two years that elapsed, sensory and articulation disorders developed, as well as walking and hearing difficulties and malnutrition. After diagnosis as Minamata disease on October 14, 1959, he developed abnormality of the joints, such as articular disease, finger deformity, and joint stiffness, and was hospitalized in 1965 for rehabilitation purposes. Gradually, he became incapacitated, with all the symptoms of Minamata disease appearing. A biopsy revealed typical Minamata disease changes, particularly fallout of nerve cells of the cerebral cortex, a microscopically spongy optic center, fallout of nerve cells in the front center), secondary changes in the spinal, conical groove section, and atrophy and fallout of anterior horn cells, and changes in posterior fibers, which usually occur after a lengthy period of Minamata disease. Since the

patient definitely had articular rheumatism and condyloma-type endocarditis, joint and finger demormity was not believed due to Minamata disease alone. However, this case was interesting in that chronic symptoms of Minamata disease were established and were gradually progressing. The fact that the patient, despite his condition, lived until age 79, indicates that death from Minamata disease is attributable only to acute symptoms, without directly affecting vital organs. The same can be said of Cases No. 5972 and No. 5952, both of whom lived until ages 81 and 85, respectively.

3. Progress of chronic conditions of Minamata disease

Even acute cases of Minamata disease, if not of the intensive type, can survive for a long time, just as chronic cases do, but will die from added complications or recurrent pathological changes. We shall not delve into long term cases, since they were the subject of previous reports a few years ago. The five cases of autopsy discussed herein fall under the chronic long-term category.

4. Mercury level in autopsy cases

Changes in the mercury level in cases subjected to autopsy may be viewed in the light of total Minamata disease-related autopsies conducted by our faculty. Generally speaking, the mercury level in most visceral organs, in the case of Minamata disease, returns to normal after 2 or 3 years, except in the brain. In Cases Nos. 5987 and 6013, however, the level had already returned to normal. In the former case, clinical records showed that three years had elapsed, but pathologically it was believed to be considerably longer. Sixteen years had elapsed in the case of No. 6013, so there is nothing mysterious about the level being back to normal.

The problem concerns the typical, complete-type Minamata disease seen in No. 5946, where the aggregate mercury level in the liver was 10 ppm wet weight, and 1.5 ppm in the brain, or two digits higher than the normal rate.

The methyl mercury value was, of course, near normal. Even though the mer-cury found in the brain was in the form of inorganic mercury, it was possible that at the outset, it was organic mercury exceeding the cerebral vascular shelf. Therefore, despite the reduction, we feel that the effects of mercury were felt not only at the onset, but for an extended period of time. Thus, we believe it necessary to consider the chronic progress of symptoms and their gradual aggravation (see Table 3).

V. CONCLUSION

Based on a study of 10 cases of autopsy covered in last year's report and the current 5 chronic cases, the following conclusions were reached.

1. At the onset of Minamata disease, not only acute but also chronic symptoms are involved.

2. In addition to the common chronic symptoms, a chronic symptom of age-accumulative, late Minamata disease exists, evoked by hidden Minamata disease striking about the time a person enters the senile period. This is different from late Minamata disease symptoms occurring as a result of mer-cury as claimed by Tsubaki.

3. In age-accumulative, late Minamata disease, senile consumptive changes occur in nerve cells, as peculiar pathological changes develop in the nervous system.

4. Neurological changes in chronic Minamata disease do not greatly differ from those of the earlier long-term Minamata disease.

5. In chronic Minamata disease, neurological changes did not appear to have a direct effect. However, neurological and mental symptoms were at times aggravated by senile changes or vascular disorders.

6. The mercury levels in long-term cases were back to normal, but in some cases were high, probably due to residence in the Minamata district.

REFERENCES

1. Takeuchi, Tadao, et al. Pathological Study of Minamata Disease After 10 Years (Part 1), Particularly Autopsy Cases of Minamata Disease AFter a Lapse of 10 Years, and Their Characteristics. Report of March, 1972. Kumamoto Medical Association Journal, Vol. 46 (11), 1972, pp. 666-705.

2. Takeuchi, T. Pathology of Minamata Disease. (Kumamoto University) Shuhan Co., 1968, pp. 141-228.

3. Takeuchi, Tadao, et al. Accumulation of Mercury in the Human Body and Its Changes — Observations on the Basis of Minamata Disease Autopsy Cases. Section on pathological study of this report.

4. Tsubaki, Tadao., et al. Personal notes.

5. Takeuchi, Tadao, et al. Two Cases of Autopsy of Minamata Disease Involving Death from Semi-Acute Conditions. Kumamoto Medical Association Journal, Vol. 33 (Supplement), 1961, pp. 602-613.

9. 2) Pathological Changes in Islets of Langerhans as Seen in Minamata Disease Autopsy Cases

Second Seminar, Pathology, Department of Medicine,
Kumamoto University

Team member: Tadao Takeuchi
Research assistants: Takaji Shigenaka, Kazuko Sato

I. PREFACE

From the results of our autopsies and numerous research reports, it is clear that in Minamata disease, the nervous system is affected the most by methyl mercury. As for the effect of mercury on common visceral organs other than the nervous system in humans and pathological changes thereof, almost no studies have been conducted, except by Takeuchi and his associates [1].

Takeuchi and his associates reported that in acute and semi-acute periods of Minamata disease, symptoms that developed were diffusive inflammation of the digestive tract, particularly, the duodenum; partial or scant formation of bone marrow; atrophy of lymph glands; and fatty changes in the liver and kidney. Shiraki [2] claimed that arteriosclerosis may be on the increase in adults with Minamata disease, since pathological changes resembling arteriosclerosis were present in fetal and infantile Minamata disease. This problem requires further study, coupled with studies on artificial nutrition and complex environmental livelihood factors.

We are currently in the stage of reviewing the effects of Minamata disease on common visceral organs. In the course of such review, we happened to observe relatively marked pathological changes in Langerhans islets of the pancreas, as reported below.

II. STUDY MATERIALS AND METHOD

From the 37 cases of Minamata disease-related autopsy, those most suit-

able for the tests following death were selected. Since chronic cases involved mostly the aged with various changes in the spleen which make it difficult to determine whether they were due to the effects of methyl mercury, it was decided to focus our attention on six cases — three infantile and an equal number of fetal cases. An additional 7 acute cases were probed, in an attempt to observe relatively new changes. Six cases with chronic symptoms were also observed. For control, infantile cases were primarily used, adult cases being considered uneventful.

References were made to autopsy records, and then microscopic specimens. From preserved visceral organs, tissue fragments were sliced and specimens made of portions of the head, body, and tail of the pancreas. Poorly stained ones were discarded. Thus, there were several instances wherein specimens made at the time of autopsy had to be relied upon.

For staining, the usual pathological-histological method was combined, primarily hematoxylin-eosin stain, Masson's trichrome stain, Azan's stain, and van Gieson's stain. In the most recent case, the Gomori stain was also used.

III. FINDINGS

1) Changes in the pancreas in fetal and infantile Minamata disease

a) Reduction of the islets of Langerhans and islet cells

In the pancreas, the number of islets of Langerhans vary depending on the area, but are generally more numerous in the tail section than in the head. Thus, observation was concentrated in the tail tissue, but as the tail tissue of each of the fetal and infantile Minamata disease cases could not be obtained, the tissue from the body near the tail was observed. The findings are shown in Figure 1.

326

In this diagram, the lateral column shows the greatest number of islets found in a 10 x 10 x 1 visual field of a single body, and the perpendicular column — the number of islet cells within an islet. From this diagram, it can be seen that the number of islets and islet cells is greatly reduced in the 6 Minamata-diseased infants as compared to the 4 control cases. While there are no distinct differences between the infantile and fetal Minamata cases, it can be generally stated that changes in the fetal group appear to be more conspicuous, there being a marked reduction in a 13-year-old.

2) Histological cases

● – Minimata diseased infant
x – Control children

Figure 1. Observation of islets of Langerhans in fetal and infantile Minamata disease — a comparison of the number of islets and islet cells

No acute changes were found in the pancreas. However, in an autopsy of an individual who succumbed around 1959, new changes were found in a part of islet cells, marked with variations and disintegration (see Photos 2-3). Since there was a time lapse of from two and one-half to 13 years in most cases, the changes were chiefly of a chronic nature, with reduction and atrophy of islets being noticeable (Photos 3, 4, 5, 6). Compensatory hypertrophy (Photo 6) was seen in many cases, making for irregularity of sizes. Relatively few scar formations were noted. Where present, it was an abnormality considering the age of the individual (Photos 4, 5). However, none

327

Photo 1*. Age, 6.3; fetal Minamata disease (No. 3794); fairly normal islets of Langerhans, with some 110 islet cells in cross section; such islets have radically decreased

Photo 2*. Age, 6.3; fetal Minamata disease (No. 3794); prominent islets of Langerhans
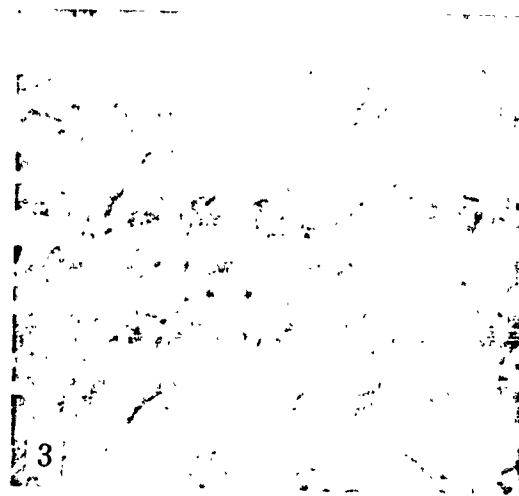
Photo 3*. Age, 6.3; fetal Minamata disease; two atrophorous islets of Langerhans, manifesting retrogressive changes in a portion

Photo 4*. Age, 6.3; fetal Minamata disease (No. 3794); Langerhans islets in pancreas undergoing atrophy and obliteration; one undergoing great atrophy; the other accompanied by scar formation

*Photos 1 - 5 — enlarged views of equal magnification.

328

Photo 5*. Same case as above (No. 3794); atrophorous islets of Langerhans showing a fibrous trend

*Photos 1 - 5 — enlarged view of equal magnification
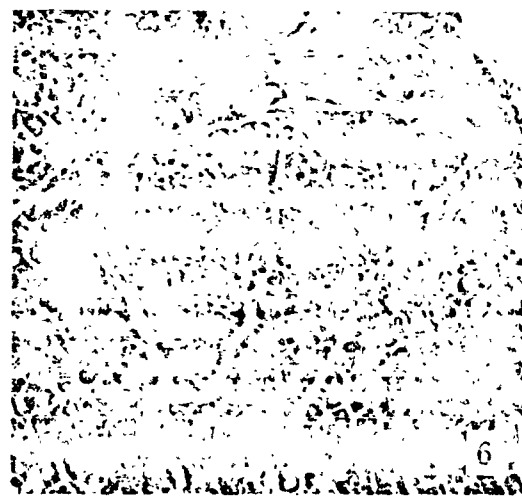
Photo 6. Weakly magnified view; age, 7; infantile Minamata disease (No. 3298); great atrophy and obliteration of islets of Langerhans in pancreas, accompanied by slight fibrous trend; conpensatory hypertrophy in the middle, and increased number of islets of Langerhans

was vitreous. Because of the irregularity of size due to reduction and regeneration of the islets, the glandular structure of the external secretory gland became unsystematic in one case (see Table, 1, Photos 7 - 12).

2) Changes in the pancreas in acute Minamata disease

From those who died of acute or semi-acute symptoms, 7 cases, all adults, who succumbed on the 45th, 53rd, 60th, 90th, 96th, and 100th day after onset, were probed. There were marked irregularities in the size of the islets, and indications of changes that had occurred in cases one and a half months after the onset of the disease. Since these conditions were observed also in younger adults aged 29 and 34, age was not found to make any difference. Variations and atrophy were seen in the islets, with many reduced in size. The number of decrease was not remarkable, except in one area; depending on the islets, there was a large reduction of islet cells. Variation and disintegration of islet cells (Photo 15) represented a new change. Although scar

TABLE 1. PATHOLOGICAL AND HISTOLOGICAL CHANGES IN THE PANCREAS IN FETAL MINAMATA DISEASE AND MINAMATA-DISEASED INFANTS

Minamata disease biopsy cases (children)	General items						Pancreas						Islets of Langerhans							Remarks
	Age	Number	Sex	Years elapsed	Date of death	Liver, ppm	External secretory gland irregularity	Fibrosis	Sclerosis of arterioles	Interstitial inflammatory changes	Fat increase	Tumor, gland increase	Mortification, disintegration	Atrophy	Decrease in number	Scar formation	Vitrification	Regeneration, hypertrophy	Size irregularity	
Stricken child	2.6	3567	♀	2.6y	3/21/61	0.3	—	—	—	—	—	—	±	##	+	—	—	±	##	*
	4	3018	♀	1.6y	1/3/58	26.0	—	—	—	—	—	.	—	##	—	—	—	±	(±)	
	6.3	379i	♀	6.3y	9/15/62	5.7	(+)	—	—	—	—	—	(+)	##	##	+	—	(+)	+)	*
	7	3209	♂	4.0y	7/24/59	5.4	+	—	—	—	—	—	(+)	###	##	+	—	(+)	+)	
	8	3216	♀	2.9y	1/2/59	6.4	—	—	—'	—	—	—	—	##	—	—	—	(±)	+)	
	13	5539	♂	13y	11/11/69	0.16	—	—	—	—	—	—	—	##	###	—	—	±	+)	*
Control child	1	5802	♀	/	3/19/71	/	—	—	—	—	—	—	—	—	—	—	—	—	—	
	3.5	5840	♂	/	5/31/71	/	—	—	—	—	—	—	..	—	—	—	—	—	—	
	4	5821	♂	/	5/10/71	/	..	—	—	—	—	—	—	—	—	—	—	—	—	
	9	5839	♀	/	5/29/71	/	..	—	—	—	—	—	—	—	—	—	—	—	—	
	12	5375	♀	/	7/21/71	/	—	—	—	—	—	—	—	—	—	—	—	—	.	

Note. By age 2.6, 6.3 is mean 2 years, 6 months and 6 years, three months, respectively.

*Fetal Minamata disease.

formation and compensatory hypertrophy denoted old changes, these could not be necessarily viewed in the same light as the underlying cause of infantile Minamata disease because of the age difference, but could be considered as attributable to growing older. A vitreous character was noted in one case (see Table 2, photos 14, 16, 17, 18).

Photos 7 - 12:

331
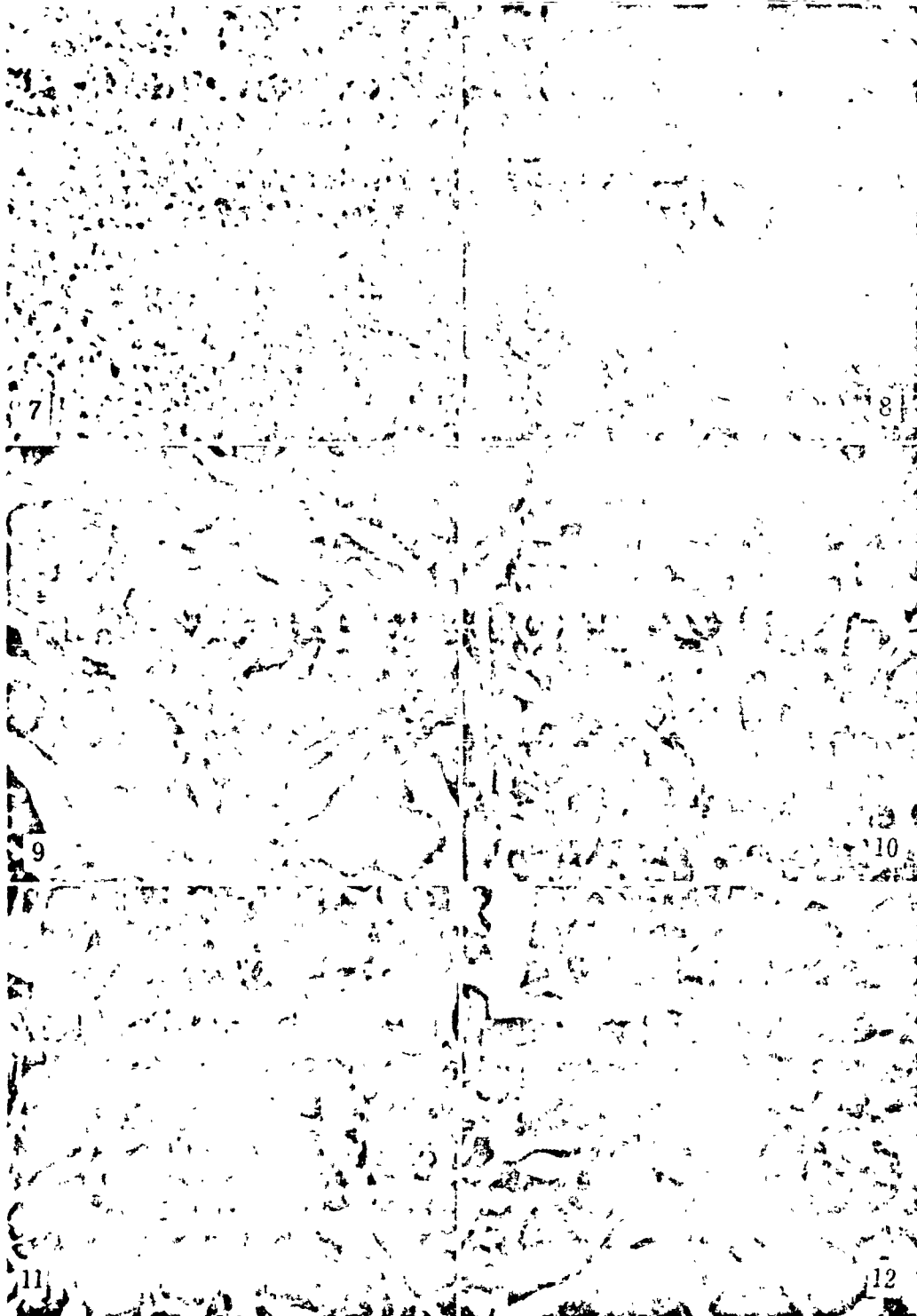
Photos 7 - 12. (continued)

Photo 7 — slightly enlarged view of pancreas of infantile
Minamata disease case (No. 3298). Relatively numerous
islets of Langerhans, the islets being small and islet
cells being few

Photos 8 - 12 — identical pancreas of No. 3298, but
greatly enlarged view; atrophy and obliteration of islets
of Langerhans in the pancreas, with some forming scars; such
changes were marked even in a child (age 7) with Minamata
disease

Photos 8 - 9 — marked atrophy of islets of Langerhans

Photo 10 — Imperfect, unsystematic regeneration following
disintegration of islets of Langerhans; indistinct boundary
with external secretory gland

Photos 11 - 12 — various images of scar formation in islets
of Langerhans; not found in children, but marked in Minamata
disease infants and accompanied by fibrous tendency

Note: Photos 12 - 18 — magnified views of same
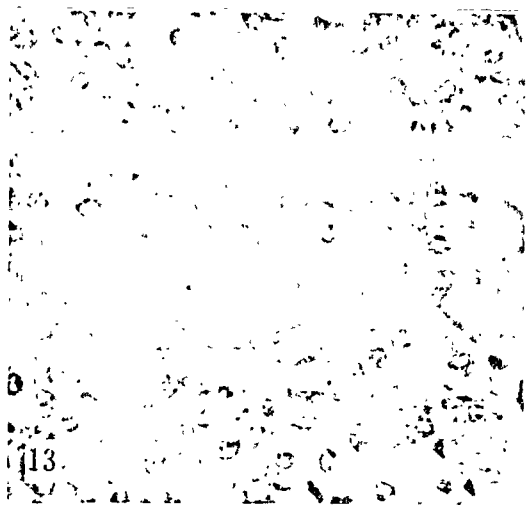
Photo 13* Islets of Langerhans of
infantile (age 6.3) Minimata disease
case (No. 3794); severe atrophy and
loss of two islets; no regeneration

Photo 14*. Semi-acute, adult Minamata
disease case (No. 3209); 90 days trans-
pired; two islets obliterated; one in
atroporous condition and in early
stages of fibrositis

*Photos 12 - 18 — magnified view of same.

332
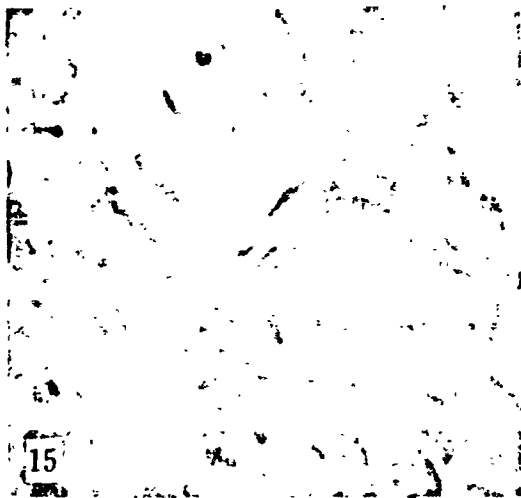
Photo 15*. Adult Minamata disease
patient (No. 3209) after 90 days;
mortification of islet cells and
fibril, vernal cells beginning to
increase
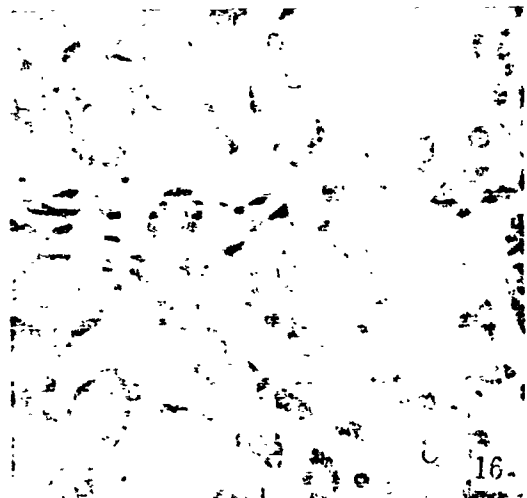
Photo 16*. Chronic adult Minamata
disease patient (No. 3497); atrophor-
ous and obliterated islet of Langer-
hans which became fibrous, and
fibrositis

Photo 17*. Fibrositis in above
case

Photo 18* Adult, semi-acute Minamata
disease patient (No. 3209); atrophor-
ous and obliterated islets of Lan-
gerhans are indistinct; unsystematic
and undergoing regeneration of ex-
ternal secretory   gland tissue

*Photos 12 - 18 — magnified view of same.

333

TABLE 2. PATHOLOGICAL AND HISTOLOGICAL CHANGES IN THE PANCREAS OF ADULT MINAMATA DISEASE AUTOPSY PATIENTS

Minamata disease biopsy cases (adults)	General items					Pancreas								Islets of Langerhans						
	Age	Number	Sex	Years elapsed	Date of death	Liver (ppm)	External secretory gland irregularity	Fibrosis	Sclerosis of arterioles	Interstitial inflammatory changes	Increase of fat	Tumor, gland increase	Mortification, disintegration	Atrophy	Decrease in number	Scar formation	Vitrification	Regeneration, hypertrophy	Irregularity of size	Remarks
Acute	29	2776	♀	53	9/3/56	39.5	—	—	—	—	—	—	—	+	—	—	—	( )	+)	
	34	3355	♂	96	12/5/59	30.0	—	(∓)	—	—	—	—	+	+	—	(—)	—	(—)	(—)	
	17	3350	♂	15	11/28/59	38.8	(∓)	(∓)	(∓)	—	—	—	—	++	(—)	—	—	(—)	(+)	
	50	209	♀	90	11/14/58	36.2	—	(—)	(∓)	—	—	—	+	—	—	—	—	(—)	(—)	*
	52	3290	♂	100	7/14/59	22.0	+	(∓)	—	—	—	—	(+)	++	(—)	(+)	—	—	(+)	
	55	3201	♀	60	11/24/58	42.1	—	—	—	—	—	—	—	—	—	(—)	—	—	(—)	*
	59	3388	♂	90	2/9/60	32.6	+	+	—	—	+	—	+	+	—	(—)	—	(—)	(+)	
Chronic	23	3139	♀	2.9	7/27/58	2.1	+	—	—	—	—	—	—	—	—	—	—	(—)	(—)	
	57	3197	♀	1.4	10/30/60	21.3	+	(∓)	+	—	—	—	—	—	—	—	—	(+)	(—)	
	60	3732	♂	2	4/19/62	8.3	+	+	+	—	—	—	++	+	—	(+)	—	(+)	(—)	
	70	4292	♀	4.3	1/15/65	2.0	+	(∓)	+	(∓)	—	—	—	+	+	—	—	(+)	(—)	*
	78	4473	♂	9.9	8/28/65	35.0	+	(∓)	+	—	—	—	(+)	(+)	(+)	—	—	(+)	(+)	
	63	4951	♀	11.3	7/9/67	0.97	+	+	—	(—)	—	—	(—)	+	—	—	—	(+)	(+)	*

Note. 2.9 for lapse of time is defined as 2 years and 9 months.

* — examination of other than tail area.

3) Changes in pancreas in chronic Minamata disease

Six cases involving a time lapse of 1 year and 4 months, 2 years, 2.9 years, 4.3 years, 9.9 years, and 11.3 years were studied, but since they were all adults and mostly of advanced age, sufficient data were unobtainable. In the chronic cases, there was no marked reduction in the number of islets, but compensatory hypertrophy and irregularity in size of islets was seen. Atrophy

334

and loss tended to be less than in infantile Minamata disease of acute or semi-acute cases. Scar formation was noted, which can be explained by the age difference.

IV. SUMMARY AND COMMENTS

As already indicated by some clinical practitioners, diabetes was sometimes found in Minamata disease patients. Whether this was due to the effects of organic mercury, or somehow developed independently, was unknown. It is known, however, that diabetes will induce diabetic neuropathy. Should peripheral nerve disorders become the main symptoms in a light case of Minamata disease, and diabetes is confirmed, it may become clinically difficult to distinguish it from diabetic neuropathy and effect the diagnosis of Minamata disease.

Okamoto and his associates [3] found in a detailed study that islet cells biologically contain zinc, which plays an important part in insulin production. Their diabetic zinc theory that obstruction of the functions of zinc will readily lead to islet type diabetes is well known. Thus, substances reacting with zinc will induce diabetic symptoms. Recently, it has been found that kinohorumu [sic] will cause nerve disorders such as SMON, and also islet-type diabetes (Miyoshi [4], Takeuchi [5], and others).

It may be possible for metals, which can be substituted for zinc to produce a chemical reaction in lieu of zinc within biologically zinc-containing cells, to influence metabolism of cells. In fact, Kakiuchi, Takahashi and Suikawa [6] reported last year (1972) before the Japan Pathological Association that cobalt chloride ($CoCl_2$) obstructs B cells of the islets of Langerhans. Thus, it is possible that mercury will affect zinc metabolism, but Ammon horns containing much zinc in the nerve tissue generally remain unobstructed by Minamata disease. Of course, obstruction by Ammon horns is sometimes seen in Minamata disease biopsy cases, but not as prevalent as in the case of disorders of the optic center or cerebellum. For the above reasons,

335

we did not then devote much attention to the disorders of the islets of Langerhans in the pancreas. However, when we realized from experimental observations that methyl mercury strongly impaired filaments of certain types of cells and not of others, we calculated that a metal would produce a different obstructive reaction, depending on the type of cell. As in the case of SMON, even substances which will cerate with metals will obstruct zinc-containing islet cells, but will not obstruct zinc-bearing Ammon horns of the nervous system. Therefore, we feel it necessary to consider each phenomenon to study the metabolism and consider the peculiarity of cells.

From the foregoing, it is highly conceivable that islet cells are obstructed during methyl mercury poisoning. In fact, the changes in the islets of Langerhans found in the autopsy of Minamata disease cases represent a phenomenon which we cannot ignore. It was interesting indeed to find that marked changes had occurred in the Langerhans islets in the autopsy of adult, fetal, and infantile Minamata disease cases.

In adult cases, the complications of diabetes can be expected because of numerous old people among the autopsy cases. However, the fact that disintegration of islet cells was found to occur in chronic cases who succumbed from adult chronic symptoms or in an environment of fish and shellfish consumption is of concern. Moreover, the verification of adult-type diabetic, pathological changes in infantile and fetal Minamata disease cases is a cause for grave concern. Its prevalence in all cases, regeneration, hyperplasia, irregularity of islet cells, and the marked reduction in the number of islets and islet cells are enough to make one suspect the effects of methyl mercury.

It is to be regretted that a detailed study on the presence of diabetes was not conducted while the patients were still alive. According to Tokuomi [7], of 11 confined patients, 1 had diabetes and 2 had a slightly high blood sugar value, but not much attention was paid to them. The existence of two cases of diabetes out of 11 means that about 20% had a high blood sugar content. Furthermore, according to last year's epidemiological report by Nomura and

336

and Matsushita [8], 24 persons (3.3%) out of 725 residents of Minamata contaminated district showed a sugar reaction in a urine test, and in Goshonoura, where there is less contamination, 21 (2.1%) out of 1009 residents showed a positive reaction. However, these were simple tests in which blood sugar was not determined. Whether the foregoing level of sugar in urine is high or otherwise compared to other areas not contaminated by mercury is not known, since there was no uncontaminated area for control purposes. We are inclined to believe that the level is not so high, on the basis of observations of islets of Langerhans in our biopsy cases, for even if the islets were destroyed, regeneration and hypertrophy would follow and their functions would be maintained. However, it is true that, because of this, irregularities of size, atrophy, and obliteration and reduction of the islets are induced. It is believed that those islets unable to sufficiently regenerate sufficiently induce an increase of blood sugar, leading to sugar in the urine. Thus, it may be correct to state that pathological changes in the islets of Langerhans take precedence, with only those severe changes inducing diabetes. This theory, however, must be established experimentally in the future.

V. CONCLUSION

A study of pathological changes in the islets of Langerhans based on Minamata disease-related autopsy produced the following results.

1) It was shown that in Minamata disease there were disturbances of islet cells, commensurate changes being noted not only in adult acute cases, but in chronic cases as well.

2) In fetal and infantile Minamata disease also, the effect of disturbances of islets of Langerhans was noted, there being a reduction and obliteration of islets and decrease of islet cells.

3. The trend of pathological changes in islets of Langerhans was relatively more marked in fetal rather than infantile Minamata disease; because of the small number of cases, further study is required in this matter.

337

4) In both fetal and infantile Minamata disease, there was shown to be regeneration and hypertrophy accompanying the obstruction of the islets, thereby resulting in a relatively marked irregularity of the size of islets. There were scar formations in some cases.

5) In autopsy cases involving the aged or those over 50, differentiation between changes caused by advanced age and those by diabetic conditions could not be made.

6) Because of the regeneration pehnomena surrounding pathological changes in the islets of Langerhans in Minamata disease, sugar in the urine cannot always be established; however, any increase of blood sugar or complications of diabetes brought on by severe pathological changes should be taken into consideration.

7) Since mercury compounds could have an effect on zinc metabolism in the islets of Langerhans, a future study is required.

REFERENCES

1.   Takeuchi, Tadao. Pathology of Minamata Disease. Minamata Disease —
     Study on Organic Mercury Poisoning. (Research team, Kumamoto Univer-
     sity) Shuhan, 1966, pp. 194-282.

2.   Shiraki, H. Discussion for Neuropathology of Minamata Disease. Sym-
     posium on Geographic Neurology with Special Reference to Geographic
     Isolates. VII Intern. Congr. Neurology, Rome, 1961.

3.   Okamoto, Kozo. Experimental Pathology of Diabetes. Japan Medical
     Publication, 1951.

4.   Miyoshi, Kazuo. SMON Symptoms and Chemical Substances. SMON Survey and
     Research Council Report No. 2, 1970, pp. 198-206.

5.   Takeuchi, Tadao, Nobuyoshi Sakurama and Takaji Shigenaga. Pathological
      Changes in Islets of Langerhans in the Pancreas as Seen in SMON
      Biopsy Cases. Path. of Medicine, Vol. 83 (7), 1972, pp. 479-480.

6.   Kakiuchi, Hiroshi, Kiyonobu Takahashi and Osamu Suikawa. Pathological
      Observations of Islets of Langerhans of the Pancreas During Adminis-
      tration of Cobalt Chloride. 61st General Assembly of Japan Patholo-
      gical Association (Tokyo), 1972.

7.   Tokuomi, Haruhiko. Clinical Minamata Disease; Minimata Disease —
      Study on Organic Mercury Poisoning. (Research team, Kumamoto
      University). Shuhan, 1966, pp. 48-76.

8.   Nomura, Shigeru, Toshio Matsushita, et al. Transition and Epidemio-
      logical Study of Minamata Disease: Results of Health survey of
      Minamata, Goshonoura District Residents (Part 1). "Epidemiological
      Clinical Medicine and Pathological Study of Minamata Disease After
      10 Years." Team report (1), 1972, pp. 6-36.

10. ACCUMULATION OF MERCURY IN THE HUMAN BODY AND ITS CHANGES AS VIEWED FROM AN AUTOPSY OF MINAMATA DISEASE CASES

Second Pathological Seminar, Department of Medicine,
Kumamoto University

Team member: Tadao Takeuchi
Research assistants: Mitsuaki Eto, Ken Sakai,
Hidetoshi Kojima

I. PREFACE

Since an accumulation of mercury within an individual is required to produce methyl mercury poisoning (Minamata disease), the level of mercury needed to precipitate poisoning has been the subject of debate centered around animal experiments and Minamata disease cases. Not long ago, Takeuchi and associates [1, 2, 3] reported on the level of mercury needed to develop alkyl mercury toxicity in cats and mice. At that time, they estimated the amount of mercury in the human body required for affliction of Minamata disease, and calculated that for an adult the amount (ingested) was 0.7 mg/kg (weight) per day until a total volume of 20 mg/kg was reached, and for a fetus — 1/4 or 0.175 mg per day for a total of 5 mg/kg. They estimated that the dangerous level would be one-tenth of the above, and added that a 2 mg/kg level would require precaution.

On the other hand, Kitamura and his associates [4] came up with figures relative to the intake and accumulation of methyl mercury. Based on findings in an MeHg^{203} intake experiment in Sweden that its biological half-life was about 70 days, they calculated the lethal and toxic levels as 1000 mg and 100 mg, respectively, for humans. They stated that a minimum of 100 mg was required to produce a toxic level in the human body, but did not discuss body weight. This meant that an individual weighing 50 kg would develop poisoning if 100 mg of methyl mercury or 2 ppm (2 mg/kg) had been uniformly distributed throughout

340

his body. This would agree with the dangerous level suggested earlier by Takeuchi and others [3]. Nevertheless, no strict comparison was possible, because Takeuchi and his associates concentrated on total mercury level, while Kitamura directed his attention to the level of methyl mercury, and further because their ratio would vary depending on visceral tissues or animal species.

Apart from such calculations, we felt the need to determine, in the case of methyl mercury poisoning, how the mercury moved in human visceral organs, in the light of related problems, such as affliction through minute ingestion, probability of chronic affliction, and multiple affliction due to late symptoms or with age.

In this report, we reviewed the movement of the mercury in visceral organs, on the basis of autopsies performed by this faculty.

II. RESEARCH MATERIALS AND METHOD

Thirty-seven cases of autopsy (30 adults, 4 juveniles, 3 fetal infants) conducted at this faculty since 1956 were reviewed, of which 23 were previously reported on and 11 more were covered in a supplemental report. Since no detailed study was made on changes in the mercury level, we decided to present an overall picture at this time.

Organs selected for review were the brain, liver, and kidney of biopsy cases. Some organs, particularly the brain, were divided for observation purposes into the cerebrum and the cerebellum, but we decided to study primarily the cerebrum in keeping with the practice in early cases.

The measurement of mercury was by the Dithizone method. The Kitamura method had been adopted in the earlier period, and then the Irukayama-Fujiki method, subsequent to 1961. Both expressed the mercury present in visceral organs by total level Hg ppm wet weight.

Table 1 gives the level of mercury found in the liver, kidney, and brain at the time of death of each of the 31 cases, out of 37, whose clinical progress was comparatively well known since the onset of the disease until death (see Table 1). A graphical presentation of same is shown in Figures 1 and 2.

Observation of acute or semi-acute periods from the nineteenth to the one hundredth day from the onset of symptoms indicated that the mercury value was high in the kidney and the liver, with the former invariably showing a higher value than the latter. On the other hand, the mercury value in the brain was very low, indicating that the degree of disorder of tissue cells and the level of mercury did not always run parallel; that, rather, some relationship existed between the properties of cell types or metabolism. However, after one and a half years or more, the mercury value gradually began to decrease. In about two and a half years, the mercury value in the liver and kidney had returned to normal, but the level in the brain was still high (0.4 ppm) compared to the normal level (0 - 0.05 ppm).

TABLE 1. CLINICAL PROGRESS AND LEVEL OF MERCURY IN VISCERAL ORGANS

No. of cases	Clinical progress	Liver (ppm)	Kidney (ppm)	Brain (ppm)
1	19 Day	70.5	141.0	9.6
1	26	18.2	47.5	15.1
2	45—48	36.7	83.6	16.2
3	53—70	38.6	70.2	12.7
5	86—100	30.2	33.9	5.8
2	1.4—1.6 Yrs	23.6	36.9	7.1
1	2.0	8.3	19.6	1.0
1	2.5*	0.3	9.4	0.4
2	2.7	4.3	8.0	0.7
2	4.0—4.3	3.7	11.8	2.1
1	6.4	5.7	11.3	0.7
1	8.8	0.4	1.0	0.3
2	9.1—9.9	17.7	7.4	1.8
1	10.2	2.3	21.6	2.6
3	11.3—11.7	0.7	7.6	1.4
1	14	1.3	10.1	1.5
1	16	0.4	0.2	0.2
1	18	0.9	3.1	2.3
15	Control	0 — 2	0 — 3	0 — 0.05

Figure 1. Recent changes in the level of mercury in visceral organs of long-term Minamata disease autopsy cases
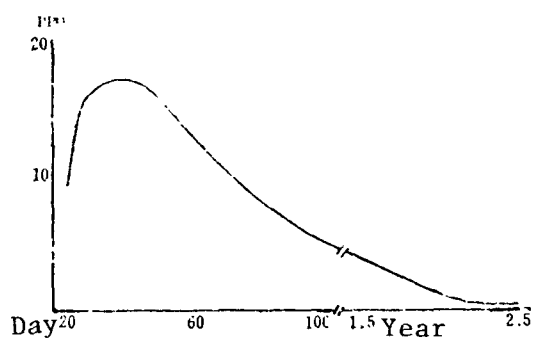
Figure 2. Changes over time in the level of mercury in the brain of Minamata disease autopsy cases

A study of the mercury value in various organs during this period showed that it differed with the organs. With respect to the liver, mercury ingested in a large amount during the initial stage decreased at a relatively rapid pace, but a large level remained until about three months, gradually returning to normal after one and a half to two and a half years. In the case of the kidney, a very high value was noted at the early stage, gradually decreasing, as in the case of the liver. However, probably because of the excretory function of the kidney, the level remained rather high, then decreasing to half in 70 days and returning, while always maintaining a higher value than the liver, to normal (0 - 3 ppm) in one and a half to two and a half years.

A unique case was that of the brain. In the early stage, the accumulation of mercury was fairly low, rising to a peak in about a month, before gradually decreasing at a very slow pace. Even after three months, the level was higher than normal by two digits. In some cases, the level was still high about a year and a half later, and did not return to normal even after two and a half years, still being one digit higher than normal (see Figure 2). Specifically, a high value of mercury accumulation was noted in the liver and kidney, but it returned to a normal value, depending on conditions. The level in the brain may be fairly low at first, but excretion of mercury, once it accumulated, was rather difficult.

It is known that methyl mercury can be accumulated in a considerable quantity, because it is easily permeated into the organ in general, and does not cause any marked disorder. In the case of the brain, it encounters difficulty because of the blood brain barrier. However, once even a small amount gains entry, a serious disorder is created in tissue cells, posing a difficulty in eliminating any accumulation.

In our cases of autopsy, we studied those in which three years or more had elapsed since affliction. Some showed a normal value in the liver and kidney, but we could not obtain definitive results because most reflected scattered mercury values, probably due to their residence in the Minamata district (see Figure 3).

As for the value of mercury in the liver, it had returned to the normal range in many cases, but some reflected an abnormally high value (35.0 ppm), with cases in which the period of affliction was unknown recording 7.99 ppm. The mercury value in the kidney likewise decreased after 3 years or more, but there were fewer cases of the level returning to normal, with most showing 10 ppm or more.

TABLE 2. MERCURY LEVEL IN VISCERAL ORGANS OF MINAMATA DISEASE AUTOPSY CASES

Case no.	Autopsy no.	Sex	Age	Time transpired clinically	Liver, ppm	Kidney, ppm	Brain, ppm
1	3159	♂	35	19 days	70.5	111.0	9.6
2	2771	♀	5	26	38.2	47.5	15.4
3	3350	♂	48	45	38.8	68.2	24.8
4	2791	♂	56	18	31.6	99.0	7.8
5	2776	♀	29	53	39.5	40.5	9.0
6	3201	♀	58	60	42.1	106.0	21.3
7	3349	♂	62	70	34.7	61.2	7.8
8	2775	♂	49	86			9.5
9	3209	♀	50	90	36.2	21.2	4.9
10	3388	♂	59	90	32.6	49.8	6.4
11	3355	♂	34	96	30.0	22.6	4.6
12	3290	?	52	100	22.0	42.0	2.6
13	3497	♂	57	Yrs. 1.3	21.3	36.5	2.8
14	3018	♀	1.5	1 5	26.0	37.4	5.3
15	3732	♂	60	2.0	8.3	19.6	1.0
16	3567	?	2.5	-	0.3	9.4	0.4
17	3216	♀	8	2.7	6.4	12.8	1.3
18	3139	♀	23	2.7	2.1	3.1	0.1
19	3298	♂	7	4.0	5.4	5.9	2.2
20	4292	♂	79	4.3	2.0	17.6	2.0
21	3794	♀	6.4	*	5.7	11.3	0.7
22	5663	♂	63	8.8	0.4	1.0	0.3
23	548?	♂	63	9.1	0.5	2.3	0.6
24	4473	?	78	9 9	35.0	12.6	3.0
25	4312	♂	66	10.2	2.3	21.6	2.6
26	4951	♂	63	11.3	1.0	10.7	2.6
27	5360	♀	68	11.7	0.1		0.1
28	5583	♂	63	11.7	0.9	4 6	2.1
29	5946	♂	79	14.0	1.3	10.0	1.5
30	6013	♂	75	16.0	0.4	0.2	0.2
31	5863	?	70	18.0	0.93	3.1	2.3
32	5346	♂	86	Un-known	8.0	28.1	2.1
33	4437	?	57	known			1.2
34	5539	♂	13	*	0.2	5.5	0.04
35	5952	♀	85	17.5	0.3	0.3	0.08
36	5972	♂	81	10.0	0.8	1.4	0.06
37	5987	♀	64	3(?)	0.4	2.5	0.03

Control (15 cases) 0—2 0—3 0—0.05

Some registered 10 ppm or more even after 10 years, or even 21.6 ppm, with a recent autopsy case in which the period of affliction was unknown recording more than 28 ppm. It was considered necessary to determine at a later date the question of whether such high values were the legacy of mercury accumulation at the onset of disease or a phenomenon created by residing in a contaminated district.
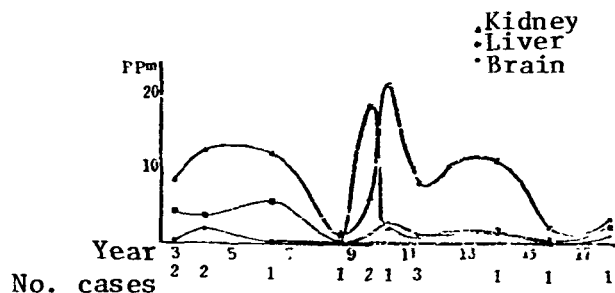

Figure 3. Changes over time in the level of mercury in visceral organs of autopsy cases

The level of mercury in the brain was noteworthy. Out of 37 cases of biopsy, the mercury level was determined in 35 cases. Of these, only two cases, i.e., one case in which the period of affliction was 13 years, and another recent case in which the period was unknown, showed a normal level, with some reflecting a one- or two-digit value higher than normal even after 10 years or more. It indicated that the mercury value in the brain could not return to normal if one resided in a contaminated district and could not be ignored, even if it were a small amount compared to that in the liver and the kidney. It should be borne in mind that the accumulation of mercury in the brain is the result of methyl mercury, and that organic mercury cannot infiltrate the blood brain barrier.

IV. SUMMARY AND COMMENTS

In their earlier, brief report on changes in the amount of mercury in visceral organs, Takeuchi and his associates [8] indicated that the brain undergoes a different sort of accumulation and change from that of the liver and the kidney. Further, Irukayama and associates [5], based on animal experiments, found that the accumulation of methyl mercury in the brain was slower than in other visceral organs during the early stage of administration, but its excretion was still slower. More recently, Shiraki and his associates

345

[6] reported that, when a monkey was administered a single dose of ^{203}Hg methyl mercury, a great amount began to infiltrate into ordinary organs immediately after administration, as observed by autoradiography, but gradually began to increase in the brain after more than a week.

We carefully studied the progress of initial symptoms by concentrating on a greater number of autopsy cases, sufficient to observe the level of mercury in visceral organs in human bodies with Minamata disease, associated with methyl mercury poisoning. In so doing, we referred to the earlier reports of Takeuchi and associates, as well as to recent information that has come to light.

1) Changes during the initial stage (acute stage)

Observation of cases in which death occurred during the early stage or acute stage of poisoning revealed that the kidney showed the highest level of mercury 19 days after affliction, followed by the liver and the brain; however, the level in the brain was extremely low as compared to that of the liver, being about 1/8, and about 1/15 of that of the kidney. That still would be about 192 times or more above the normal level of the brain, assuming it to be 0 - 0.05 ppm. Organic mercury and inorganic mercury can accumulate in the liver and the kidney, but inorganic mercury cannot infiltrate the brain. Since only alkyl mercury can cross over the blood brain barrier, it can be assumed that the accumulation in the brain of biopsy cases consists of mercury from methyl mercury. Thus, the effects of mercury accumulation on the brain should be fully studied, even though its level may be lower than in the liver or kidney. Moreover, methyl mercury is known to strongly disturb and even cause the death of brain tissue, especially nerve cells, but does not kill liver or kidney cells, even though it may cause damage (Takeuchi [7], Eto [9], Suko [10]). This was the cause of death of our case.

Twenty-six days to one month after affliction, it was found that the mercury level suddenly declined in the liver and the kidney, but still

346

remained high in the brain. This reaffirmed the findings of Takeuchi, Irukayama, and Shiraki, indicating that a similar trend existed in the human body, as in animals. It can be assumed from pathological observations of the brain that the accumulation of mercury in the brain during this period has a decisive effect on cranial nerve cells.

About two months after affliction, the level of mercury in the liver and the kidney was about half that of the early stage, which agrees with the findings of a Swedish academic group about a biological 70 day half-life. However, there were little or no changes in the level of mercury in the brain, showing virtually no decrease after two months.

Three months after affliction, a considerable decrease was noted in the kidney and the liver, but some differences were seen in the degree of change in both, indicative of the difference in the nature of the organs. The level of mercury in the brain finally started to decline, reaching almost one half between two and three months after affliction. However, its level was more than 116-fold over the normal level, causing concern about its effect on cranial nerve cells over a three-month period.

2) Changes during semi-chronic period after one to two and a half years

The cases during this period do not necessarily mean semi-chronic cases in which acute cases who survived later died of recurrent diseases, but that the symptoms persisted throughout that period. The case extending almost two and a half years involved a fetal infant who was initially stricken while the mother was 8 months pregnant, for a total affliction period of 2 years and 7 - 8 months. In this case, the level of mercury in the liver had returned to normal, and to virtually normal in the kidney, indicating that the mercury accumulation in visceral organs, in general, can be eliminated after at least two and a half years. The level of mercury in the brain at this time was 0.4 ppm.

347

It should be remembered, however, that even a small level of mercury develops symptoms in fetal cases as compared to adults, as has been established by comparison of clinical symptoms of the mother and through the study of affliction of fetal-type Minamata disease.

In the case of adults, even methyl mercury in an amount sufficient to precipitate affliction and death will decrease and return to normal in visceral organs after two and a half years, except in the brain. Even in fetal-type cases, where a relatively small amount is involved, mercury continued to persist in the brain.

3) Cases three to four years later

Cases in which the patients were stricken for a period of three to four years developed during the days when the mercury issue was at its peak, and the intake of fish and shellfish was strictly prohibited. Thus, the level of mercury in the liver and kidney was slightly higher than normal, being near the upper limits of the normal value. The level of mercury in the brain during this time showed a high value, 14 - 42 times that of the normal value, in both adults and children.

This was generally true, even after six or eight years.

4) Changes after ten years or more

With the alleged decrease of mercury contamination since around 1965, and the cessation in 1968 by the Chisso Corporation of the acetylaldehyde process, coupled with lax enforcement of regulations against consumption of fish and shellfish, some people of the contaminated districts again began to eat them, which probably was the reason for further changes in the level of mercury in visceral organs. According to the survey of Fujiki and his associates [11, 12], the mercury level had decreased to 1/20 of that during the prevalent period of Minamata disease. Neverthelss, a high value was still

348

noted in some fish and shellfish, and examinations of human navel regions revealed that methyl mercury level which reached its peak in 1951 during the prevalent period continued to show a high value until 1968. It gradually decreased thereafter, but was found to still persist in 1970. This confirmed that the return of the mercury level to normal range in certain cases was not true in all cases. With the foregoing as background, there is a need to study cases in which 10 years or more have transpired since affliction. Most were either chronic cases or mild cases at the time of prevalence which later were accompanied by complications; there were very few serious, acute cases which took on added complications.

Hence, there were many who died not from recurrent ailments, but from other types of disease. The knowledge thus acquired should benefit a future study of patients with mild or moderate degrees of affliction.

Considering such phenomena, we examined the level of mercury in visceral organs 10 years after Minamata disease affliction. Although there were individual differences, a high level still remained in the liver, kidney, and the brain. The fact that a high level persisted even after 10 years, although it was supposed to return to normal in the liver and kidney after about two and a half years from the early to middle stages, raised a question as to whether it was the legacy of mercury ingested 10 years ago. Irregularity in the curve reflecting mercury level changes was also peculiar. It may be that the accumulation of mercury observed 10 years later reflected the recent dietary habits of the residents. This theory is supported by a case (No. 4321) having a level of 20 ppm or more in the kidney, and by the recent case (No. 5346) in which a level of 28.1 ppm was recorded.

Notwithstanding, the mercury level in the liver was normal in many cases, and slightly greater in the kidney than in the liver, exceeding the normal levels in some cases. The level of mercury in the brain continued to be 10 - 150 times greater than normal even after 10 or more years.

349

Mercury in the brain is the result of entry of methyl mercury, and once there is an accumulation, it is very difficult to remove, based on observations two or three years after the initial stage. What does it portend if there is hardly any decrease in succeeding periods? Since it has been established that a small amount may be excreted in two or three months, it would be difficult to believe that it would persist even after 10 years. It could be that, even though the biological half-life in the brain may be drawn out, an accumulation could manifest itself through a balance between new accumulations and excretion. At any rate, it should be recognized that such a level of mercury is found today in the brain of Minamata disease cases among residents of contaminated districts.

Cerebro-biological half-life

The foregoing was a description of the level of mercury found in the brain, liver, and kidney of biopsy cases. In an attempt to seek the average base point between the lapse of time and the number of cases of autopsy, we established the attenuation curve over time of the mercury level by the exponential functive curve and, using the latter as base line, mathematically obtained the biological half-life. There was apprehension that the use of an average method when there was a high initial value in the mercury level in the liver and the kidney would mathematically distort the true biological significance, because of the obscure treatment of the base point. We decided to calculate the biological half-life of mercury in the brain, because the average values are stable in the case of the brain, the ingestion of mercury in the brain is similar to that in animals during experiments, and the base point is stable. Another point of consideration was that the biological half-life in the brain was tied closely to the disease, because methyl mercury caused primarily disorders of the cerebro-nervous system; further, that methyl mercury compounds entered the blood brain barrier slowly in the initial stage, accumulated slowly, and were excreted slowly.

Additionally, it was a good chance to calculate the half-life, because in cases of acute stages (19 - 100 days) or early stages of Minamata disease,

the intake of fish and shellfish was not so free as now for therapeutic and other reasons. Accumulation of mercury attributed to new contamination has been observed in long-term cases of autopsy, due to recent, renewed ingestion of fish and shellfish at the affected sites. It was found that it took about 230 days from the onset of disease to half-life.

The case of the brain is described (see Table 1).

TABLE 1

x_i (no. of days)	y_i (p.p.m)	z_i	$x_i - \bar{x}$	$z_i - \bar{z}$	$(x_i - \bar{x})^2$	$(x_i - z)^2$	$(x_i - \bar{x})(z_i - \bar{z})$
46.5	16.3	2.791	−339	1.523	114.921	2.236	− 513.65
61.5	12.7	2.541	−324	1.277	104.976	1.631	− 413.75
93	5.8	1.758	−292.5	0.494	85.556	0.224	− 111.50
510	4.1	1.411	126.5	0.147	16.002	0.022	+ 18.60
720	1.0	0	334.5	−1.264	111.890	1.598	− 422.8
880	0.4	−0.916	494.5	−2.156	244.530	1.645	−1066.14
total (t)					677.873	19.469	−2546.19
av. value $x=385.5$ $t, 6$	$z = 1.264$				$\sigma_x = 336.12$	$\sigma_x = 1.320$	− 424.37

A graphical presentation of the distribution of specimens provided (xi, yi) indicates ppm attenuation of Hg over a period of time (Figure 1, line A). The exponential function curve

$$y = b'e^{ax} \quad (b' = e^b, \; \therefore b = \log b')$$

close to the broken line formed by the specimens (xi, yi), is obtained.

The manner in which this is obtained is to estimate an approximate curve ' ' ' ', and then determine b', a.

Take the coefficients of both areas of the approximate curve $y = b'e^{ax}$ as y = log z. In other words,

$$z = \log y = \log b'e^{ax} = \log b' + \log e^{ax} = b + ax$$

Thus, the equation developed by this manipulation, z = ax + b, represents the straight line at the plane formed by the axis of x (number of days) and z (log y = z).

Against the specimen (xi, yi), zi = log yi is placed. Thus, a new specimen (xi, zi) is obtained (dark line, Figure 2). We can determine a, b
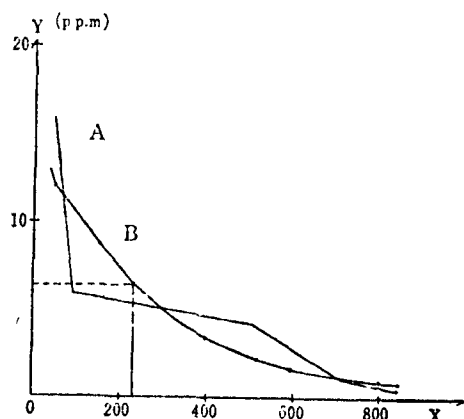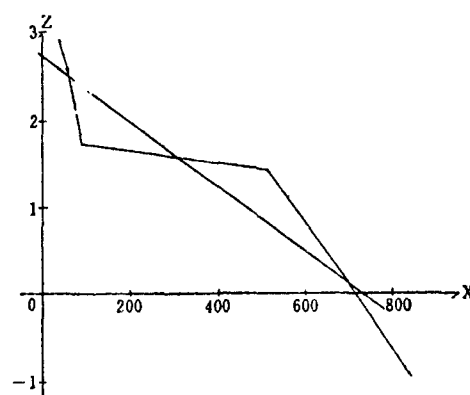

Figure 1   Figure 2

so that the distance will be the minimum between the straight line y = ax + b, having x, z as the axis of coordinates and various specimens (xi, zi). (Refer to the section on minimum self-multiplication straight line, Introduction to Easily Accessible Statistical Mathematics.) In other words,

$$a = r \frac{\sigma z}{\sigma x}, \quad b = -r \frac{\sigma z}{\sigma x} \overline{x} + \overline{z}$$

However, when (xi, zi) is indicated as $1 \leq i \leq N$:

$$\overline{x} = \frac{1}{N} \sum_{i=1}^{N} x_i, \quad \overline{z} = \sum_{i=1}^{N} z_i$$

(average value)

$$\sigma x = \frac{1}{N} \sum_{i=1}^{N} (x_i - \overline{x})^2, \quad \sigma z = \frac{1}{N} (z_i - \overline{z})^2$$

(standard deviation)

$$r = \frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})(z_i - \bar{z})$$

(coefficient of correlation)

In the case of the brain, $x = 355.5,\ \bar{z} = 1.591,\ \sigma_x = 46.1,\ \sigma_z = 1.321,\ r = 0.976.$

From the foregoing, the minimum approximate straight line z = ax + b relative to (xi, zi) was obtained. In the case of the brain, it is $a = -0.0256,\ b = 2.719$ (the straight line graph is indicated as the straight line in Figure 2). As log y = z = ax + b, thus,

$$y = e^{ax+b} = b' e^{ax} \quad (e^b = b')$$

This is one of the approximate curves according to the exponential function relative to the broken line graph (Figure 1, line A) of specimens (xi, yi) (see Figure 2).

The method of obtaining B.H. (the period from onset to half life)

If x_0 were the number of days of maximum ppm of Hg, the maximum value y_0 is determined by:

$$y_0 = b' e^{ax_0}$$

In the case of the brain, $x_0 = 46.5$ resulted in $y_0 = 2 \div 6.325$. Next, solution of

$$\frac{y_0}{2} = b' e^{ax}$$

is made for x. In other words,

$$\log \frac{y_0}{2} = \log (b' e^{ax}) = \log b' + \log e^{ax}$$

$$= b + ax \quad (\log b' = b)$$

$$\therefore \quad x = \frac{1}{a} \left\{ \log(\frac{y_0}{2}) - b \right\}$$

From the foregoing, BH x_0 was

$$x_0 = \frac{1}{a} \left\{ \log(\frac{y_0}{2}) - b \right\}$$

In the case of the brain examined, $x_0 = 230$ was obtained.

In other words, the maximum value Y_0 is determined by:

$$a = r \frac{\sigma z}{\sigma x} = (-0.956) \times \frac{1.321}{336.12} = -0.00376$$

$$b = -r \frac{\sigma z}{\sigma x} \bar{x} + \bar{z} = -(-0.956) \times \frac{1.321}{336.12} \times$$

$$335.5 + 1.261 = 2.712$$

$x_0 = 46.5$.

$$y_0 = e^{ax_0 + b} = e^{-0.00376 \times 46.5 + 2.712} = e^{2.538} = 12.649$$

$$\therefore \quad y_0 / 2 = 6.325$$

Thus, x_0 is

$$x_0 = \frac{1}{a} \left\{ \log\left(\frac{y_0}{2}\right) - b \right\} = \frac{1}{-0.00376}$$

$$\left\{ \log(6.325) - 2.712 \right\} = 239.84$$

(Above by Shoichi Sato, Assistant Professor, Department of Physics, Kumamoto University.)

Comments: Methyl mercury damages visceral organs to a relatively mild degree. Pathologically, several disorders have been attributed to it, but not as clinical symptoms. However, it affects the nervous system tremendously, almost beyond comparison. This indicates that methyl mercury is closely related to metabolism, existing only in either the brain or the nervous system, and we are pursuing a separate study to clarify its structure. Such matters are associated with the fact that a relatively large amount of mercury in the liver or the kidney produces little disorder, whereas a very small amount in the brain would have a marked effect.

Kitamura and his associates have linked the level of mercury accumulation in human beings with poisoning symptoms. This may be fine from a hygienic concept, but we thought that, in order to uncover the relationship between the occurrence of cranial changes known as poisoning symptoms, it was better to concentrate on the intake level of alkyl mercury. Thus, based on our earlier findings, an examination of the relationship between the intake level of alkyl mercury and cranial changes disclosed the following (see Table 3).

354

TABLE 3.　ALKYL MERCURY INTAKE LEVEL REQUIRED TO PRODUCE POISONING SYMPTOMS
(MINAMATA DISEASE)　(TAKEUCHI AND ASSOCIATES)

Humans and animals		Daily intake level, mg	Aggregate amount causing affliction mg
Acute, semi-acute affliction (previously) [3]	Cats, mg/kg	1.5 - 2.0	20.6 - 25.7
	Mice, mg/100 g	0.5 - 1.0	10 - 20
	mg/kg	5.0 - 10.0	100 - 200
	Humans (presumed)		
	Adults, mg/kg	0.7	20.0
	Fetus, mg/kg	0.175	5.0
	Dangerous level in human beings		
	Adults, mg/kg	0.07 mg	2 mg
	Fetus, mg/kg	0.0175	0.5 mg
Chronic affliction (current)	Adults (50 kg)	0.25 mg	Affliction in several years; > 10 years
	mg/kg	0.005 mg	
	No effect level	(1/10)	
	Adults (50 kg)	0.025 mg	Maximum no effect
	mg/kg	0.0005 mg	level

According to this table, a person 50 kg　in weight will develop an acute affliction by daily intake of 35 mg of alkyl mercury when the aggregate dose reaches 1000 mg.　A dangerous level would be reached when a daily intake of 3.5 mg reaches a total of 100 mg.　According to Kitamura, 100 mg would cause affliction in human beings, and when biological half-life is considered, it would require 10.5 days at a daily dose of 10 mg to attain a 100 mg level; 28.7 days — at 4 mg; 40 days — at 3 mg; 465 days — at 1 mg; further, that a daily dose under 1 mg would never reach 100 mg, because of half life.　In other words, Takeuchi's dangerous level and Kitamura's affliction-poisoning level are in virtual agreement.

355

Since serious cases of Minamata disease have developed acute symptoms within one to two months, such findings would mean that a daily dose of three to four milligrams was being ingested by the individual, and that chronic patients had taken about one milligram per day for over one year. The level of mercury in the brain of patients after death averaged about 10 ppm (10 mg/kg). As the weight of the brain of the patients was about 1100 g, that would mean about 11 mg accumulation in the brain. A minimum value of 2.8 mg would cause death.

On the other hand, one tenth of the level is said to cause affliction. This would mean that approximately 1 ppm or a minimum of 0.28 ppm would develop poisoning symptoms in the brain of human beings in biopsy cases. Thus, this affliction level is more than 20 times the normal level of mercury (0 - 0.05 ppm) in ordinary persons, a significant matter when considering the level of mercury in the brain of Minamata disease autopsy cases.

Observations in the foregoing manner lend credence to the claim that methyl mercury infiltrates the brain and slowly accumulates therein, causing affliction when the level reaches an average of 1 ppm or more. Such observations can probably be used to demonstrate that such developments are also possible in recent chronic affliction cases as well.

As a result of our latest study, chronic and late symptom cases are continuing to be found in the Minamata district, after it was established that affliction can be due to contaminated fish and shellfish in Minamata Bay. The wet level of methyl mercury in such marine life averaged 0.25 ppm; therefore, if an afflicted person had consumed a large quantity, he would have ingested an average of 300 g in the same district. Even assuming that he ingested a maximum of 1000 g (possible for a fisherman), the daily intake would be 0.25 mg (mercury). In other words, a person ingesting 0.25 mg methyl mercury daily would develop chronic affliction after several years, or more than 10 years.

If 1/10 of the amount represents a no effect level, that would mean 0.025 mg is the limit. Assuming that the average weight of a Japanese is 50 kg, that would mean 0.0005 mg/kg, much less than the 0.07 mg/kg which we believed to be the case in acute or semi-acute affliction, making even 1/140 dangerous. We must, therefore, change our thinking concerning mercury.

This means that the safe permissible level for an individual weighing 50 kg is 0.175 mg (mercury) or less of methyl mercury per week.

V. CONCLUSION

Study on the accumulation and level of mercury in visceral organs based on autopsy of methyl mercury poisoning cases (Minamata disease) disclosed the following facts.

1. The level of mercury in visceral organs in the case of Minamata disease depends on the type of organ; it is greatest in the kidney in the early stage, and then in the liver. There was very little in the brain.

2. The level of mercury in visceral organs changed with the passage of time, the type of change being different for each organ.

3. In the kidney, there was a marked accumulation in the early stage, which rapidly decreased and reached one half in about 70 days. At least two and a half years were required to return to almost normal, but accumulation remained in many cases.

4. In the liver, the initial marked accumulation rapidly decreased, and was reduced to half sooner than in the kidney. The level returned to normal after two and a half years, and there were fewer cases of accumulation remaining as compared to the kidney.

5. Initial accumulation in the brain reached a peak after about a month and decreased very slowly thereafter. It took 230 days from the date of affliction to be reduced to half.

6. Accumulation of mercury in the brain is thought to be due to methyl mercury. Because of the blood brain barrier, the level of accumulation was very low when compared to those of other organs. However, when compared with a normal brain, it was 200 times or more in the early stage, and one to two digits higher in value thereafter.

7. The level of mercury in visceral organs of recent autopsy cases in which the patient had been stricken for 10 or more years underwent no definite changes. This was thought to reflect the living habits of residents of contaminated districts at that time.

8. The high mercury level in visceral organs of recent autopsy cases was not thought to be due to mercury contamination more than 10 years ago — rather, a reflection of subsequent contamination.

9. With reference to comments, an average wet weight of 1 ppm or more with a minimum of 0.26 ppm mercury in the human brain will develop an affliction, and 5 ppm or more — death.

Thus, the level of mercury in the brain should be treated separately from that in other visceral organs.

10. The no effect level of methyl mercury in the human body is thought to be about 0.025 mg (wet mercury weight)/day.

358

REFERENCES

1. Takeuchi, T., et al. A Pathological Study of Minamata Disease in Japan. Acta Neuropath., Vol. 2, 1962, pp. 40-47.

2. Takeuchi, T. Pathology of Minamata Disease.; Experiments with Organic Mercury, Particularly with Methyl Mercury Compounds: Similarities Between Experimental Poisoning and Minamata Disease. In: Minamata Disease. Shuhan Co., 1968, pp. 141-252.

3. Takeuchi, T. Biological Reactions and Pathological Changes in Human Beings and Animals Caused by Organic Mercury Contamination. Environmental Mercury Contamination, ed. Hartung and Dinman. Ann Arbor Sci. Publ., Inc., 1972, pp. 247-289.

4. Kitamura, Masaji, et al. Accumulation of Methyl Mercury in the Human Body and Its Effect. Research Council Report Concerning Internal and External Reasons for Toxic Neurological Afflictions, 1971.

5. Irukayama, K. Minamata Disease — Methyl Mercury Poisoning. Assem. Formosan Med. Assoc. (Taipei), 1970.

6. Shiraki, H., et al. Time-Dependent Distribution of ^{230}Hg-mercury Compounds in Rat and Monkey as Studied by Whole Body Antoradiography. J. Hyg. Chem, Vol. 17 (2), 1972, pp. 93-107.

7. Takeuchi, Tadao. Pathology of Minamata Disease; Study on Minamata Disease — Organic Mercury Poisoning Symptoms (Research Team, Kumamoto University). Shuhan, 1966, pp. 194-282.

8. Takeuchi, Tadao, et al. Minamata Disease after 10 Years and Its Pathological Changes. Japan New Medical Bulletin, No. 2402, 1970, pp. 22-28.

9. Eto, Mitsuaki, et al. Unpublished.

10. Suko, Shuji, et al. Unpublished.

11. Fujiki, Shiroshi, et al. Survey and Study Concerning Mercury Contamination and Its Change in the Vicinity of the Minamata District: Epidemiological, Clinical Medicine and Pathological Study Concerning Minamata Disease at the end of 10 Years. Team Report, 1972, pp. 1-5.

12. Fujiki, Shiroshi, Shizuko Tajima and Akiko Omori. Changes in the Level of Mercury-Caused Contamination in the Minamata Region. Japan Hygiene Journal, Vol. 27 (1), 1972, p. 115.

359

11. CLINICAL, PATHOLOGICAL OBSERVATIONS ON ABNORMALITIES AND PATHOLOGICAL
CHANGES IN THE AREAS OF MOUTH AND TEETH OF MINAMATA DISEASE PATIENTS, PARTICU-
LARLY ABNORMALITIES OF FETAL DEVELOPMENT

Second Seminar, Pathology, Department of Medicine,
Kumamoto University

Team member: Tadao Takeuchi
Research assistant: Guniku Aoki

I. PREFACE

The fact is gradually being established that methyl mercury adversely
affects newborn infants and adults, especially their nervous system and a
part of the visceral organ tissue. Further, upon discovery of fetal Minamata
disease as one form of Minamata disease, it was established that methyl mercury
permeated the placenta and affected the fetus. Takeuchi and his associates
[8] assumed, from the standpoint of four types of methyl mercury disorder ——
fetal, embryonal, gene distorders, or hereditary —— that only the fetal type
existed in human beings, and that only disorders occurring subsequent to the
4 - 5 month fetal period made their appearance. Gene disorders have been
proven [5, 6] expermentally, using drosophilae and onions. Further, the
obstruction of synthesis of DNA or RNA was shown through the studies of Otsuka,
Sakai and Kojima [4]. Sakai [10] established that, in the initial stage of
sperm formation in mice, there was an inhibition of division of the mother
cell. Whether this is true in the case of human beings is still unknown.

Meanwhile, Murakami theorized, on the basis of an experiment using
phenlyl mercury and teeth abnormalities in fetal Minamata disease, that teeth
disorders may also be caused by methyl mercury.

Faced with the urgency of clarifying whether such a phenomenon occurred in Minamata disease patients, we decided to conduct a study focused on Minamata disease, particularly on congenital Minamata disease. Since Murakami [3] had indicated that embryonal disorders among congenital Minamata disease cases may be in the area of dentistry, we directed our efforts especially to the mouth and teeth.

In the area of the mouth and teeth, no extensive studies have yet been conducted on pathological changes and disorders precipitated by organic mercury, being limited to matters involving saliva flow or teeth misalignment among Minamata disease patients [9]. Ikejiri and his associates indicated [1], on the basis of examination of the mouth of fetal Minamata disease patients, that a high incidence of decayed teeth and incomplete enamel formation was seen in those patients.

Hence, we tried to uncover the abnormalities of the mouth of fetal, juvenile, and adult Minamata disease patients by examining patients clinically diagnosed as having Minamata disease, and creating and studying study models. Bsed on our findings, we reviewed the abnormalities of the mouth and teeth, areas affected and their conditions.

II. OBJECT OF SURVEY AND METHOD

The object of our latest survey was 18 Minamata disease patients hospitalized at Minamata Municipal Hospital, Myosuien. Of these, 8 were diagnosed as either juvenile or adult Minamata disease patients (hereinafter termed simply as Minamata disease patients) and 10 fetal Minamata disease patients. Of the latter, two were excluded from our survey, as one of them was a 15-year old male youth who was uncooperative and excited during our examination, and the other was a 65-year-old woman who had all dentures and appeared to have no abnormalities in the soft mouth tissue. Therefore, the actual number of patients examined was 16: 7 Minamata disease patients (1 male, 6 female; aged 22 - 72), and 9 fetal Minamata disease patients (4 male, 5 female; aged 15 - 18).

361

Examination of the patients' teeth and of the hard and soft tissue of the inner mouth was made. Study models of the teeth of three Minamata disease patients and six fetal Minamata disease patients, all of whom had been co-operative, were completed so that, together with findings related to the mouth, we could conduct a detailed study. For comparison and reference purposes, we referred to school health statistics for 1970, and a survey report on the people's dental disease situation for 1969.

III. FINDINGS

1. Results of examination of the mouth of Minamata disease and fetal Minamata disease

Table 1 and 2 reflect the results of examination of the mouth of adult and juvenile Minamata disease patients and fetal Minamata disease patients, ba based on diagnosis and study models. An explanation of each item follows.

1) Hard tooth tissue

Initially, studies were made of those with decayed teeth, and whether treatment was received or not. Decayed teeth were classified into 3 categories as to whether or not treatment was received and/or teeth were missing (filled teeth excluded), and overall results were expressed by the acronym DMFT — for decayed, missing, and filled teeth. They are shown in Table 1.

All or 100% of those examined had decayed teeth. The number of un-treated decayed teeth ranged from 1 to 16 in all of the cases. Out of 9 fetal Minamata disease patients, 4 had not received treatment for 10 or more teeth, and 6 of this group had never received any treatment. It was a fairly high rate, compared to ordinary individuals in the same age bracket. In the adult Minamata disease patients, a greater number had received treatment, but there was also an increase in the number having missing teeth. DMFT was 5 – 19 teeth in the fetal patients, or an average of 10.5 per person, a very high

362

Table 1. OBSERVATIONS CONCERNING THE MOUTH OF MINAMATA DISEASE PATIENTS (a)
HARD TOOTH TISSUE, SOFT TISSUE, OCCLUSION

Case number	Name	Age, sex	Hard tooth tissue					Incomplete enamel formation			Soft mouth tissue	
			Untreated teeth	Treated teeth	Missing teeth	DMFT	Grinding surface deterioration	Milky	Reddish pits	Yellow-brownish discoloration	Inflammation of edge of gums	Swollen gums
1	Naka, Chi	15, F	11	0	1	12	–	+	+	+++	+++	–
2	Mori, Hi-e	15, F	7	0	1	8	+	–	–	+	+	–
3	Iwa, Su-ko	15, F	14	0	0	14	–	–	–	++	++	–
4	Naga, Isamu	16, M	4	0	0	4	+	+	–	+	+++	++
5	Mori, Tomo	16, F	5	0	0	5	–	–	+	+++	+++	–
6	Oni, .Yu	16, M	16	3	0	19	–	+	–	+	++	–
7	Ka, Ta-ko	17, M	6	0	1	6	+	+	–	–	+	–
8	Kane, Yu	17, M	3	6	2	11	+	–	+	+	+++	++
9	Han, Kazu	18, M	13	2	0	15	+	–	++	+++	++	–
10	Kita, Sa-e	22, F	3	7	1	11	–	–	–	++	+++	+
11	Kita, A-ko	24, F	2	2	0	4	–	+	–	++	++	–
12	Fuchi, Ki-no	55, F	11	6	4	21	+	–	–	–	+	–
13	Yama, Sada	62, F	2	0	3	5	–	–	–	++	+++	–
14	Mori, -me	68, F	1	10	17	28	+	–	–	+	++	–
15	Yama, U	68, M	1	0	0	1	–	–	–	–	+++	+
16	Saka, Ma-yo	72, F	4	1	16	21	–	–	–	–	++	–

Cases 1 - 9 involve fetal Minamata disease

TABLE 1. (continued)

Case number	Front teeth area				Occlusion — Molar area		Abnormality in arched alignment of teeth	Uses tooth-brush
	Lower jaw center rel. to upper jaw	Over bite	Over jet	Malocclusion	Position, front and rear	Position arrow-shaped surface		
1								No
2								No
3								No
4	Left	++	+	Deep overbite	Right} Left } centrifugal	N	Upper: V Lower: constriction	No
5	Right	-	++	-	Right} Left } centripetal	Left molars, opposite	Upper: V	No
6	Right	+++	-	Deep overbite	Right} Left } centrifugal	Right molars, opposite	Upper: V	No
7	.N	+	-	-	N	N	Lower: gap	O*
8	Left	+	-	-	N	N	-	No
9	Right	+	+++	Upper jaw protrusion	Right: centrifugal Left: centripetal	N	Upper: V	No
10	Right	+	-	-	Right: N Left: centrifugal	N	Upper: V Lower: constriction	No
11	Left	+	-	-	N	N	-	No
12	Left	-	-	End removed	Right: centripetal Left: N	Left molars, opposite	-	O*
13								No
14								No
15								No
16								O*

Cases 1 - 9 involve fetal Minamata disease

*O — occasionally.

TABLE 2. OBSERVATIONS CONCERNING THE MOUTH OF MINAMATA DISEASE PATIENTS:
(b) ABNORMALITY OF POSITION OF TEETH AND OTHERS

Case no.	Observations concerning the mouth
1	⎯⎯ , filled; ⊓ missing and unrestored.
2	⊓ missing and unrestored; filled.
3	⊔ ⊔ remaining; ⊔ root remaining; ⎯⎯ filled.
4	³¹ ¹ centripetal distortion; ₇₅ ₁₂ ₂₅₇ shift to tongue side; ⎯ shift to lip side; centrifugal shift of arched teeth alignment of lower jaw; ₂ ₂ conical teeth; ⎯⎯ filled.
5	₁₁ shift to lip side; ₇ shift to cheek side; ⎯⎯ filled.
6	₅₄₂ ₂₅ shift to tongue side; ⁴ centripetal distortion ⎯⎯ filled.
7	5 mm gap between ₄ and ⎯ʝ, between ₄ and ₃ ; ⁶ missing and unrestored; ⎯⁵ centripetal distortion; ₅ shift to cheek side; ℯ root remains; ⎯⎯ filled.
8	⎯ shift to lip side; ᵇ missing; ⎯ bridge affixed; ⎯⎯ filled.
9	⁴ centripetal distortion; ³ centrifugal inclination; ⁴ ¹ shifts to tongue side; ⎯¹ centrifugal inclination; ₂₃ ₁₂ shift to lip side; ℯ conical teeth; ⎯⎯ filled.
10	¹ centripetal distortion; ¹ shift to lip side; ⎯⎯ centripetal inclincation; ⎯ centifugal inclination; ₇₆₅₄ shift to tongue side; ℯ filled; ₆ missing and unrestored.
11	₁₁ centripetal distortion; ⎯ℯ ₂ shift to tongue side; ₃ centripetal inclination; ₆ ᵃ missing and unrestored.
12	⁴ centripetal distortion; ₅ ₃₅₆ missing; ⎯⁵³ bridge; ⎯⁷ shift to check side; ⎯⎯ filled.
13	Heavy tartar coat; ₇₅₅₄₃₂₁ ₁₂₃₄₅₆₇ p, ⎯¹ ⎯ missing; ¹ denture; ⎯⎯ filled.
14	⎯¹⎯¹¹⎯²⎯ bridge affixed; ³² succeeding teeth damaged; ⎯₅⎯₆₃₂⎯₂₃⎯ p, ⎯²⎯⎯⎯ missing.

(Table continued on following page)

365

TABLE 2. (continued)

Case no.	Observations concerning the mouth
15	! C.
16	74 ?, P. 651 127? missing; bridge damaged.

rate compared to 2.5 teeth among middle school students of "S" district, as
reported by a survey of the Mouth Hygiene Academy [2]. Adults also reflected
a high DMFT rate, but theirs cannot be compared with that of the fetal
patients because of the age factor.

As for tooth enamel, we looked for the degree of milkiness or color
and for the presence of reddish pits. We found that incomplete enamel forma-
tion existed in almost all of the fetal patients. Specifically, yellowish-
brownish discoloration of the enamel was seen in 8 out of 9 cases, and almost
half manifested either milkiness or pits in the enamel (Photos 1, 2, 4, 7).
In Case no. 9, a shallow pit was clearly seen in 1/2 of the neck of the tooth
(Photo 7). Among the adults, one case manifested a milkiness of enamel, but
none had pits. Four out of 7, however, showed yellowish-brownish discoloration,
3 of whom had moderate degress of discoloration (Photo 8). As described above,
incomplete formation or development of the enamel was easily visible, the
degree of incompleteness and incidence of being being marked in fetal Minamata
disease patients.

Other abnormalities of the hard tooth tissue were deterioration of the
grinding surfaces and indentation or extension of the roots of the teeth.
These were detected in more than half of the fetal patients and in two adults.
They may be due to convulsive actions of the Minamata disease or bad habits,
such as teeth grinding, but we shall not discuss them here as to their link
with clinical symptoms.

2) Soft mouth tissue

As for the soft tissue, we directed our efforts to inflammation of the gums, swelling and degree. Gingivitis was noted in all of the cases, although its degree varied (Photos 1 - 8). Serious gingivitis was seen in 4 out of 9 fetal patients, and in 3 out of 7 adult cases, there being no marked difference degree between the two groups. Compared to ordinary patients, it was very serious and of great incidence. Considering that 45.19% of ordinary patients examined (1970) had periodontal problems, it can readily be recognized that the rate is considerably higher for Minamata disease patients. A moderate degree of swollen gums was noted in 2 fetal patients, and a mild degree in 2 adult cases. No other significant changes were noted.

3) Occlusion

We looked for malocclusion, if any, and its degree, using the study model prepared from 6 fetal Minamata disease patients and 3 adult Minamata disease patients. Here, we classified occlusion into the three categories of front teeth area, molar area, and teeth arch alignment for convenience.

a) Front teeth area

First of all, in the front teeth area, we studied the degree of delineation, as measured from their center, of the lower jaw from the upper jaw. Normally, they are positioned exactly in the center. However, in 8 out of 9 Minamata disease patients examined, there were deviations either to the left or right. An almost normal position was seen in only one fetal patient. The proportion of deviation was 4 cases each on both right and left sides.

Next, we studied whether the front teeth of the upper jaw overlapped the front teeth of the lower jaw. Although there were differences as to degree, overlapping was noted in 7 out of 9 cases. In 2 of the fetal patients, a marked overlapping, or so-called deep over bite, was noted. In 6 of such
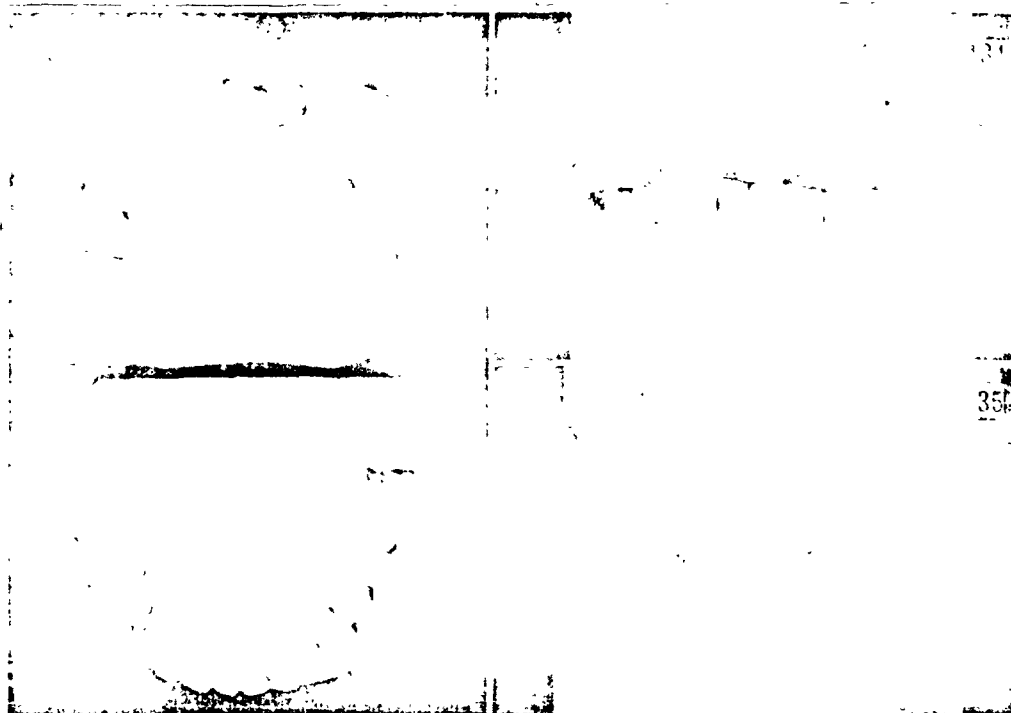
367

9 12
10 13
11 14

16

17

19

20

21

22

25

23
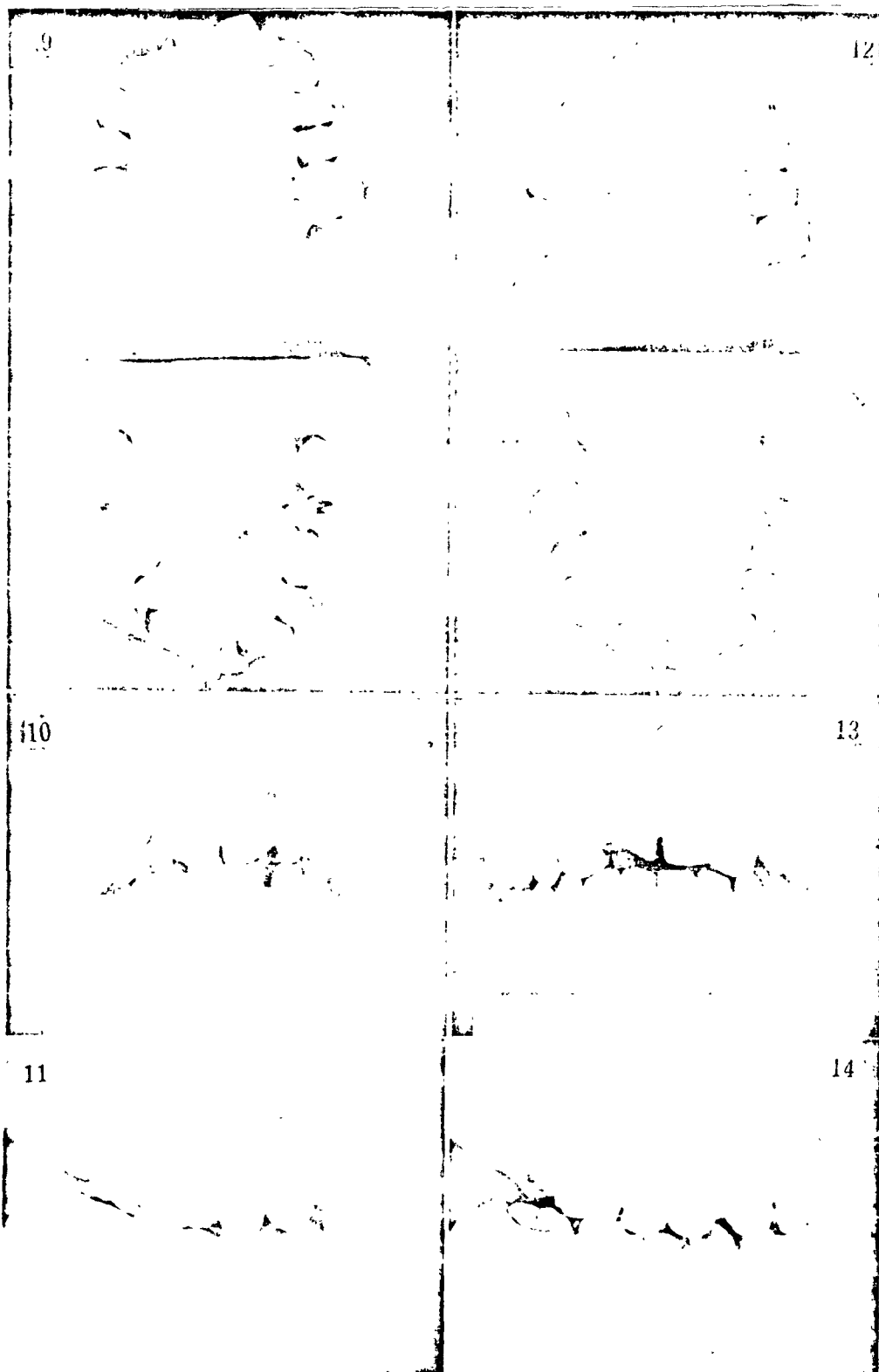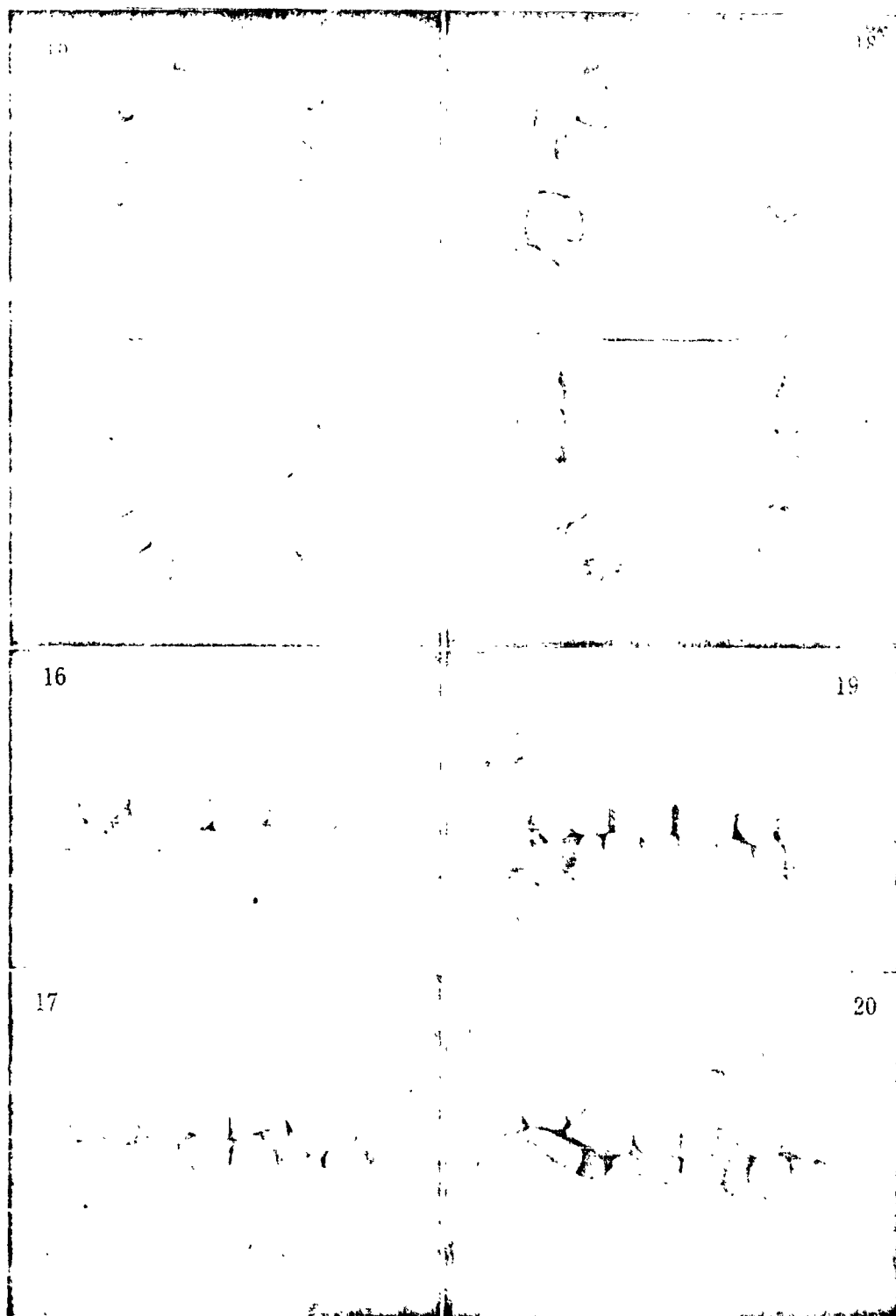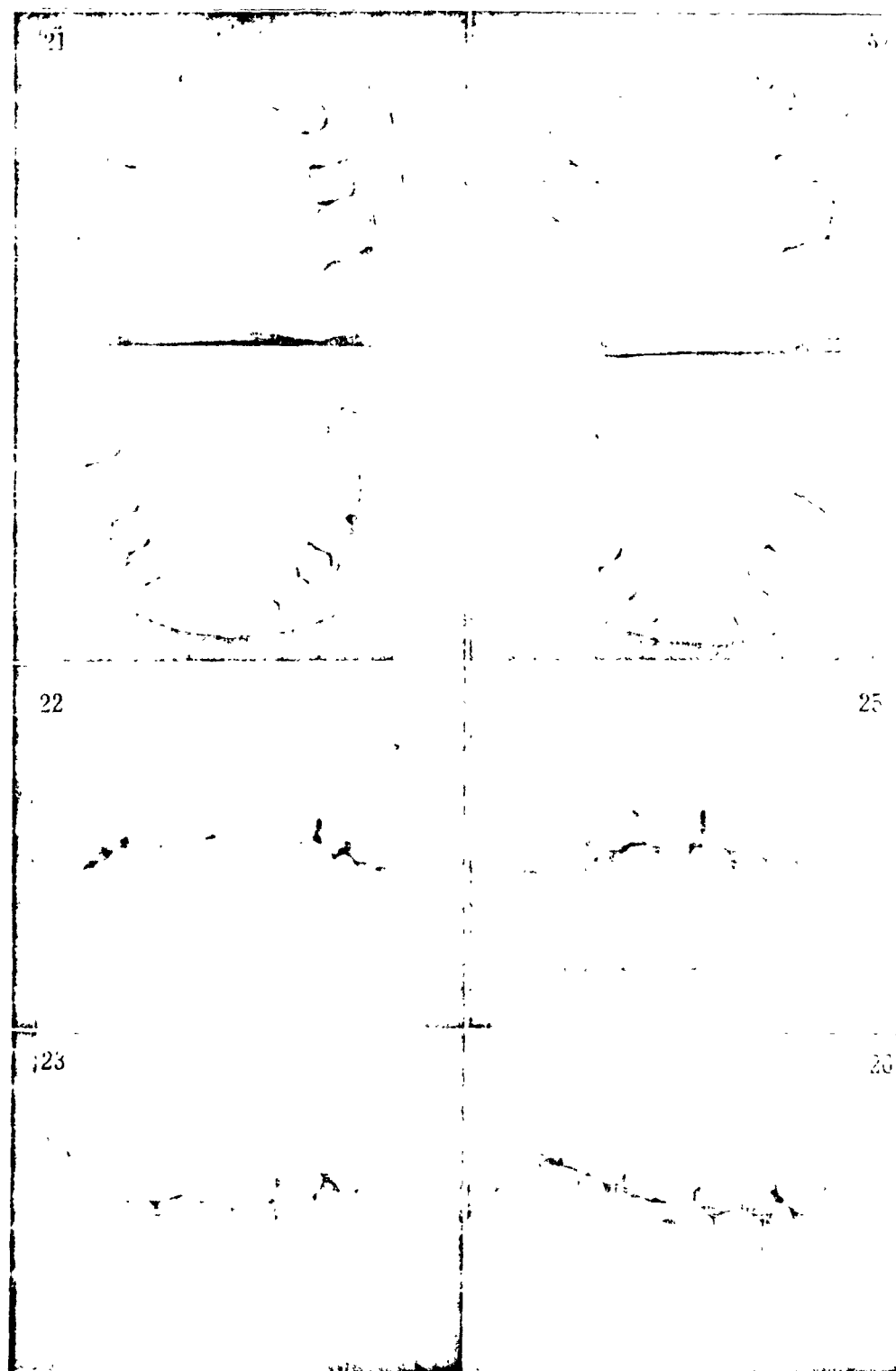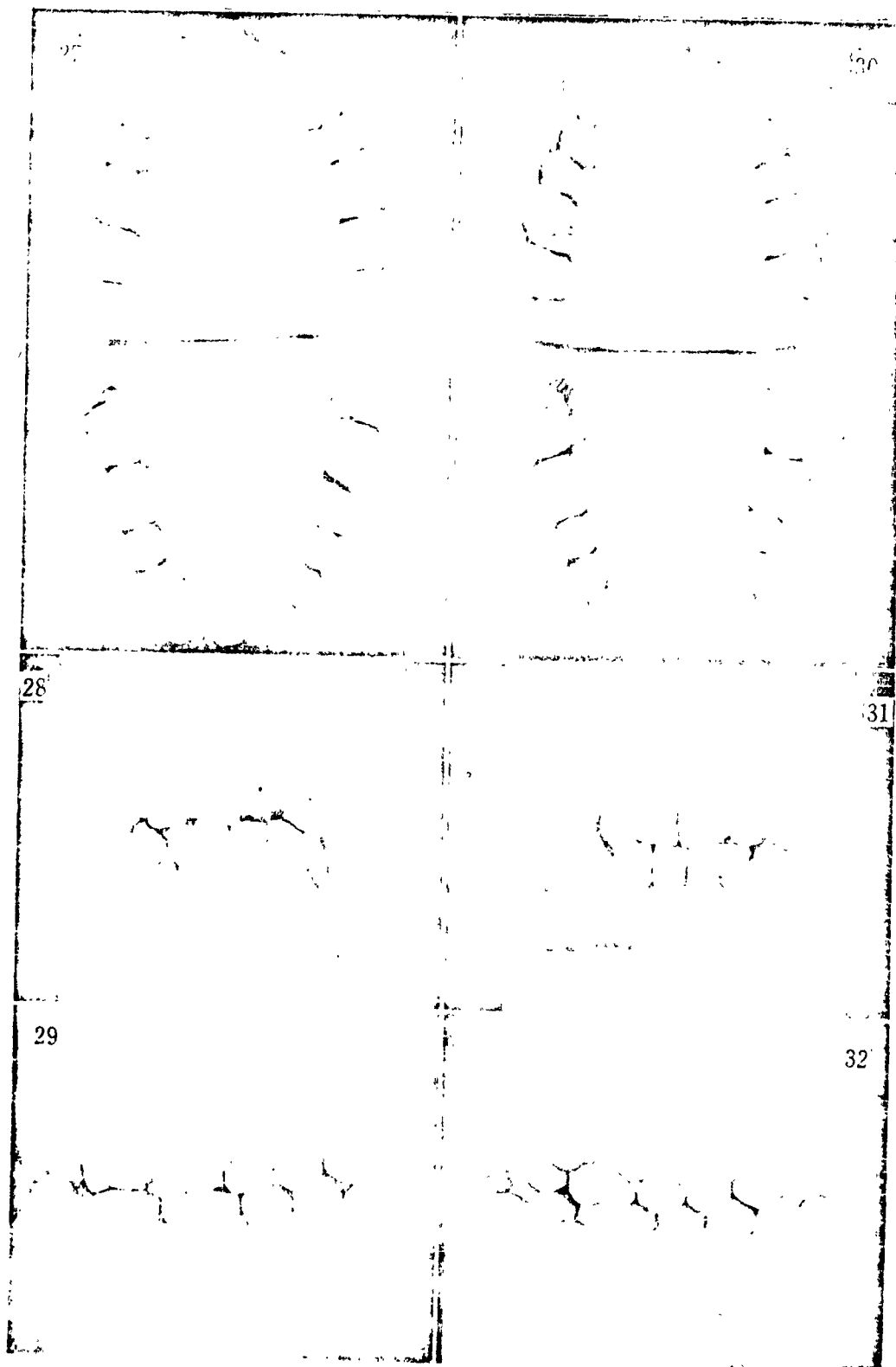
Description of Photos

Photos 1 - 2. Case No. 1. Milkiness of teeth and reddish, medium pits; heavy
yellowish-brownish discoloration; serious inflammation of
edge of gums.

Photo 3. Case No. 3. Moderate yellowish-brownish discoloration; moderate
inflammation of edge of gums; mild deterioration of grinding
surfaces; b|b, root remaining; 2|2 filled.

Photos 4 - 5. Case No. 4. Serious inflammation of edge of gums; moderate
overbite; 2|2 conical teeth.

Photo 6. Case No. 5. Moderate, reddish pits; heavy yellowish-brownish dis-
coloration; serious inflammation of edge of gums; moderate over-
jet, |7 filled.

Photo 7. Case No. 9. Moderate reddish pits and serious yellowish-brownish
discoloration; serious over-jet accompanied by open bite;|c
conical teeth; 2|2 filled.

Photo 8. Case No. 13. Moderate yellowish brownish discoloration; serious
inflammation of edge of gums; marked tartar coat.

Photos 9 - 11: Case No. 4. For this case and cases indicated below, molds
give view of bite area with mouth open, frontal occlusion, and
bite on left side. Observations are outlined in Tables 1 and 2.

Description of photos (continued)

Photos 12 - 14 — Case no. 5; Photos 15 - 17 — Case no. 6;
Photos 18 - 20 — Case no. 7; Photos 21 - 23 — Case no. 8;
Photos 24 - 26 — Case no. 9; Photos 27 - 29 — Case no. 10;
Photos 30 - 32 — Case no. 11; Photos 33 - 35 — Case no. 12

cases, the front teeth on the lower jaw were covered so much that they could
hardly be seen (Photos 15 - 17).

Observation of the degree of protrusion of the front teeth of the
upper jaw relative to the front teeth of the lower jaw revealed 2 cases of
serious over-jet among the fetal patients. In other words, Case no. 5 has
a 5 mm gap between the front teeth of the lower jaw and those of the upper
jaw, and 7 mm in Case no. 9 (Photos 12 - 14, 24 - 26). Also, a moderate over-
jet was noted in an adult Minamata disease patient.

Other malocclusions in the front teeth area involved frontal projection
of the upper jaw in 9 fetal patients, wherein the molars were in contact dur-
ing the bite, but the front teeth did not and were open. These cases also
were accompanied by a deep over-jet (Photos 24 - 26). Further, in 12 adult
cases, the front teeth in the upper jaw came in contact with those of the
lower jaw at their ends (Photos 33 - 35).

b) Molar area

Assuming that the arched teeth alignment of the upper jaws was in a
normal position when the molars were in a biting position, we studied the
position of the lower jaw in relation to the upper jaw. At this time, our
concept was that the normal pattern consisted of the centripetal apex on the
cheek side of the first molar of the upper jaw fitting in the groove on the
cheek side of the first molar on the lower jaw; that forward position of the
lower jaw constituted centripetal occlusion of the lower jaw, and that the
backward position of the same constituted centrifugal occlusion of the lower
jaw. In the case of fetal patients, 4 out of 6 manifested either centrifugal

374

or centripetal occlusion on the left or right sides, with only 2 cases being normal. A somewhat lighter degree was noted in adult cases, with 2 out of 3 showing centrifugal or centripetal occlusion on either the left or right side.

Next, in determining the surface of the molars, our concept was that the normal position was for the upper jaw to overlap the lower jaw, and that the opposite occlusion position was for the molars on the lower jaw to protrude toward the cheek. Among the fetal and adult patients, there were 2 cases and 1 case, respectively, of only the molars on the left side manifesting opposite occlusion (Photos 13, 16, 34).

   c) Arched teeth alignment

In normal persons, the arched alignment of teeth is naturally parabolic. Occasionally, however, an abnormality develops due to the shift of the front teeth to the lip side, and of the molars to the tongue side. In other words, the arched alignment appears to be V-shaped, constituting a so-called V-type arched alignment (Photos 9, 12, 15, 24). Such an abnormality was noted in 10 adult cases, but noteworthy was the fact that one individual had conditions similar, age-wise, to those of fetal cases (Photo 27).

Other abnormalities of the arched alignment of the teeth were constricted arched alignment due to shift of the molars to the tongue side, as observed in Cases 4 and 10 (Photos 9, 27). These 2 cases were accompanied by V-shaped arched alignment in the upper jaw. In Case no. 7, a gap was noted in the arched alignment, due to uneven growth of the jawbone and teeth (Photos 18 - 20).

Among the fetal Minamata disease patients, only 8 were entirely free of abnormality of arched alignment of the teeth, most of them showing V-shaped arched alignment of the upper jaw, or occasionally constricture of the lower jaw (Photos 9 - 11, 27 - 29), or accompanied by a gap in the arched alignment (Photos 18 - 20). In adult cases, all except Case No. 10 were completely

free of abnormality. In other words, a morphological abnormality was noted primarily in fetal patients, which was rather interesting from the standpoint of tissue formation.

4) Filled teeth and milk teeth

It has been generally stated that eruption of new teeth ends with the second molar around the age of 10 to 14 [2]. Of those over 15, 4 out of 9 fetal patients had filled teeth; specifically, Case nos. 3, 5, 6, and 9 had filled teeth $\frac{2}{2}$. $\overline{7}$. $\overline{7}$, and $\frac{7}{2\ 2}$, respectively. The average number of teeth among the fetal patients were 27.1. Three cases — Case nos. 3, 7, and 9 — still had milk teeth — $\overline{b}\ \overline{b}$. $\overline{c}$, and $|\underline{c}.$

5) Other abnormalities

With respect to abnormal positions of teeth, centripetal or centrifugal distortion was noted in 4 fetal patients and 3 adults. As for shifts to the tongue side or to the lip-cheek side, there were 3 cases each among the fetal patients, and 2 cases each among the adult patients. Another abnormality was centrifugal or centripetal inclination, involving 2 or 3 cases.

Morphological abnormality of the teeth was seen in fetal Cases nos. 4 and 9, manifesting conical teeth at $\underline{2|2}$ and $|\underline{c}$, respectively (Photos 9 and 24).

Pyorrhea was not observed in any fetal cases, but was found in 3 adult cases.

2. Ailments of the hard tissue, according to area
   affected, and abnormal teeth positions in Minamata
   disease patients

1) Hard tissue ailments and their treatment

Teeth ailments, according to the area affected, and treatment were re-
viewed on a statistical basis on all cases examined. Because of occasional
difficulty in determining whether a tooth not existing in the arched align-
ment was a missing tooth or a filled tooth, we treated it as a filled tooth
for convenience. The results are shown in Table 3. We are presenting the
data merely as information, because of the small number of cases involved.

Among the Minamata disease patients, an average of 48%, or nearly half,
had teeth ailments, primarily decayed teeth. Teeth (including filled teeth)
most susceptible to disorder were $\frac{8\ 7\ 6\ 6\ 7\ 8}{8\ 7\ 6\ 6\ 7\ 8}$, the most sound were $\frac{3}{5\ 1\ 3\ 2\ 1}$ $\frac{3}{1\ 2\ 3\ 1}$,
with other in between. The rate of treatment for decayed teeth was 26.4%
(12.7% for fetal cases alone), with untreated cases running 20%. No compari-
son was made of these findings between fetal and adult cases, because of the
small number of cases and age differences.

2) Abnormal position of teeth

All the teeth of 9 persons on whom models were prepared were examined
by classifying the abnormality into the three groups of shift, distortion,
and inclination. The results are outlined on the right side of Table 3.

In all Minamata disease cases, the abnormal position involving a shift
to the tongue side was preponderant, registering 10%, followed by a shift
to the lip-cheek side, with 4.5%, manifested primarily in fetal patients.
This can be regarded as a highly unique abnormality in fetal cases. Centri-
petal distortion was noted in 4.5% of all cases, and either centripetal or
centrifugal inclination — about 1%. There was believed to be a need for a com-
parative study of these abnormal positions by examining more cases.

377

TABLE 3. HARD TISSUE AILMENTS AND ABNORMAL POSITION, ACCORDING TO TEETH, IN MINAMATA DISEASE PATIENTS

Area		Sound teeth		Missing teeth		Filled teeth		Treated teeth		Untreated teeth C_1-C_3		Untreated teeth C_4		Abnormal position Shift Tg		Abnormal position Shift L-C		Distortion Cp		Distortion Cf		Inclination Cp		Inclination Cf	
		T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F
Upper right	8	1	0			14	9	1	1	2	1	1	0	1	0										
	7	4	2	2	0	1	1			3	1	7	3	2	1		1			1	1				
	6	4	2	3	1					4	3	1	1	2	1		1								
	5	9	5	2	0					2	1	0	4	3	2					3	2	1	1		
	4	9	4	1	0					1	0	1	0							1	1				
	3	14	9							1	0	1	3												
	2	8	5			1	1			1	0	5	3	2	1	1	2			3	2				
	1	5	4	3	0					1	0	6	5												
Upper left	1	9	6	2	0					0	4	3	1					2	2	2	1				
	2	10	6	1	0	2	1	1	0	2	1	2	0	1	1		1	1	1						
	3	11	7	2	0	1	1	1	0	1	1	1	0												
	4	10	6	1	0			1	0	2	2	2	1	1	1			1	0					1	1
	5	10	6	2	0					4	3	1	1	1	1	1	1								
	6	6	3	3	1			1	0	5	4	1	1	1	1			1	0						
	7	7	3	3	0			1	0	5	4														
	8	5	1			11	8			5															
Lower left	8	1	0			13	9	1	1	1	0	1	0												
	7	1	1	2	0					2	1	7	6	1	1	1	2	1				1	0		
	6	3	1	5	2					1	0	1	1	6	5	1	0							1	0
	5	10	6	2	0					2	1	2	2	1	0					1	1				
	4	13	7	1	0							1	1	1	0							1	0		
	3	14	8							1	0	1	1												
	2	13	8	1	0	1	1			1	0	1	1	2	0										
	1	13	8	2	0									1	1										
Lower right	1	14	9	2	0											1	1								
	2	13	8	1	0	1	1			2	0	1	0	2	1										
	3	13	9							1	0	2	2												
	4	12	7	1	0					1	0	2	1	1	1										
	5	13	7	1	0					1	1	1	1	2	1										
	6	3	0	2	1					3	1	2	1	1	0										
	7	3	1	1	1	1	1	3	3	1	7	5		2	1										
	8	3	0			12	9					6	1	2	0									1	0
Total		267	151	46	6	59	43	37	11	77	60	26	17	29	15	13	11	13	7	1	1	3	0	2	1
% of Total (512)		52.4		9.0		11.3		7.2		14.8		5.3													

% of total no. teeth (288) of 9 persons on whom molds were made: 10.0 | 4.5 | 4.5 | 0.3 | 1.0 | 0.7

Notation: Tg — tongue side; L-C — lip-cheek side; Cp — centripetal; Cf — centrifugal; T — total; F — fetal

378

IV. SUMMARY AND COMMENTS

Minamata disease patients examined consisted of 9 fetal cases, aged
15 - 18, and 7 adult cases, aged 22 - 72. Case No. 10, a 22-year-old woman,
exhibited conditions similar to those of fetal cases, age-wise, including the
condition of her mouth. There were relatively few disorders of the teeth
in adults, not considering changes due to age. But several abnormalities were
noted in fetal cases. We decided, therefore, to study each aspect of our
findings. Table 4 is a comparison of statistical findings concerning the
mouth of Minamata disease patients with those reported in other references.

TABLE 4. STATISTICAL COMPARISON OF MOUTH AILMENTS OF MINAMATA DISEASE PATIENTS
AND OTHERS

Item	Minamata disease (including fetal type)	Ministry of Welfare survey (1969)
% decayed teeth	100%	85.66%
% treatment of decayed teeth	26.4% 12.2% (only fetal type)	55.0% 21.30% (middle school students)
DMFT	10.5 teeth (only fetal type)	5.8 teeth ("F" middle school, acc. to Ikejiri and others) 2.5 teeth (elementary and middle school students, Survey Comm., Dental Hygiene Academy)
Those with periodontal tissue ailments	100%	45.19%
Malocclusion (front teeth area)	50% (only fetal type)	13.36% (age 21 or below)

Use of toothbrush
  Brushed daily
  Brushed occasionally
  Never brushed
	
	0%
	18.75%
	81.25%
		
		79.68%
		11.81%
		8.13%

Among ailments of the hard tooth tissue, decayed teeth was the highest among Minamata disease patients, with 100%. This was remarkably high, compared to 85.66%, registered in the survey of human teeth ailments for 1969 [2]. DMFT per person among all Minamata disease patients examined was 11.8 teeth, a breakdown of which was fetal patients — 10.5, and adult cases — 13.0. According to Ikejiri and his associates [1], DMFT in 1970 was 5.8 teeth at "F" middle school in a Minamata disease prevalent area. Further, according to a report prepared by the Survey Committee of the Dental Hygiene Academy [2], DMFT of middle school students of "S" district, which we used for comparison, was about 2.5 teeth. This showed that the rate was highest in fetal patients, and also high among "F" middle school students in the disease prevalent area. Considering that the district is mercury contaminated, there could be some relationship between decayed teeth and organic mercury contamination. The percentage of decayed teeth treated was 12.2% in fetal cases, or almost 1/2 of that of 21.30% of middle school students, according to school health statistics for 1970 [2].

With respect to enamel, there was at least one case among fetal patients of milkiness, reddish pits, and yellowish-brownish discoloration. Since the figure was high as to the degree of incompleteness and incidence when compared to ordinary patients, it was noteworthy in relation to the previously described incidence of decayed teeth.

As conditions for occurrence of decayed teeth, there is believed to be a complicated decaying process involving pathogenic, environmental, and host (property of teeth) factors, as in other ailments. It is wholly possible that secondary factors, such as inability to use toothbrush because of accompanying disorders of the motor function or mental-intellectual disorders of Minamata disease patients, lack of sense of hygiene or awareness of decayed teeth, or decreased concern for treatment, would be a cause for decayed teeth or gingivitis. On the other hand, serious incomplete formation of enamel was noted, especially in fetal patients. Aside from the participation of secondary factors, it is well known that incomplete enamel formation abets

380

tooth decay. The question, then, was whether, during the formation of devel-
opment of the fetus, the mercury contamination had some effect on tooth for-
mation and growth, particularly on enamel formation. Even if there were
such a possibility, we do not possess sufficient data to prove this point
at this time.

Based on observations of the condition of the bite from study models,
it can be stated that serious malocclusions exist in fetal Minamata disease
patients. Most noteworthy was that in the front teeth area there was a
serious degree of overlap of the front teeth in the upper jaw in 5 out of 6
cases in fetal patients, two of whom also manifested a serious overbite
(Photos 9 - 11, 15 - 17). One case also indicated a serious over-jet, along
with open bite (Photos 24 - 26). Serious malocclusion of the front teeth
area, as viewed from the side, was indicated in 50% of the fetal patients —
a remarkably high rate when compared to 13.4% (age 21 or below) cited in the
survey report on human teeth ailments for 1969. In addition, virtually all
of the cases disclosed eccentric bite to either the left or right.

As for malocclusion in the molar area, abnormal positions in the front
or back or with respect to the arrow-shaped surface were noted in almost half
of the Minamata disease patients examined, with two having a combination of
the above.

With respect to the abnormality of the arched alignment of the teeth,
most noteworthy was the V-shaped arched alignment in the upper jaw of 4 out
of 6 fetal patients (Photos 9, 12, 15, 24). Some manifested a gap or con-
stricture in the arched alignment in the lower jaw, with 1 case showing a com-
bination of the latter and V-shaped arched teeth alignment. In adult cases,
the degree of malocclusion was slight as compared to fetal cases, but the
condition of Case no. 10, a 22-year-old woman, was serious and comparable
to that of the fetal patients (Photos 27 - 29).

While we have discussed malocclusion in Minamata disease patients, the
distinction between normal and irregular cases was occasionally extremely

381

difficult because even a normal bite showed some changes. Our method of morphological classification of the arched teeth alignment, for example, may be inaccurate because of visual measurement. We therefore wish to defer consideration of the question of measurements until another time.

The cause of malocclusion can generally be classified into hereditary and environmental factors [7]. Today, even hereditary aspects have been clarified with respect to certain cases of malocclusion, but we wish to direct our attention to the latter factor as the cause of malocclusion. Amidst the environment are congenital and a posteriori causes, each containing numerous other causes. It would be clinically difficult to determine which one of these factors is responsible for malocclusion. However, when considered in the light of Minamata disease, we tend to believe that disturbance of nutrition and growth of the fetus by methyl mercury contamination naturally retards the formation and growth of the teeth, and also precipitates uneven development of both upper and lower jaws and peripheral soft tissue. Additionally, a posteriori factors would emerge, abetting various forms of malocclusion. We wish to caution that, among fetal patients, there are still some who have bad habits, such as toying with their fingers, tongue, and lips [9]. There probably is a need to consider these matters as well.

Next, filled teeth were noted in four of the fetal patients, who had an average number of 27.1 teeth. We consider that this number of teeth represents no major difference when compared with an average of 27.1 teeth among "F" middle school students, as reported by Ikejiri and his associates [1]. Three of the fetal patients still had milk teeth — ', ', and . The fallout of milk teeth was late in these fetal patients, and beyond the average length of time. It may be due to the absence of succeeding permanent teeth or other abnormalities, but we could not probe further on the basis of clinical examination alone. Moreover, it is known that the presence of milk teeth is a factor responsible for the aforementioned malocclusion [7]. In addition, various degrees of abnormal teeth positions and morphological abnormality of conical teeth were noted in Minamata disease patients. As explained in the case of malocclusion, we believe that we cannot disregard the direct or

382

indirect effects of methyl mercury as a continuing factor. A comparative study of these abnormalities in position, according to area, and morphological abnormalities would probably be necessary by examining a greater number of cases.

As explained above, it was found from the study of the mouth and study models of the Minamata disease patients that several characteristics exist in Minamata disease patients. Those characteristics appearing in fetal Minamata disease were: 1) malocclusion, especially overbite, over-jet, and abnormal arched teeth alignment; 2) incomplete enamel formation; 3) existence of milk teeth. Among them, there was nothing to indicate any unusual abnormality attributable to abnormal development. However, there were many which could be considered abnormal growth in the course of teeth formation. Factors in the course of development during the fetal period played an important role. However, embryonal factors could not be determined. Abnormal development of the fetus is still believed to be an important factor.

Thus, it can be pointed out that complete disturbance of development of the arched teeth alignment and the recurring effects of nerve disorders combined to precipitate various secondary pathological changes. Such secondary pathological changes probably were manifested as numerous cases of decayed teeth, gingivitis, and deterioration of grinding surfaces.

Conclusion

Examination of the mouth and of study models of 9 fetal patients and juveniles and 8 adults among Minamata disease patients led to the findings described below:

1) We found characteristics peculiar to Minamata disease patients, particularly in fetal patients, but were unable to pinpoint any unusual pathological changes attributable to abnormal development.

383

2) We found, in fetal and juvenile Minamata disease patients, abnormal developments in the teeth and mouth structure in the course of growth during the fetal and subsequent periods. The abnormalities consisted of:

i) Serious malocclusion. Overbite, over-jet or open bite was found in 3 out of 8 fetal and juvenile patients. In the molar area, centrifugal, centripetal, and opposite occlusion were noted in about half of those examined. With respect to abnormal arched teeth alignment, V-shaped arched alignment in the upper jaw was found in 4 cases.

ii) Indications of incomplete enamel formation were noted, although its degree varied, in all fetal and juvenile cases, being marked especially in fetal patients.

iii) Among fetal patients, 3 cases still had milk teeth, 4 cases had filled teeth, and 2 cases had conical teeth. However, the average number of teeth was 27.1, showing no great difference from that of the control group.

3) Also, secondary pathological changes were noted in Minamata disease patients, due to a combination of motor function and mental-intellectual disorders.

i) In these cases, a high incidence of decayed teeth was noted in all. DMFT 10.5 teeth among fetal patients was rather high compared to the control group.

ii) Quite serious cases of gingivitis were noted in all Minamata disease patients — 2 or 3 having swollen gums.

iii) Deterioration of grinding surfaces was noted in nearly half of all Minamata disease patients; also, a heavy tartar coat was seen among adult patients, the incidence being greater than in other symptoms.

We wish to express our appreciation to Professor Noboru Ohashi, Chief, Minamata Municipal Hospital, the staff of Myosuien and patients for their assistance and cooperation in this study. We also wish to thank Professor Yoichiro Otsuka, parttime lecturer, Second Seminar, Pathology, of this university, for his advice and supervisior.

REFERENCES

1. Ikejiri, Shigeru, Hisayasu Uji and Shunichi Ito. Observations of the Mouth of Fetal Minamata Disease Patients. Dental World Outlook, Vol. 37 (1), 1971, pp. 49-53.

2. Japan Dental Association. Data and Commentary for the Supervision of Mouth Hygiene. Japan Dental Association Journal, Vol 24 (12), 1973, pp. 33-52.

3. Murakami, U. The Effect of Organic Mercury on Intrauterine Life. Drugs and Fetal Development. Ed., Klingberg, Abramovici and Chenke. New York, Plenum Publ. Co., 1972, pp. 301-336.

4. Otsuka, Yoichiro, Ken Sakai, Hidetoshi Kojima and Tadao Takeuchi. Effects of Methyl Mercury Chloride on Cell Multiplication, Particularly on Cell Fission and Nucleic Acid Synthesis. Unpublished.

5. Ramel, C. Genetic Effects of Organic Mercury Compounds. I. Cytological Investigations on Allium Roots. Hereditas, Vol. 61, 1969, pp. 208-230.

6. Ramel, C. and J. Magnusson. Genetic Effects of Organic Mercury Compounds. II. Chromosomes Segregation in Drosophila melanogaster. Hereditas, Vol. 61, 1969, pp. 231-254.

7. Takahashi, Shinjiro. Orthodontics. New Edition. Tokyo, Nagasue Bookstore, 1966, pp. 74-105.

8. Takeuchi, T. Biological Reactions and Pathological Changes in Human Beings and Animals Caused by Organic Mercury Contamination. Environmental Mercury Contamination. Ed., Hartung and Dinman. Ann Arbor Sci. Publ., Inc., 1972, pp. 247-301.

9.   Tokuomi, H. Minamata Disease. Study Group of Minamata Disease, Kumamoto University, 1968.

10. Sakai, K. Effect of Methyl Mercuric Chloride on Rat Spermatogenesis. Kumomoto Medical J., Vol. 25 (3), 1972, pp. 94-100.

12. OPTICAL-ELECTRON MICROSCOPIC STUDIES ON TASTE BUD DISORDERS CAUSED BY METHYL MERCURY CHLORIDE

Second Seminar, Pathology, Department of Medicine

Kumamoto University

Team member: Tadao Takeuchi

Research assistant: Morio Sonoda

I. PREFACE

Takeuchi and his associates have reported on characteristic and unusual pathological changes, primarily in the central nervous system, at every opportunity [1]. Takeuchi and his associates [6, 7, 10] and also Eto [8] have further reported on pathological changes in the peripheral nerves, while Matsumoto and his associates [3, 4], Kameda [9], and Miyagawa [5] provided a detailed, clinical, microscopic report, all on disorders of the sensory nerve system.

Apart from disorders of the nervous system, certain pathological changes in visceral organs in general were said to appear in Minamata disease (Takeuchi). About the same time as this study, it was clarified by Takeuchi, Shigenaga, and Sato [11] that there was also a disorder of the islets of Langerhans. Meanwhile, many of the Minamata disease patients complained about a disorder of the sense of taste, and Nozaka and his associates [2] have reported that about 80%, clinically, have disorders in the sense of taste. Since a disorder of the cerebral cortex is involved, judging from Minamata disease cases, the taste nerve cannot be said to be dissociated. Yet, generally speaking, there were not serious changes in the neurons in the brain stem or the thalamus. However, a disorder of the taste nerve endings was believed possible; therefore, we decided to study its receptor, the taste bud, and nerve endings. Since, from our experience with the gastrocnemius muscles in the human body, we knew that optical microscopic studies cannot

be fully accomplished unless electron microscopic changes were observed, we experimentally conducted an optical and electron microscopic study of methyl mercury on the taste buds.

II. EXPERIMENTAL MATERIALS AND METHOD

For experimental animals, we used Wistar strain white mice, weighing about 100 g, and orally administered 3 mg/day (mercury volume 2.4 mg/day) methyl mercury chloride mixed with margarine to each animal, continuously or intermittently. For control, healthy white mice of the same strain were fed the same feed, but without the methyl mercury chloride.

On six mice used for optical microscopic studies, the total mercuric dose administered successively was 7 mg (A), 26 mg (B), 55 mg (C, D, E), 58 mg (F). When about a 40 mg level was reached, the animals were stricken (see Table 1 and Figure 2). The tongues of the animals that had died therefrom or were slaughtered were fixed in formalin, and an ordinary microscopic specimen prepared therefrom was examined.

Animals used for the electron microscopic studies were treated similarly, but in separate experiments. Four mice (Nos. 1 - 4) were examined. No. 1 was daily administered 2.4 mg/day for six days for an aggregate dose of 14 mg; No 2 — 2.4 mg/day for a total of 58 mg; No. 3 was administered intermittently whenever there was a decrease of body weight, and was slaughtered 16 days after it was stricken on the 25th day — aggregate dose was 43 mg. Like No. 3, No. 4 was treated intermittently until the 87th day, and left alone for the ensuing 16 days. On the 37th day, an intersecting phenomenon occurred. The total dose was 77 mg (see Table 1 and Figure 3)

Preparation of the electron microscopic specimens was done according to Mr. Kitamura's [12] method. The experimental animals were anesthetized for about 5 minutes with ethyl, and just before they reawakened they were injected with 0.2 cc/100 g of hexabarbital natrium in the dorsal muscles.

TABLE 3. METHOD OF ADMINISTRATION, DOSE AND NUMBER OF DAYS ELAPSED

	Method of administration	Onset of affliction*	Total number of days elapsed	Aggregate dose	
				MeHg	Hg
A	Daily	–	5 days	15 mg	7 mg
B	Daily	9th day 27 (21 mg)	12	36	26
C	Daily	16th day 48 (38 mg)	23	69	55
D	Daily	22nd day 66 (53 mg)	23	69	55
E	Daily	21st day 63 (50 mg)	23	69	55
F	Daily	16th day 48 (38 mg)	24	72	58
No. 1	Daily	– 18 (14 mg)	6	18	14
No. 2	Daily	16th day 42 (38 mg)	24	72	58
No. 3	Intermittently	25th day 48 (38 mg)	41	54	43
No. 4	Intermittently	37th day 75 (60 mg)	103	94	77

*Milligram numbers in parentheses ( ) for MeHg have been converted to indicate amount of Hg.

Ten minutes later, the stomach was cut open, the aorta and cervical vein exposed, the blood flow stopped on the descending aorta at a point just above the branching area of the kidney artery, the artery wall incisioned, about 300 cc of 3% glutalaldehyde solution (0.05M, pH 7.4, with cacodyalte buffer) was pressure injected in reverse flow, and when the previously exposed cervical vein became inflated, it was severed with a razor and the head was biotically irrigated (see Figure 1). Following irrigation, the tongue was removed and severed into small fragments lengthwise. After 1 hour of fixing in glutalaldehyde solution, post-fixing was carried out with 1% osmium
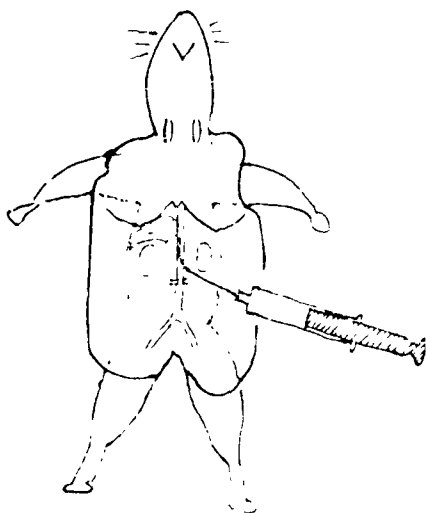
Figure 1
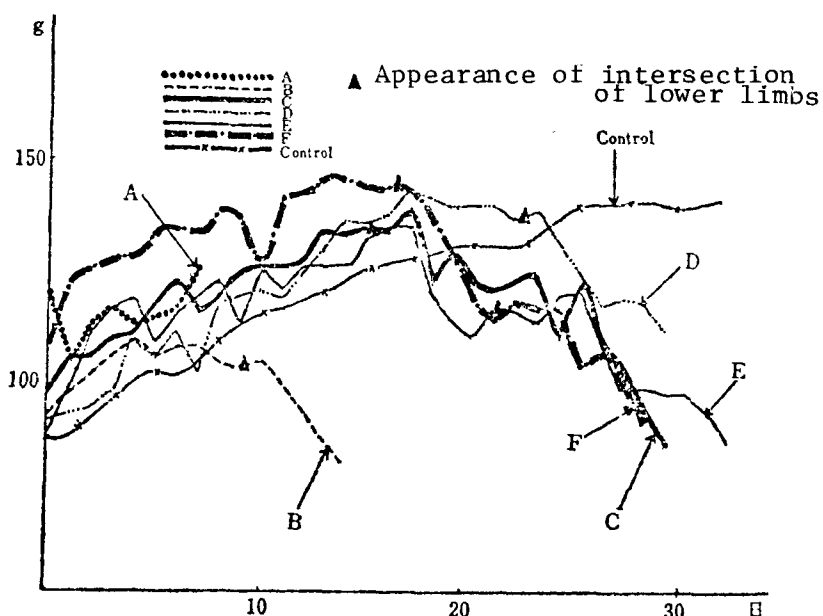
Figure 2. Changes in body weight of animals for electron microscope use
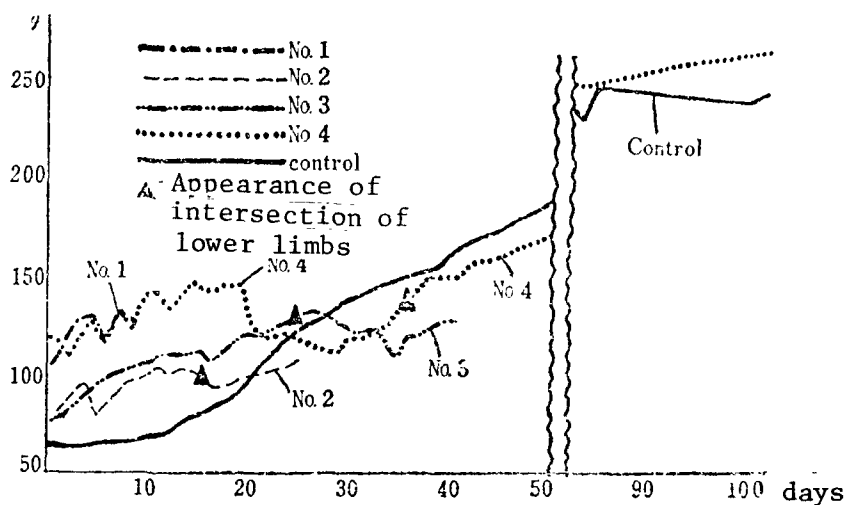
Figure 3. Changes in body weight of animals for electron microscope use

tetroxide (0.1M, pH 7.4, with veronal buffer). Then water was removed, enveloped with epon, a 1 micron slice was made with ultrotome of LKB, and toluidine blue-stained optical microscopic specimens were prepared. After observing them, the required portion containing the taste bud was trimmed

390

and a thin slide was obtained. Upon double staining according to the Raynolds method, it was examined with Hitachi electron microscope HU-11A

III. EXPERIMENTAL FINDINGS

1) Optical microscopic observations

A (total dose, 7 mg): As compared to the control (Photos 1, 2), the epithelial and innate layers were marked by mild atrophy, as a result of which the groove of the taste bud was somewhat wider. Swelling and vacuolar changes of nuclei of taste bud cells and mild nuclear fusion were noted. As a result, some irregularity in the size of the nucleus, as well as a mild degenerative fusion of cytoplasm, was seen. Partial changes were observed in nerve fibers in the innate layer, and the Schwann's cells were enlarged (Photos 3, 4). Some of them on the epithelium of the mucous gland in the muscular layer were swollen (Photo 15).

B (total dose, 26 mg): No marked changes were noted in the epithelium in general; however, mild atrophy was seen in the epithelium of the taste bud and the groove was somewhat wider. As for taste bud cells, the size of nuclei varied, some were swollen, and chromatin condensed into granular forms or were concentrated. Also, cell bodies undergoing fusion were scattered, and interstitial cells were scanty (Photos 5, 6). Partial changes were noted in nerve fibers in the innate layer, as well as an increasing trend of Schwann's cells. Partial changes were seen in glandular cells of the mucous gland, and a mild change in the epithelium of the serous gland.

C (total dose, 55 mg): There was marked degenerative atrophy in the epithelium of the taste bud area, and the groove was considerably wider. Degenerative changes in the taste buds were serious, there being great atrophy. In the taste bud cells, there was an increase of small nuclei into which chromatin had concentrated, but, on the other hand, there was a marked degenerative enlargement of the cytoplasm. There was also atrophy of the innate layer, and a mild increase of nuclei substances. While not serious,

391

there were partial changes in the nerve fibers between the surface and deeper area of the innate layer (Photos 7, 8). Serious changes were observed in the epithelium of both mucous and serous glands.

D (total dose, 55 mg): Moderate atrophy was seen in the epithelium of the taste bud area, with degenerative atrophy being marked in the taste bud. Taste bud cells were undergoing pyknosis or fusion and obliteration, thus making the inside of the taste bud brighter and sparse. Fat tissue was on the increase in the innate layer; there was degenerative enlargement of nerve fibers, and enlarged Schwann's cell nuclei. The nerve fibers in the basal portion of the innate layer were fairly intact, but there was an enlargement of Schwann's cell nuclei (Photos 9, 10). Moderate degree of degenerative swelling of the epithelium of the nucous and serous glands, as well as fusion, was seen.

E (total dose, 55 mg): There was marked atrophy in the epithelium of the taste bud area, and a rather severe epidermoid keratosis in the surface layer. Atrophy of the taste buds was even more serious, there being a space between the surrounding epithelium and its border of the taste bud. Most of the nuclei of the taste bud cells were undergoing serious pyknosis or fusion and obliteration; the cells themselves were in the process of disintegration and obliteration, and the taste bud was contracting markedly (Photos 11, 12). Degenerative disintegration of some glandular cells was observed in the epithelium of both mucous and serous glands.

F (total dose, 58 mg): Mild atrophy of the epithelium of the taste bud area, and a relatively marked irregularity in the size of taste bud cells were noted. A considerable number of taste bud cells was swollen. Interstitial cells were scanty and light. There was atrophy in the innate layer (Photos 13, 14). Nerve fibers were visible in the lower area of the innate layer, the medullary sheath being swollen. Moderate changes were noted in the epithelium of mucous and serous glands (Photo 16).

392

TABLE 2. DEGREE OF PATHOLOGICAL SYMPTOMS OF TONGUE

Pathological changes / Animal code	Upper surface of membrane		Taste bud			Innate layer			Secretory gland		Cell infiltration
	Atrophy	Hornification	Atrophy and reduction in number	Nuclear degeneration and disintegration	Irregularity of size of nucleus	Atrophy	Degeneration of nerve fibers	Increase in fat	Epithelial degeneration of mucuous gland	Epithelial degeneration of serous gland	
A	(+)	—	+	++	±	+	(±)	--	--	(-)	—
B	+++	+	+++	+++	++	+++	++	—	--	(±)	—
C	++	—									
D	++	(±)	+++	+++	+++	+++	--	—	--		—
E		++									
F	±	++	++	++	++	++	++	—	--	--	±
Control	—	—	—	—	—	—	—	—	—	—	--

TABLE 3. AVERAGE NUMBER OF NUCLEI IN A TASTE BUD

Animal code	Observations	Number of taste buds	Number of cells nucleus	Average number of nucleus
A	28	254	9
B	21	228	11
C	21	177	8
D	22	168	7
E	16	86	5
F	26	195	7
Control	27	271	10

393

Table 2 is a summarization of the foregoing (see Table 2). It shows the extent of differences in marked pathological changes, depending on the level of methyl mercury administered. Also, as Table 3 reflects, a remarkable difference was noted when we counted the average number of nuclei in a single taste bud, in view of their decrease in the taste buds (see Photo 3).

2) Electron microscope observations

Following epon enveloping and preparation of a 1 micron slide, we examined the toluidine blue-stained specimens. In No. 1 (total dose, 14 mg; Photo 17), the boundary of each cell was relatively distinct. In No. 2 (total dose, 58 mg; Photo 18), however, all taste bud nuclei were moderately transparent and swollen, the nuclei had no difference in shade, presented a uniform oval appearance, and cell boundary was indistinct; compared to No. 1, the taste bud was in a state of atrophy. Changes similar to those described under optical microscopic observations in 1), above, clearly became evident as dosage was increased. The taste bud area of this specimen was trimmed, and an electron microscopic photograph was made thereof.

We wish to describe below the pathological changes in the taste bud as they occurred, depending on the period of administration and dose.

In the initial stage of administration (No. 1, 14 mg Hg, administered for 6 days), there was no marked change in Class I cells (dark cells, supporting cells), and Class II cells (light cells, taste cells) among taste bud cells, but a small vacuolar space was seen between cells, and electron density of cell nuclei appeared to be somewhat increasing, making differentiation between Class I and Class II cells somewhat difficult (Photo 20). In the spaces between cells, changes in the movement of and increases of microvilli was seen (Photo 21). An irregular but distinct pathological change was noted in a part of the ground substance of filaments. About this time, a similar change was observed in the axon of the peripheral neurite, but no changes were seen in neurotubules.

394

13

14

15

16

17

18

19
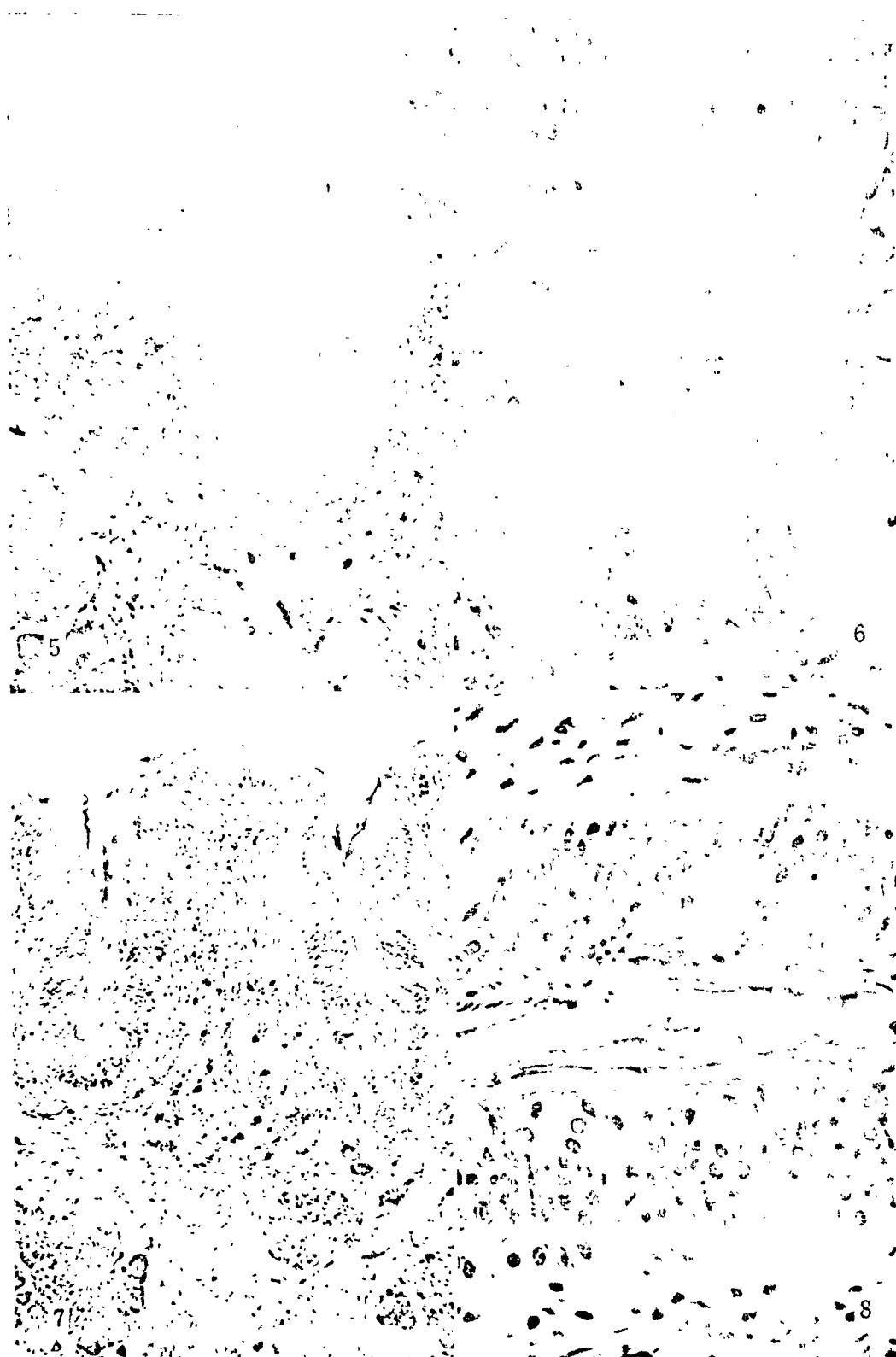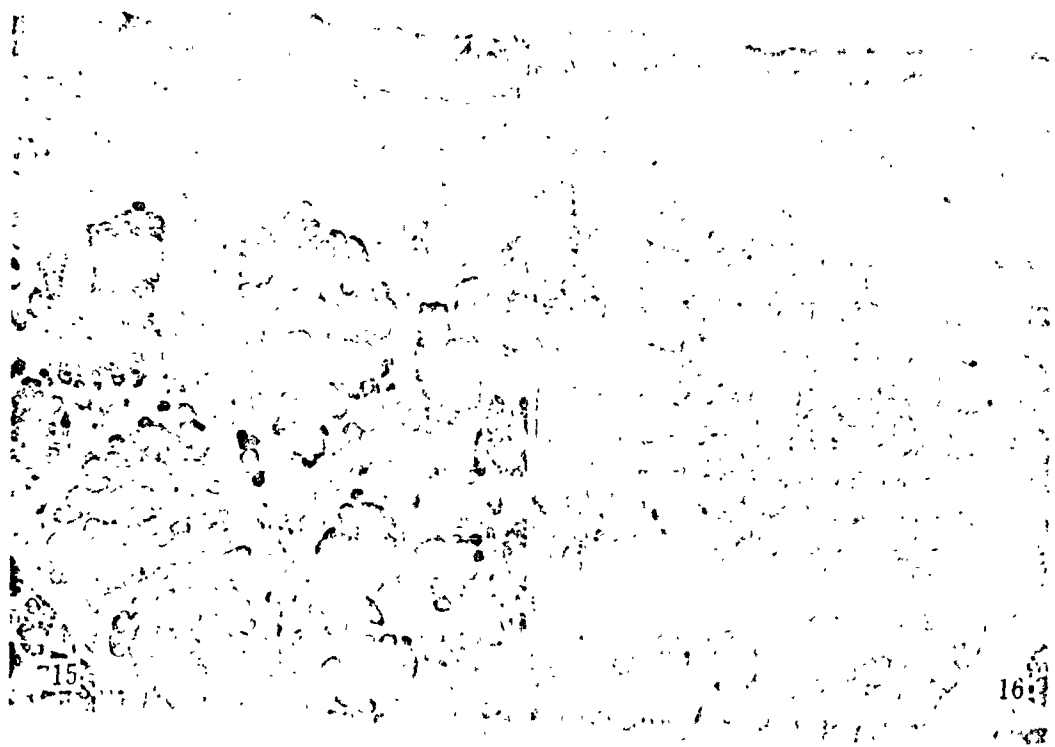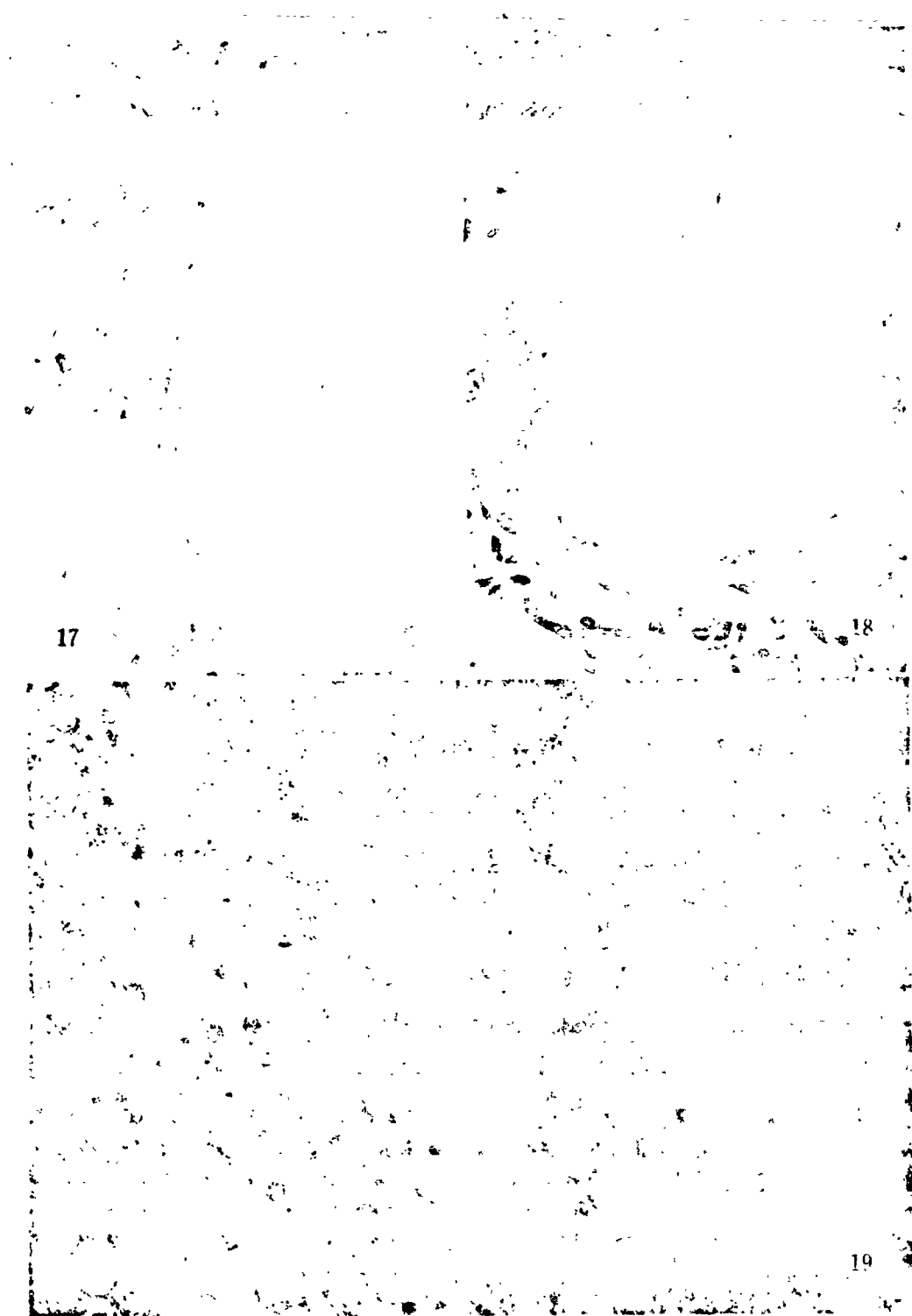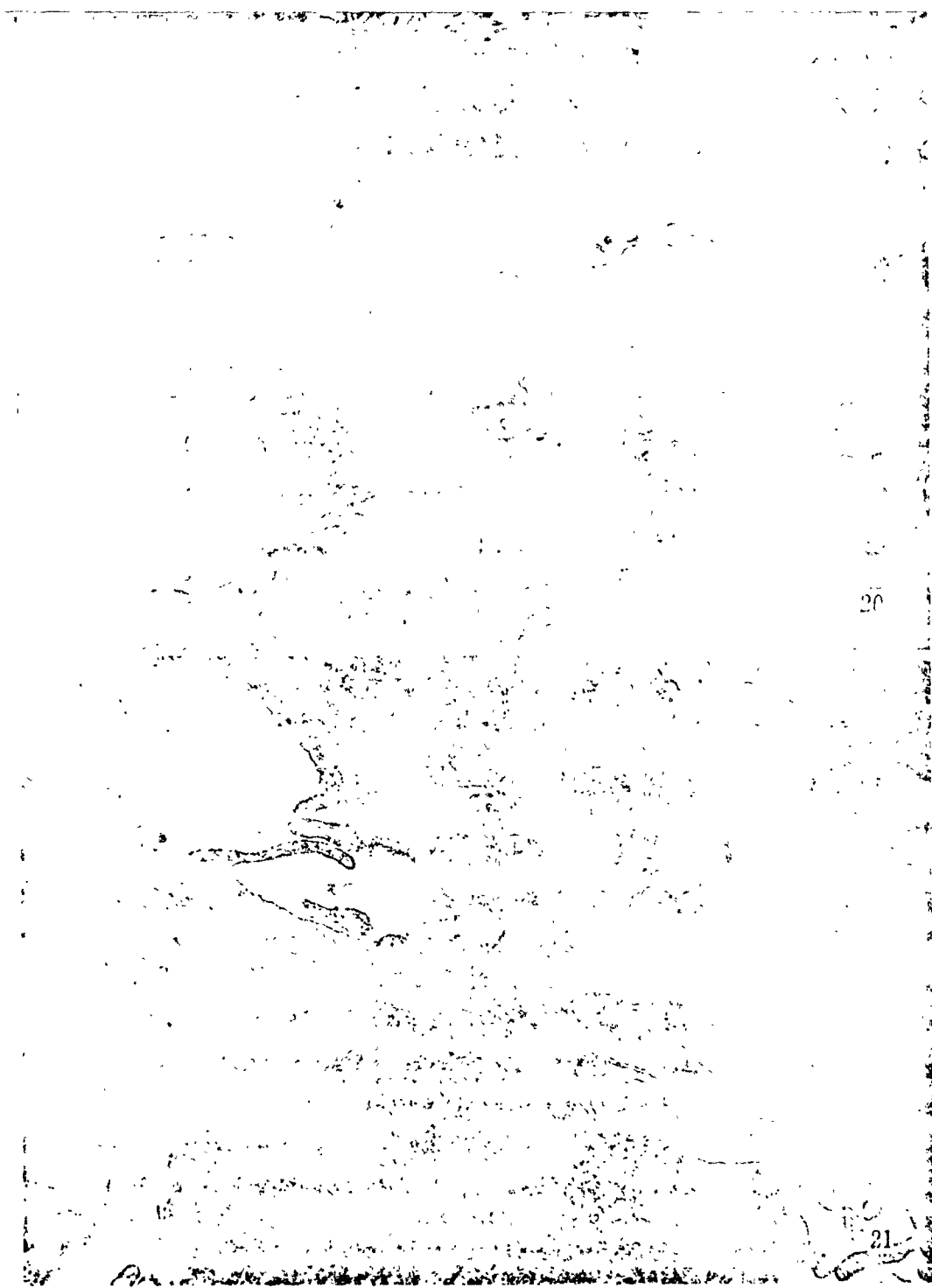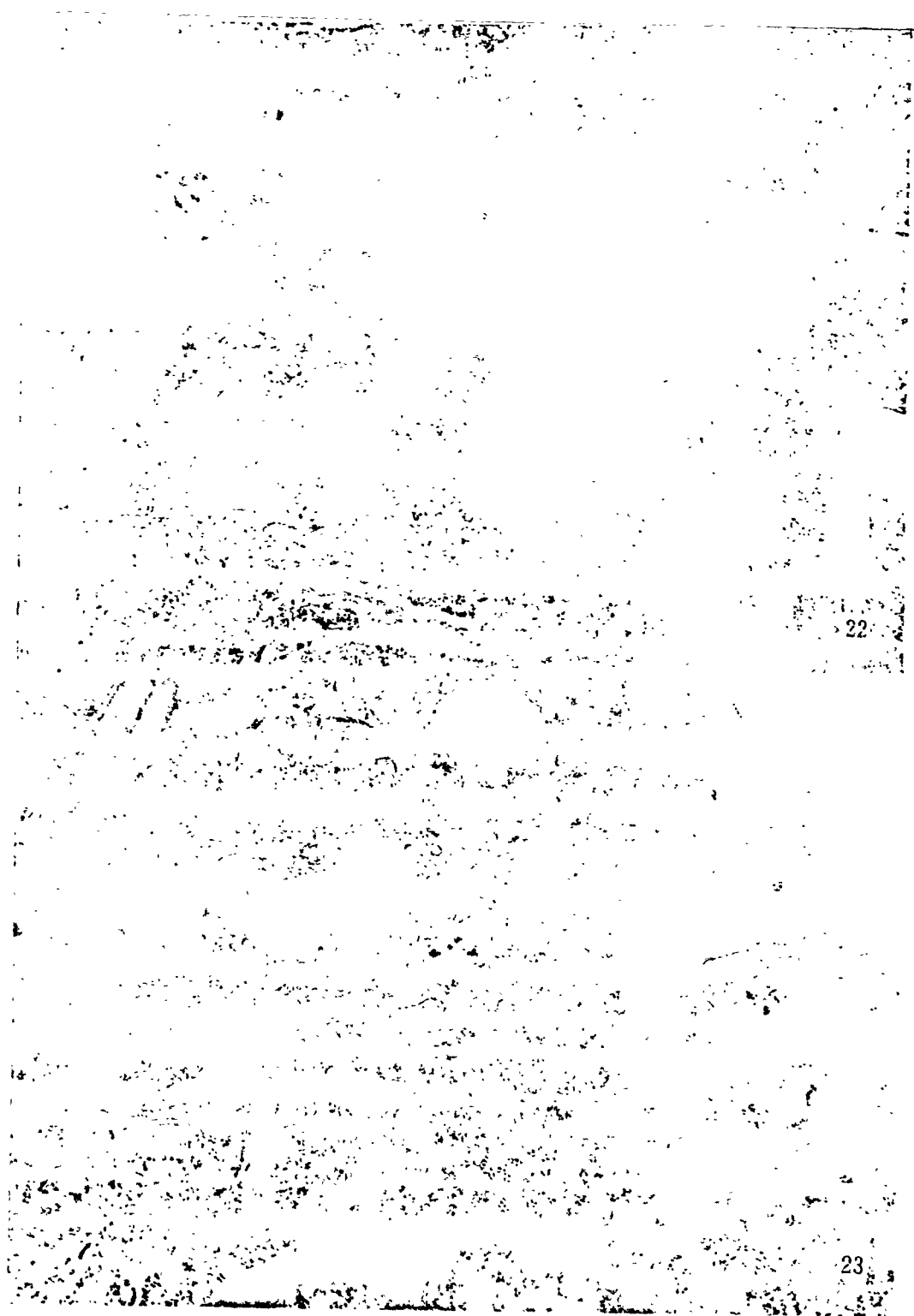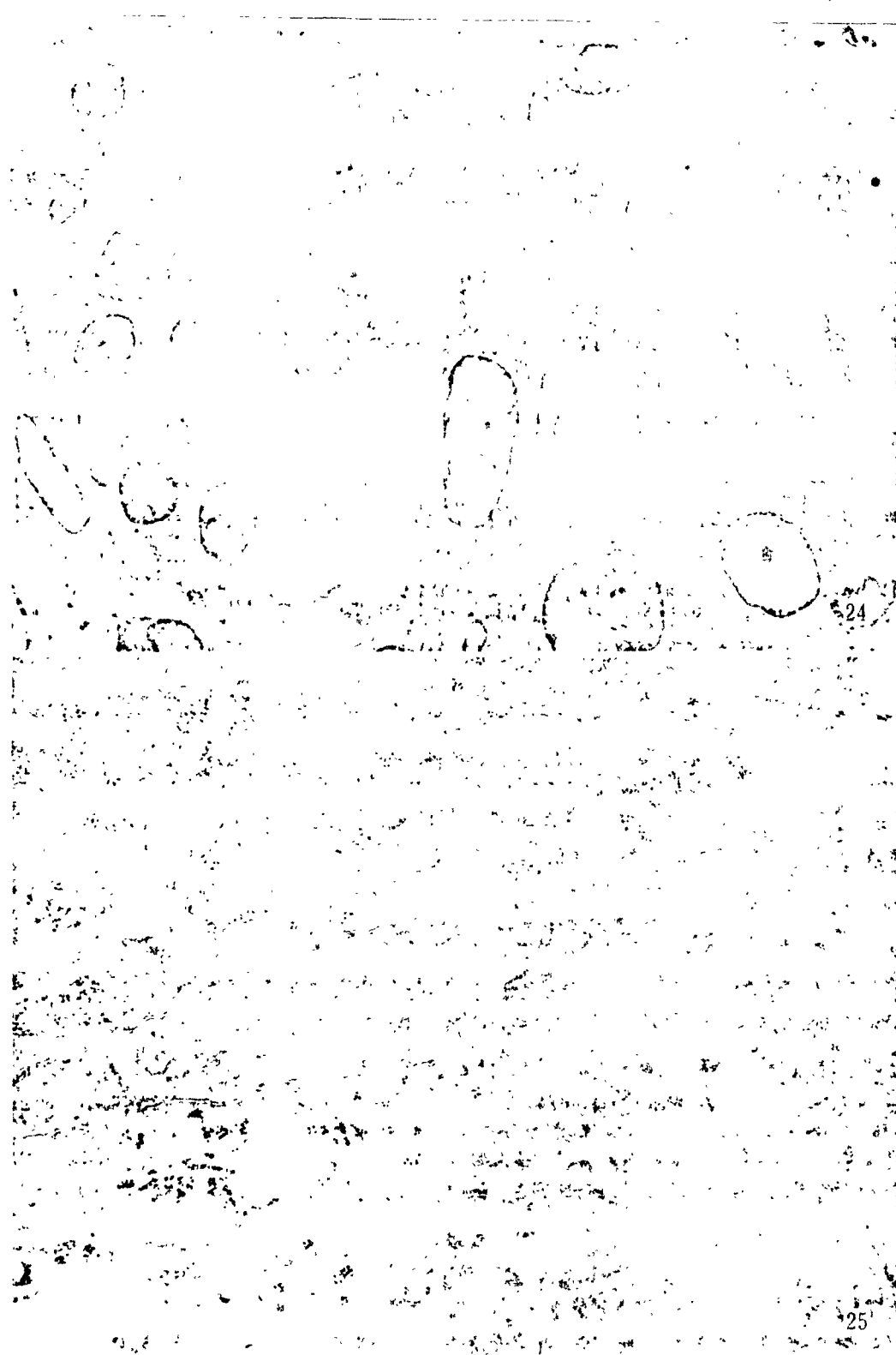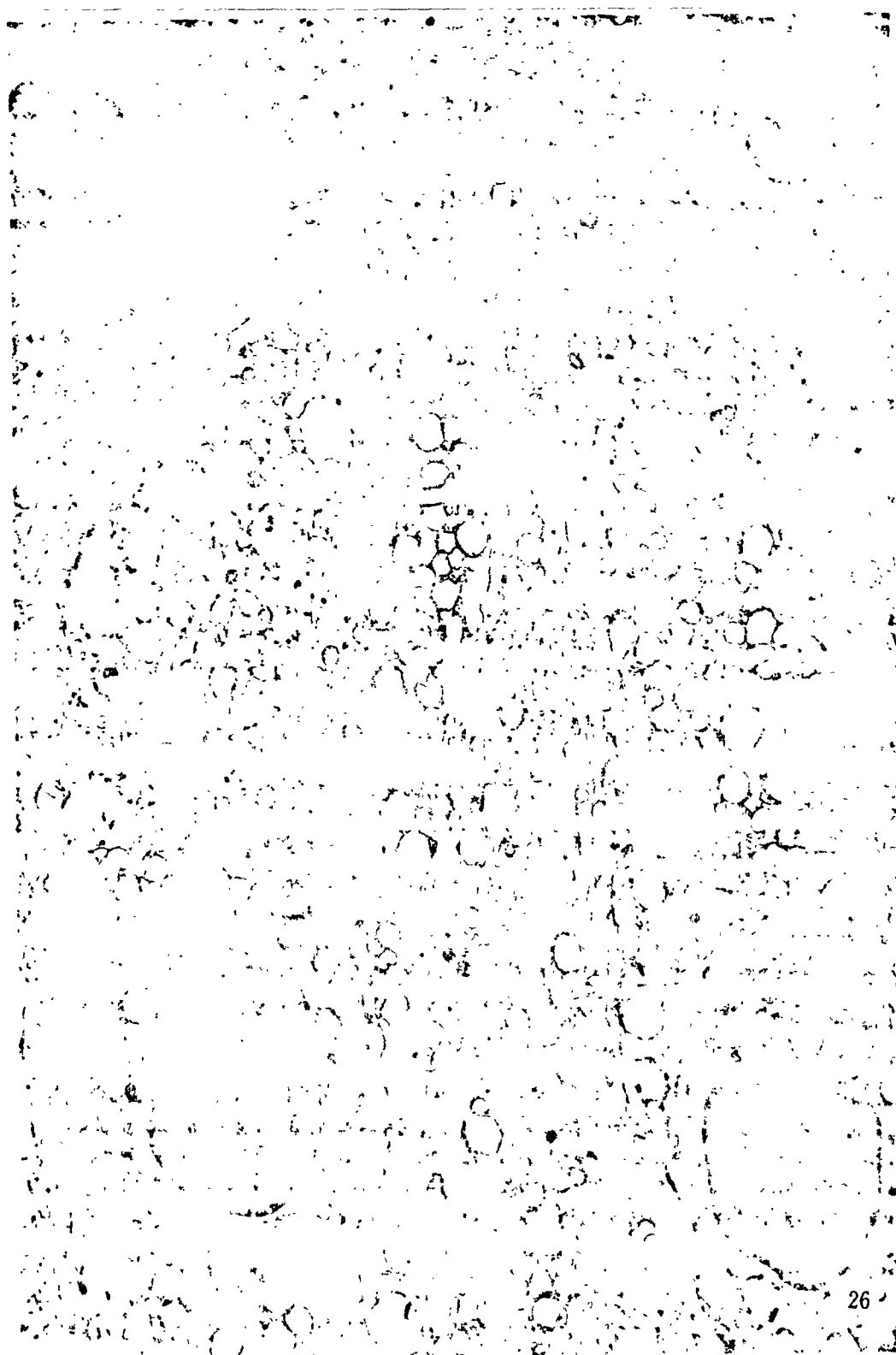
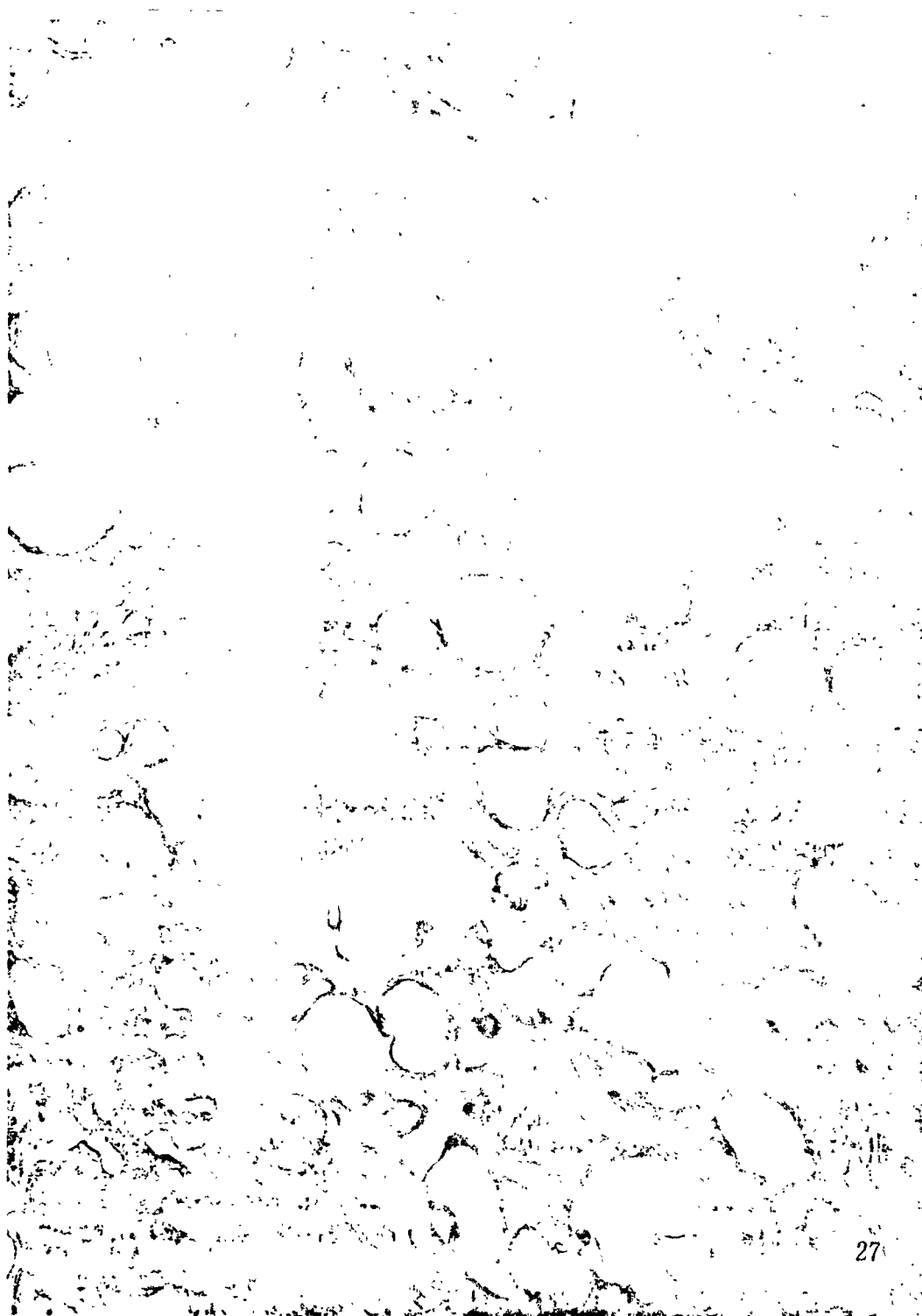## Description of Photos

Photos 1 - 2:   Control case (low and high magnification of the taste bud)

Photos 3 - 4:   Case A (fifth day); nuclear enlargement and fusion is seen in the taste bud cell; the cell body is swollen

Photos 5 - 6:   Case B (twelfth day); degenerative change is fairly distinct in the taste bud; in the taste bud cell (primarily taste cell), there is pyknosis of the nucleus and nuclear destruction; some are undergoing degenerative disintegration as cell bodies become swollen

Photos 7 - 8:   Case C (23rd day); marked degenerative atrophy of the taste bud; taste bud has been reduced in size because of degenerative disintegration of taste bud cells

Photos 9 - 10:   Case D (23rd day); although the same number of days has elapsed as in the case above, there is greater degenerative atrophy, probably owing to larger mercury dose; degeneration and disintegration marked in taste bud cells; nuclei undergoing pyknosis in remaining taste cells; vacuolar degeneration in cell bodies

Photos 11 - 12:   Case E (23rd day); virtually identical observations can be seen in the taste bud as above; some atrophy in the surrounding epithelial cells; relatively serious keratosis in the surface layer

Photos 13 - 14:   Case F (24th day); milder changes than above; the process of degeneration to disintegration of taste bud cells can be seen in each

Photo 15:   Salivary gland (tongue gland) of Case A; degenerative swelling is seen in part of the mass in the epithelium of the serous gland

Photo 16:   Salivary gland (tongue gland) of case F; prominent degenerative swelling on serous gland, extending in the mouth

Photo 17:   Control case of toluidine blue-stained, epon-covered, one-micron slice; Class I and II cells seen in the taste bud

Photo 18:   Toluizine blue-stained, epon-covered slice from Case No. 2 (24th day); degenerative atrophy of the taste bud; distinction between Class I and II cells (taste cells) impossible; nucleus of taste cells transparent and border indistinct

Photo 19:   No. 3 (41st day); part of apical, formative substance of taste cells near the tip of the taste bud; vacuolation of filaments; partial vacuolation of smooth-surfaced vesicle; possibly serious disorder of receptor structure (electron microscope, 10,000 x 2.5)

Photo 20: Deep area of taste budy of No. 1 (sixth day); basal membrane of taste bud normal, but some spaces between taste cells· increased electron density of nuclei (electron microscope, 2000 x 2.5)

Photo 21: Enlarged view of No. 1; slightly irregular transparency in ground substance of some of the filaments; neurites partly visible, but no marked changes; identical changes in their filaments (electron microscope, 10,000 x 2.5)

Photos
22 - 23: No. 2 (24th day); most serious changes seen in filament, with ground substance becoming transparent and appearing to be worm-eaten, and there being obliteration of cristae; among marked cases, some filaments undergoing vacuolation, leaving only their external membrane. In Photo 22, no change was seen in coarse-surfaced vesicles, but as shown in Photo 23, there were maceration and obliteration of ribosomes in some cells. Vacuolation was also seen in smooth-surfaced vesicles (electron microscope, 10,000 x 2.5).

Photo 24: No. 4 (103rd day); two taste buds; increased electron density of nuclei of taste cells evident in comparison with that in surrounding epithelium; marked vacuolation of taste cells also seen (electron microscope, 2500 x magnification).

Photo 25: No. 2 (24th day); marked vacuolation of filaments in taste bud cells: also vacuolation of vesicles; part of neurite running laterally between two taste cells (center); increase as well as destruction of filaments, with some becoming transparent and appearing to be worm-eaten (electron microscope, 10,000 x 2.5)

Photo 26: No. 4 (103rd day); shows 4/5 of single taste bud; mild changes during this period, but marked vacuolation visible through electron microscope; decrease of taste cells; increased electron density of nuclei and concentration thereof; vacuolation of filaments; enlargement of vesicles; decrease and obliteration of ribosomes; irregularity of Golgi apparatus and enlargement of Golgi sac; formation of dense body (electron microscope, 2500 x 3)

Photo 27: Enlargement of taste cells of No. 4; marked vacuolation of formative substance of cells; sound filaments are few, with most being transparent and undergoing vacuolation; enlargement and vacuolation of vesicles; irregularity of Golgi apparatus; enlargement of Golgi sac; concentration of nuclei; decrease of filaments (electron microscope, 6000 x 3).

As the dose was increased and days passed (Nos. 2 - 3), Class I cells disappeared in the taste bud, and only Class II cells were visible. Further, there was some increase of electron density of the cells, clearly manifesting pathological changes when compared with the surrounding epithelial cells of the taste bud. In Class II cells, marked changes appeared in the filaments; there was a loss of cristae, and the ground substance of the filament was transparent and appeared to be worm-eaten. They were gradually undergoing vacuolation, forming large vacuoles covered by a filament membrane (Photos 22, 23). As for vesicles, microvacuolation of smooth-surfaced vesicles appeared, and irregular enlargement of Golgi vacuoles was seen in the Golgi field. No marked changes were noted in the cells, except for increased electron density. There was an irregular increase of microvilli located between the cells, and a wider space between such cells. No marked changes were observed in the basal membrane of taste buds, and some irregularity was noted in the filaments.

In neurites, similar changes were seen in filaments in the axons, but no marked change was noted in neurotubules.

When the dose of methyl mercury was further increased and as days passed (No. 4), marked changes generally appeared in the taste buds and no distinction could be made between Class I and II cells; there was a greatly increased electron density as compared to the surrounding epithelium, as well as vacuolation (Photo 24). In taste cells, the nuclei were concentrated, chromatin was changed, and the double structure of nuclear membrane was distorted and spaces were created. There was a loss of cristae in the filaments, enlargement of vacuoles due to transparency of the stroma, and creation of many large vacuoles. Destruction or fusion of vacuoles was also noted, there being great changes in vacuoles (Photos 24, 26). Their changes were remarkable when compared to those in surrounding epithelial cells. There was a great increase of microvacuolation in the vesicles, especially in smooth-surfaced vesicles, forming relatively small spiny cells in some places. The structure of the Golgi field was irregular, and spiny cells were seen (Photo 27). There was no irregularity of RNA granules. Although no marked changes were

407

seen in the dense body of lysosomes, they indicated a tendency to increase
to some extent. A marked decrease and irregularity was noted in filaments,
and in taste cells under going great changes, the process of cell deteriora-
tion caused by spiny cell formation was seen (Photo 24). Mild changes were
also noted in peripheral cells, with some becoming spiny.

In neurites, degeneration of spines in the filament was noted, with
neurotubules being indistinct.

IV. SUMMARY AND COMMENT

Nozaka and his associates have clinically indicated that there were
both olfactory and gustatory disorders in Minamata disease patients. However,
no light was shed on how such disorders developed. In the cerebral cortex,
thinning and fallout of nerve cells occur, but not completely, with most
nerve cells remaining except in extremely serious cases. Therefore, a study
was required to determine whether peripheral factors were involved in
gustatory disorders.

Using mice, we studied the changes in the taste bud, which is the taste
receptor, by orally administering methyl mercury chloride and experimentally
eliciting toxic symptoms similar to those in Minamata disease.

In eliciting poisoning symptoms, we considered the three features claimed
by Takeuchi and his associates. Symptoms appeared when the dose of mercury,
starting at a minimum of 21 mg per animal, was increased to a maximum of 60 mg.
In the case of continuous administration, symptoms developed even at a level
of roughly 38 mg per animal. As for the period of administration, the minimum
was 9 days, but symptoms developed usually after 2 to 3 weeks, and in one case,
after more than a month. The administration of organic mercury was continued
even after the initial symptoms appeared. The total mercury level administered
was a minimum of 26 mg per animal to a maximum of 77 mg per animal, over
a period of 12 to 77 days. Pre-symptom cases examined were those which had
been administered 7 mg per animal for 5 days.

Morphological changes in the taste bud tissue of these animals were visible under an optical microscope. After 5 days, the inside of the nucleus of the taste bud cell was already transparent, and the cell body was enlarged. At the end of 12 days, degeneration of these cells was apparent, with emergence of nuclear pyknosis, partial fusion, spiny formation on the cell body, enlargement and swelling. After a lapse of 3 weeks, there was degenerative disintegration of the taste bud cells, nuclear pyknosis, nuclear destruction, fusion and obliteration, with some cell bodies becoming swollen and disintegrating. As a result, there was a decrease of taste bud cells, marked degenerative atrophy, and even contraction and loss of the taste bud.

Compared to the relatively profound pathological changes in the taste bud, the surrounding epithelium was hardly affected. Only vacuolar changes and some atrophy were noted. In some cases, there were fairly strong instances of keratosis.

In peripheral nerves in the innate layer, changes could not be seen using ordinary stains, especially HE, but through the use of KB stain, swelling of the axons were visible. Changes in Schwann's cells could not be determined, optically.

No serious changes were noted in the salivary gland. However, a future study is needed, since some glandular epithelial changes and degenerative disintegration of glandular cells was seen.

An electron microscopic study showed changes in neurites of the taste bud and nerve endings. In the taste bud, changes were apparent at the end of 5 days, progressively becoming serious. In other words, Class I cells (dark cells, supporting cells) could not be differentiated from Class II cells (light cells, taste cells), and taste cells occupied a larger portion of the taste bud. In the taste cells, localized light areas in the electron density in the stroma of filaments appeared, and as the light area enlarged, the stroma had holes as if eaten by worms. Simultaneously, the cristae was

obliterated, and spiny formation on filaments increasingly developed. The filaments formed spines completely, leaving only the outer membrane, became enlarged, and then burst. As for vesicles, the ends of smooth-surfaced vesicles became enlarged, underwent microvacuolation, and gradually became vacuolized. In the coarse-surfaced vesicles, RNA granules had fallen and free ribosomes were disarrayed and decreasing. On the other hand, changes also occurred in the membrane structure in the Golgi field, with disconformity of the membrane, vacuolotion, and gradual obliteration. In the nucleus, its electron density generally increased at the outset, but there was a change in homogeneity and its structure was obliterated, with the double structure of the nuclear membrane being ruptured. In such a fashion, the taste cells tended toward degenerative disintegration, as the filaments decreased.

While peripheral cells of the taste bud have offered some resistance, degenerative changes commencing with vacuolation of filaments also occurred in taste cells.

Neurites on nerve endings were faily well preserved, but were affected as taste bud orders progressed. With respect to changes in the neurites, the axon structure was clearly visible at the outset, but there was a gradual vacuolation in the filaments as in the case of taste cells. This was later followed by changes in neurotubules, and swelling of the entire neurite was seen.

Outside of the taste bud, changes were generally mild. While there was a little pitted vacuolation of filaments in epithelial cells, most filaments were sound, and no serious disorder was seen in the cells.

As explained above, it was found that in the case of methyl mercury poisoning, pathological changes were induced in the sensory receptor, which is the final apparatus manipulating the sense of smell. It was also found that a cell disorder commencing with the destruction of filaments in the taste cells, which are most important to the taste bud, ultimately induced cell disintegration and obliteration, causing a decrease in the number of

taste buds. At the same time, it caused chages in neurites, making it impossible for taste buds to perform their functions as taste receptors.

Although it had been known that sensory disorders were involved in Minamata disease, little was known concerning receptor disorders in the periphery. Cell disorders caused by methyl mercury in the retina, the visual receptor, and the Corti's organ, the hearing receptor, have not yet been established. There is believed to be no great change, but further study will, of course, be required. The revelation of cell disorders in taste receptors, as described above, will probably necessitate a study of other sensory receptors.

V. CONCLUSION

A study was made of pathological changes induced in taste receptors by experimentally evoking organic mercury poisoning symptoms in mice by oral administration of methyl mercury chloride. The following results were obtained:

1. Methyl mercury developed a disorder in the taste bud of taste receptors, and induced degenerative disintegration of taste cells. As a result, there was a decrease and obliteration of taste cells, and the taste bud contracted and was obliterated.

2. Optical microscopically, taste cells developed changes such as nuclear pyknosis, nuclear destruction, nuclear fusion, as well as enlargement, swelling, and fusion of cell bodies.

3. Electron microscopic differentiation between Class I and Class II cells was difficult. In taste cells, which are Class II cells, there was transparency with holes as if eaten by worms of the stroma in filaments, a loss of cristae, ultimately vacuolation and disintegration of the filament. Changes also appeared in the vesicle and Golgi apparatus, with greater electron density, uniformity, and finally, vacuolation of the entire cell.

411

4. In nerve endings, degenerative changes appeared at the outset in filaments, similar to those in taste cells, with changes in neurotubules following later.

5. Outside of the taste buds, there were few changes in the epithelium. Vacuolation was induced in part of the filaments, but there was no cell disorder. As a result, the taste bud disorder was clearly visible.

Observations concerning the inducement of disorders in the sensory receptor caused by methyl mercury in the taste bud were significant. Our study was the first to explain pathological changes in the receptors.

REFERENCES

1. Takeuchi, Tadao. Pathology of Minamata Disease, Minamata Disease (Compiled by Research Team, Dept. of Medicine, Kumamoto University). Shuhan, 1966, pp. 194-282.

2. Nozaka, Yasuji. Taste Disorder, Minamata Disease (Compiled by Research Team, Dept. of Medicine, Kumamoto University). Shuhan, 1966, pp. 147-148.

3. Matsumoto, Fuo, and Kameda. Pathological Study of Toxic Polyneuropathy (I); Experimental Formation of Polyneuropathy Through Methyl Mercury. Progress of Neurology, Vol. 13 (3), 1969, pp. 660-669.

4. Matsumoto and Kameda. Pathological Study of Toxic Polyneuropathy (II); Electron Microscopic Observations Concerning Degeneration and Regeneration of Nerve Fibers Caused by Methyl Mercury Chloride Poisoning. Latest Medicine, Vol. 25 (4), 1970, pp. 939-950.

5. Miyakawa, T., et al. a) Experimental Organic Mercury Poisoning, Pathological Changes in Peripheral Nerves. Acta. Neuropath., Vol. 15, 1970, pp. 45-55. b) Regeneration of Peripheral Nerves. Acta Neuropath., Vol. 17, 1971, pp. 6-13.

6. Takeuchi, et al. Electron Microscopic Observations During Biophia of Peripheral Nerves Under Conditions of Methyl Mercury Poisoning (Minamata Disease) in Human Beings. Twelfth Meeting, Japan Neuropathological Association (Tokyo), 1971.

7. Taeuchi, T. Biological Reactions and Pathological Changes of Human Beings and Animals Under Condition of Organic Mercury Contamination. Intern. Congr. Environ. Mercury Contamination, 1970. Ann Arbor, 1970, pp. 1-30.

8. Eto, Mitsuaki. On Pathological Changes of Peripheral Nerves in Minamata Disease in Human Beings, Especially on Its Electron Microscopic Observations. Progress of Neurology, Vol. 15 (3), 1971, pp. 606-618.

9. Kameda, Kosei. Pathological Study of Toxic Polyneuropathy (III). Optical Microscopic and Electron Microscopic Study on Regeneration Following Peripheral Nerve Disorder Caused by Methyl Mercury Chloride Poisoning. Kumamoto Medical Hournal, Vol. 45 (10), 1971 pp. 985-999.

10. Takeuchi, et al. Electron Microscopic Observations During Biophia of Gastrocnemius Nerve in Fetal Minamata Disease. Kumamoto Medical Journal, Vol. 46 (11), 1972, pp. 706-719.

11. Takeuchi, Shigenaga and Sato. On Pathological Changes in Islets of Langerhans in Minamata Disease Biopsy. In this report.

12. Kitamura, Katsuya. Neurohistological Study on Degeneration and Regeneration of Taste Buds. Kyushu Dental Association Journal, Vol. 18 (3, 4), 1965, pp. 1-40.

VI SUMMARY

These research studies on Minamata disease in the Minamata district and the surrounding area have clarified somewhat the conditions of mercury pollution, health conditions of the inhabitants, the incidence of the disease, the progress of the disease, and the fundamental knowledge of clinical treatment for the disease. Following is a summary of the important results in this report.

1. The situation of mercury pollution in Minamata district and the
    surrounding area.

Fish and shellfish from outside or around Minamata bay have recently
served as the main protein source of food for the residents. Seafood taken
from the area was, therefore, examined for mercury content. The mercury
content in the fish taken from outside Minamata bay (around the Koiji island),
is remarkably decreased from the value measured in 1961. The highest mercury
content in the fish by wet weight was 0.530 ppm. and the highest methyl
mercury content was 0.440 ppm. The lowest value was 0.182 ppm which was
higher than those of other districts. If 200g of the seafood containing the
highest mercury levels were eaten daily, the diet would exceed the level
estimated to be harmless by Dr. Kitamura as presented in his table on the
toxic effects of mercury; however, it would remain below the level of
poisoning. In the polluted area, generally, shellfish were more contaminated
with mercury than fish. In the heavily polluted area in Minamata district,
there have been patients having mild symptoms of Minamata disease from 1965
to the time of this survey. Visceral organs from autopsy cases with Minamata
disease had high mercury levels, considering the bio-half life of mercury
in viscera. It is also known that the bio-life of mercury in the brain is
longer than in the viscerral organ. Therefore, it will be recommended that
no more than 200g of fish and shellfish contaminated with mercury from the
area of Minamata bay can be eaten daily.

In Minamata district, the relationship between the distance from
Minamata bay and the methlymercury content of fish, taking into account fish
species, was examined. Even though there were some differences of mercury
content among fish species, in general, methyl mercury content was highest
in fish taken from Minamata bay and its neighboring sea. Next high values
were found in fish from the mouth of Minamata river, the hairtail fishing
area in Minamata bay, and the Kurata area. According to the distance from

Minamata bay, methylmercury content in fish was gradually decreased in the Yatushiro Sea. Fish from Midoriura and Yatushiro districts had remarkably low levels of mercury. Therefore, fish from these areas seem to be less dangerous than that from the Minamata bay area. In contrast to these areas, mercury pollution in the Ariake sea area is a new problem. In the Ariake sea area, fish and shellfish taken from the coast of Yushima Island and the mouth of Midori River contained somewhat high values of methylmercury as compared with those from other areas. The value was not so high as the value estimated for Koiji Island, but nearly equal to that for the mouth area of Minamata River. This suggests that there has been an additional source of mercury pollution in the area when the low value of mercury content in the fish from Yatushiro area and the ocean stream is considered. Even though the number of people studied and the experimental error for the survey were taken into account, the mercury content of hair from many residents of Minamata district was high while the mercury content of hair from people living in the Goshonoura area was relatively low. In Minamata district, the highest value of total mercury found in the male was 14.82 ppm of which 11.37 ppm was methyl mercury; in the female, the highest value for total mercury was 10.44 ppm of which 9.21 ppm was methyl mercury. These values indicate that the mercury pollution in the area is below the poisonous dose level at this time. However, many people having high mercury contamination were found. This fact is indicative that the people in this area have been exposed to mercury pollution. In contrast with the Minamata area, only one person having 4.65 ppm methyl mercury in the hair in the Goshonoura area was detected and all the others were found to have less than 1.35 ppm. The results suggest that Goshonoura area has not been polluted with mercury recently.

2. Health hazard of residents in Minamata district.

From the epidemiologic survey, two remarkable points were recognized.

1) Death statistics in the Minamata district during the past 24 years from 1948 to 1971, show that the average span of human life in the Minamata area is lower than that of the Ariake district, chosen as a control. The average age of death was 50.36 ± 28.91 in the male and 52.50 ± 29.49 in the female. The average age of death in females in the Minamata area is remarkably younger than that of females in other places. This trend is also observed in statistics for every five year period. The age of death in females observed in Minamata district was younger than that at any other place in Kumamoto prefecture. The average life span in Goshonoura area from 1963 to 1967 was significantly shortened. The rate of death in infants and the stillbirth rate were observed to be the highest in the Minamata area, second highest in the Goshonoura area, and third in the Ariake area. From the classification of the causes of death in the area, many deaths by external factors or by infectious diseases were reported in the Minamata area. The external factor category included many accidental deaths by drowning.

2) The screening method used for the detection of Minamata disease included examinations for sensory impairment, constriction of the visual field, disturbances of movement, and hearing and speech disturbance. The method was effective with about 80% sensitivity and 60% specificity for detection of the disease. It was obvious that some patients were not identified; therefore, some problems remained in the method of the clinical screening for diagnosis of the disease. Later clinical examination of the patients who did not appear to have Minamata disease did demonstrate Minamata disease or health injury.

3. Incidence and distribution of Minamata disease

For the survey for detection of Minamata disease, Yudo, Dezuki, and Tsukiura in Minamata district were selected as the heavily polluted areas and Arashiguchi in the Goshonoura district was selected as a slightly polluted area. For control areas, Akazaki, Sunoko, and Oura in Ariake district were chosen as non-polluted areas, but they were found to be polluted areas by this survey. For the first year survey, 1119 persons (520 male, 599 female) of 304 families in the Minamata district; 1871 persons (902 male, 969 female) of 459 families in the Goshonoura district; and 1180 persons (570 male, 610 female) of 278 families in the Ariake district were examined.

Upon screening by questionnaire and general clinical investigation for symptomatic analysis, persons who needed further precise clinical examination for Minamata disease were found to number 315 (33.7%) in Minamata district, 135 (8.5%) in Goshonoura district, and 29 (3.1%) in Ariake district. There were some changes in the above recorded numbers up to the second year's survey.

Persons who had been clinically examined by neuropsychiatrists before the general clinical examination included 245 in Minamata, 134 in Goshonoura, and 26 in the Ariake district. Among the above recorded persons, the numbers diagnosed as "probable" cases of Minamata disease, were 191 persons (78.0%) in Minamata, 25 in Goshonoura (19.4%), and 8 (30.8%) in Ariake district. After the further clinical examination, the persons who were diagnosed as "very possible" cases of Minamata disease, were recorded as 20 persons in Minamata district, and 34 persons in Goshonoura district.

When the persons with the "probable" or "very possible" Minamata disease

diagnosis were summed up in these areas, there were 211 persons (86.0%)

in Minamata district and 59 (44.0%) in Goshonoura district. We found

10 persons (38.5%), including two questionable cases, having very similar

symptoms of Minamata disease in Ariake area in the survey. Furthermore,

20 persons in Minamata, 30 persons in Goshonoura, and 9 persons in Ariake

district were reserved for precise examination.

(Note: The number recorded above as having Minamata disease is going to

increase later, because the neuropsychiatric clinical survey has been

continued to detect the disease as a follow-up to the general clinical

diagnosis).

Of the persons who underwent precise general clinical examination,

there were 195 in Minamata, 39 in Goshonoura, and 33 in Ariake district.

Some people who lived in the local area in places inaccessible to the

transportation of clinical instruments were called in and diagnosed at

the university hospital. There were 150 persons out of 195 persons

(77.0%) who were diagnosed at the hospital as having Minamata disease.

(Minamata disease was confirmed in 64 persons at survey time, 81

persons at the end of March, 1972). Minamata disease was suspected in

20 persons. Therefore, when the number of suspected cases was added to the

number of persons diagnosed as having Minamata disease, the total number

became 170 patients (87.2%). In Goshonoura district, 16 patients (41.0% out

of 39 persons were diagnosed as having Minamata disease. One patient was

confirmed and 8 suspected cases were found. The total number of patients was 24

persons (61.5%) in this area. The number of persons reserved for

further precise clinical examination was 24 in Minamata and 9 in Goshonoura

district. The numbers may be increased when further examinations are

completed. In the survey, 5 patients (21.7%) having the same symptoms as those for Minamata disease were found in Ariake district. Out of the 5 persons, 3 persons were diagnosed as similar to Minamata disease cases and 2 persons had suspicious symptoms. In addition to these, 9 persons were reserved for further clinical examination. The Ariake area had been thought to be a non-polluted area until the survey was undertaken. However, from this survey, it was clear that the area was also polluted with mercury. The pollution in this area seems to be slight at the present time; the methylmercury content in fish taken from the area was not so high. The fact also suggests that it seems to be difficult to think about the disease as constituting an outbreak. By epidemiological survey, if the patients of the Ariake area have a disease similar to that of patients in Minamata caused by organic mercury poisoning in the past, the incidence of the disease in Ariake would be called the third outbreak of Minamata disease, following the second outbreak of Minamata disease as reported in Niigata prefecture in Japan. As this is an important finding, the problem in the Ariake area has to be made clear and be resolved by future work. At the same time, it will be necessary also to detect the source of methylmercury pollution in the area. On the coast of Yatsushiro-sea, many persons having health disorders were found in the fishing village or around the area, and many persons having complaints of nervous symptoms were affected with mercury pollution in the Minamata area which had been heavily polluted with mercury. These incidences were fewer in the Goshonoura area located on the coast opposite the Minamata area. This fact suggested that the mercury poisoning in the area depended on the eating of much fish and shell fish contaminated with mercury. To the contrary, other points made by the research survey concerning mercury poisoning in the Ariake area might be considered.

Concerning the change of incidence and of Minamata disease itself, the situation is not so clear, because the disease was mainly diagnosed by patients' complaints. There were some diseased persons before and after the great outbreak during the time from 1953 to 1960. After 1965, diseased patients have appeared every year. This fact is indicative that the chronic or delayed type of the disease would be present. These problems shall be examined and clarified by autopsy of this type of patient.

In the field of pediatrics, 9 children (9 in Minamata and 0 in Goshonoura) were suspected of having the disease. Adding to this number, 2 children were in the "probable" category of diagnosis, and 5 children were reserved for policlinical diagnosis. In total, 76 children related to 29 patients as offspring or as brothers or sisters of children born with Minamata disease were recorded. Thirty-six patients out of the 79 children were examined and abnormalities were found in 13 children. Six out of 13 children has sensory impairments or disturbance of movements.

4. The clinical medical study on Minamata disease and the detection of new clinical symptoms.

To diagnose Minamata disease or methylmercury poisoning, it is important to find the characteristic and specific clinical symptoms of the disease and also to develop new methods for detection of the symptoms. It is impossible to diagnose the disease with the simple combination of the individual symptom or of several nervous symptoms. For example, our studies showed that there were about 30 chemicals which can cause constriction of the visual field, and there were many chemicals which cause sensory impairments of peripheral nerves. There are also many poisons which involve disturbance in movements. Therefore, it is important that the combination of the appropriate characteristic symptoms be used to diagnose the disease.

In addition to those observations, the most important thing is the application of an epidemiological survey or chemical examination for mercury pollution before clinical medical diagnosis. In our recent experience it was possible to diagnose the disease. With the data collected from epidemiological surveys and from accurate chemical analyses for mercury pollution, we were able to diagnose Minamata disease, even if patients' symptoms were slight. The fundamental, new findings of electron microscopical observations of biopsied tissue of the peripheral nervous system are also helpful for the diagnosis when used in parallel with the epidemiological survey.

The characteristic main symptoms of Minamata disease are recognized as sensory impairments, constriction of visual field, cerebellar antaxia, dysarthria, and hearing difficulty. Some other symptoms were also reported. The research group, however, found some additional important symptoms in ophthalmology. In addition to centripetal constriction of the visual field, we found the subsided visual field, abnormality of eyeball movement, and disorder of short sight reflex. The patients having centripetal constriction of the visual field were also found to have scattered disintegrated dark points located in the center of the constriction of visual field in high incidences. Squint eye is characteristic in children born with Minamata disease. Lateral squint caused by visual disturbance in the diseased children was found and was effectively treated by new clinical treatment. These neurophthalmological symptoms are caused by disintegration of nerve cells in the cerebral postcentral-cortex. This symptom is important for the detection of the presence of cerebral cortex disturbance. Therefore, the symptom is an important reference for diagnosis. For these ophthalmological symptoms, we designed a new instrument that detects centripetal constriction of the visual field and the movement of the eye ball at the same time. The new instrument

developed by us was named Kumamoto University's *Neuro-ophthalmometer* and it can be used for the diagnosis of many people in a short time. The machine will be very useful for mass diagnosis and for clinics for the residents in the area. The otorhinolaryngological survey showed that hearing ability, sense of equilibrium, and lingual, taste, and smell senses in cases of Minamata disease were functionally disturbed in general. The impairment of hearing associated with the disease appears to be caused mainly by the disturbance of the retro-labyrinth, of its auditory center in the brain, and of the peripheral auditory nerve. However, this presumption has not been confirmed up to the present time, because in our histopathological examination of the inner ear, especially the organ of Corti, no change was found in these organs. Recently, cats experimentally poisoned with methyl-mercury were examined and it was found on histopathologic examination that the cells in the organ of Corti remained intact. Another finding in the case of poisoned cats was that methylmercury could not pass into the fluid of the cochlea. These results help to clarify the origin of auditory disturbances and will be helpful also in clinical treatment. However, in the case of chronic Minamata disease, the source of disorder in the auditory organ still remains obscure.

Taste sense is very important for daily eating habits. The origin of the dysgensia in Minamata disease is not only related to the loss of nerves in the respective cortical center, but also related to the damage of peripheral gustatory cells such as gustatory buds, a finding which was clarified by histopathological examination in this experimental study. In another study using autoradiography with 203 Hg-methylmercury, it was demonstrated that 203 Hg was incorporated into gustatory bud cells. In the course of experimental animal study, the gustatory bud cells were degenerated, atrophied, and decreased in their number. The kind of

experiment in the acceptor of peripheral nerve damaged by methylmercury was the first study. In future work it will be examined whether damage to acceptors of the other sensory peripheral nerves is present or absent. Through study up to the present time, no damage was found in the cells of Corti organ in the inner ear and in the cells of retina in the eye. Therefore, peripheral acceptors such as smell, pain, the pressure, and thermal sense will be carefully examined in future study. It is already known that severe damage to peripheral fibers of sensory nerves were observed in the intoxications. Other important neurological symptoms were mental and emotional disturbances in sensation which were generally recognized in almost all cases of the disease which were referred for diagnosis. These symptoms were reflected by cortical disturbance of the cerebrum, particularly of the frontal cortex. The disintegration of nerve cells in that area cannot be negligible. In mild cases of the disease, it is difficult to differentiate from geriatric change. However, in such cases, methylmercuric poisoning must be suspected because other neural symptoms such as defect in character and other mental disease were observed. Epilepsy was also observed in rare cases of the disease. In the severely diseased case with marked damage to the patient, the progressive paralysis and total disability was observed. On autopsy, including the cases which had been studied during the first year of this survey, it was found that the symptoms seemed to be caused by the damage of a wide area of the cerebrum, mainly from the loss of nerve cells of the cortex and diffuse atrophy in the central cerebrum. When the change in the cerebral cortex due to aging is added to Minamata disease, it is possible that the delayed type of the disease will appear. Persons whose nerve cells were slightly affected and injured by methylmercury show earlier geriatric changes. This tendency was also confirmed by the epidemiological survey and by the autopsy. The premature geriatric changes caused by methyl-

424

mercuric poisoning will be reflected in the clinical diagnosis of the disease. All symptoms revealed by this survey were put in a computer system and were used to facilitate diagnosis. It could be pointed out by the survey that a new standard of clinical diagnosis for slightly affected patients will be necessary.

Among the clinical symptoms, pain in the joints and muscular atrophy were also found in this survey to be common characteristic symptoms of the disease. However, we did not have adequate study of the causal factor for these symptoms. These symptoms will be analyzed and examined with comparison to the neuromuscular disease in future work.

5. Could the methylmercury disease be inherited by descendants of patients in the Minamata district?

There were cases of congenital Minamata disease. However, the congenital disease is a kind of fetal disease, not an embryonic disease. Therefore, it cannot be called true congenital disease. However, molecular biology experiments and other studies suggested that methylmercury inhibited RNA syntheses and DNA syntheses. In experimental mice exposed to methylmercury, the division of spermatocytes into the spermatids was inhibited by the chemical effect on the process of spermatogenesis. Researchers in Sweden, who studied the root of onion and drosophila, found that methylmercury damaged chromosomes, resulting in genetic variation in descendants. They also found that chromosomal change in le cocytes appeared in the methylmercury poisoned animal. However, in our research survey in the Minamata district at this time, we could not find any evidence of genetic damage or genetic variation. Through the survey, we found some instances of structural chromosome breakage in congenital Minamata disease. However, the occurrence seems

to be in the natural range, since such breakage found in the diseased children were also recognized in normal control children. Deformity was not found in the congenitally diseased children. Dental examination revealed much abnormality in the oral cavity. However, the abnormality was only determined as abnormal growth in the period of a fetus, but not as abnormal embryonic development. During our survey of congenitally diseased children and newborn babies from the families affected with Minamata disease in the area heavily polluted with mercury, no abnormal development and no deformity were found. The results mentioned above indicate that contamination of pregnant women with methylmercury could not cause genetic or embryonic abnormality, but could damage the fetus. These facts could be explained by postulating that methylmercury could pass through the mother's body and could not affect the embryo in the early stage, prior to the formation of the placenta, and then the methylmercury could pass through the formed placenta into the fetus and damage the nervous system of the fetus. For this reason, therefore, it is reasonably acceptable to call this disease infantile or fetal Minamata disease rather than congenital Minamata disease.

6. Relationship between the mercury content and the occurrence of Minamata disease.

There are several findings concerning the relationship between the amount of mercury intake and the occurrence of symptoms of poisoning. The relationship between methylmercury intake and mercury or methylmercury accumulation in the body has to be considered.

From the results of experimental poisoning and the findings of autopsy of the disease, Dr. Takeuchi estimated that the total amount, 1,000 mg, of mercury compounds by the oral administration for several months would be the toxic dose which essentially caused Minamata disease for the person having 50 kg of body weight. The total amount 100 mg of mercury (one-tenth of 1,000 mg) will be a risk dose for the disease in the same way. In Dr. Kitamura's study, poisoning symptoms could appear when mercury accumulation in a body reached 100 mg and the lethal dose seemed to be 1,000 mg of mercury in a body, considering about 70 days estimated as the biological half-life of methylmercury.

Our research study in this time showed that there were many differences among the mercury accumulation in various organs. Therefore, it suggested that the extent of damage in the organs by the contamination with mercury could be varied depending upon the amount of mercury accumulation in these organs. From the examination of mercury content in the case of autopsy of the human body receiving methylmercury by oral administration, it was found that the highest content value was in the kidney, the second highest content in the liver and remarkably low content in the brain. In the early stage of the disease, the mercury content in the brain was less than about one fifteenth of the content in the kidney and one-eighth of the content in the liver. This value in the brain of the diseased persons, was high, being about 192 times the values of $0 \sim 0.005$ ppm detected in normal brains. In spite of the various mercury contents in organs, the fatty degeneration in the kidney and the liver was only observed by the histopathological examination in the cases of acute and subacute disease. In contrast to these visceral organs, pathological changes in nervous tissue were especially severe. Among the nervous tissues, the visual central nervous area in the cerebral cortex and the deep area of the cerebellar cortex were commonly damaged. In severe cases, the damage spread out into the entire area of the cerebral cortex,

but some extent of the first slight changes were scarcely observed in diencephalon, in mesencephalon, and in spinal cord. Therefore, there were some special relationships between the amount of mercury intake, the accumulation in the individual organ, and the response of tissue cells. However, it is not so simple to discuss the relationships among them, such as the relationship between the appearance of symptoms and the total mercury content. Mercury accumulation in the brain can be considered as the total amount of mercury which is derived from the methylmercury compounds which have passed through the brain blood barrier. From the findings in autopsy cases, poisoning symptoms will appear when the mercury content in the brain reaches beyond 1 ppm, and the patient's death will occur when the content reaches beyond 5 ppm. Dr. Tsubaki indicated that the disease would appear in persons having more than 40 ppm mercury content in their hair.

In the case of humans, biological half-life time of mercury content in the kidney was estimated as about 70 days and in the liver was a little less than 70 days. Contrary to the findings in these organs, bio-half-life of mercury in the brain was remarkably different. Mercury accumulation in the brain was very slow and delayed after mercury intake into the body. The maximal content was found at about the 40th day after mercury intake. The decreasing pattern of mercury content in the brain was different from other organs. After two months from the date of maximal accumulation, little decrease was observed, after the disease was apparent. One half decrease of mercury content in the brain was found approximately 230 days after the disease was apparent and one-third decrease was found one and one-half years later. After two and one-half years, mercury content in the liver and in the kidney of diseased patients had generally returned to normal level, but in the brain, mercury still remained ten to one hundred times higher than the normal value. Therefore, this observation requires a revision of the table presented by Dr. Kitamura

428

which estimated the relative value between the mercury accumulation
and the intake on the basis of 70 days as mercury bio-half-life period.
It means that a lesser amount of mercury contamination in the body
than the dose of mercury poisoning reported by Dr. Kitamura seems to
be enough to cause the disease or the death. It will be revised to
show that if a person had been taking 1 mg mercury daily, it would take
465 days to reach 100 mg of the total amount of mercury accumulation in a
body according to the table presented Dr. Kitamura, but a shorter period
would be enough to cause the appearance of disease symptoms by our
estimation as mentioned above. Dr. Kitamura's other suggestion that the
daily intake of 1 mg mercury would be harmless for the human body, must
be changed. From the recent autopsy cases of the Minamata disease patients
previously recognized or other diseased patients in the Minamata district,
the mercury content in the liver was found at normal levels and somewhat
high values were found in the kidney, but high values in the brain of
1 - _2 ppm mercury were found in many cases. This fact indicates that the
condition of the human body exposed to mercury pollution still remains
above the poisoning dose level.

Epidemiologic surveys also support that there have been many Minamata
disease patients in every year since 1965. Therefore, contamination by
very small amounts of methylmercury for a long period, would make it
possible to accumulate mercury in the brain in large enough amounts to
cause poisoning, nevertheless significant mercury accumulation in the liver
and in the kidney could not be detected. For this reason, a more precise
study will be necessary to establish the relation between the intake of
small amounts of mercury for a long period and its poisonous effects.

7. Occurrence of a chronic type of Minamata disease--a chronic Minamata disease.

It is essential for the occurrence of Minamata disease that the accumulation of methylmercury in the brain has to reach the toxic dose level. Therefore, when a person is given a large amount of methylmercury, the mercury accumulation in the brain will be rapid and an acute type of disease will appear. Severely ill patients have mostly died within a period of 100 days after onset. In such cases, mercury content in the brain at death was found to range from a high of 24.8 ppm to a low of 2.6 ppm, with 10.31 ppm being the average value. When a person receives lesser amounts of mercury, the latent period of the disease will be prolonged and the subacute type of the disease will appear. In this case, no deaths will be found due to mercury poisoning. With still less mercury contamination than that mentioned, it will take a longer period for symptoms of mercury accumulation in the brain to appear as described above. Since 1961, patients with Minamata disease seemed to be almost entirely of the chronic type. Among the autopsy cases, many have been recently found of the chronic type and very few cases were found of the subacute type. In these chronic type cases, it cannot be clearly recognized when they were suffering from the disease and the typical symptoms appeared later. Such chronic type of the disease is called chronic Minamata disease. In cases of the slightly ill patients, we found that many were cured after the clinical treatment in the early stage of the disease. However, in the two years survey in this time (the first and the second year's research), some cases showed a satisfactory progress into a cured condition, but some cases took a turn for the worse. These facts suggest that the problem about the relationship between a small amount of methylmercury pollution for a long period and its accumulation

in the brain still remains obscure. Another study was made to detect subclinical or inapparent Minamata disease and to clarify the picture by examination of autopsy cases and the estimation of mercury content in organs. Subclinical Minamata disease was sometimes revealed by detectable symptoms during the aging process. This type of case is different from the delayed type of Minamata disease reported by Dr. Tsubaki. The subclinical Minamata disease was detected only in middle-aged people, but not in young people, and could be called a delayed type of Minamata disease in aged people. This type of Minamata disease should be differentiated from the symptoms of the diseases of old age.

The general pathological changes of chronic Minamata disease in the nervous system, such as brain, spinal cord, and peripheral nerves, were precisely described in a separate report. In the cases of chronic Minamata disease, pathological changes were observed in the nervous tissue cells, similar to those generally observed in mercury poisoning. The secondary degeneration in the nervous tissue was generally recognized as corresponding to central nervous system damages of a chronic nature. For example, a marked disturbance in the precentral cortex caused secondary degeneration in the pyramidal tract. Secondary degeneration in the cornuposterior of the spinal cord was found in a case with severe disturbance in the sensory nerve, especially in the posterior tract of the peripheral nerve. The severe disintegration of the cerebral cortex cells caused diffuse demyelination in that area. These pathological changes were the same as those reported earlier.

In cases with high mercury content in the brain, because of progressive thinning and disintegration of the cerebral cortex atrophy or more often the loss of cells was seen. During these pathological changes, active chromato-phagocytes appeared and chromatin increased in the nerve cells. Therefore, these pathological changes are difficult to differentiate from aging plagocytic

431

changes. For differentiation between aging and Minamata disease, the locality of changes and the demonstration of mercury will be helpful.

8. Additional findings of pathological changes in organs---diabetic changes

General disturbances in organs in cases of acute or subacute Minamata disease were observed as fatty degeneration in the liver and in the kidney, hypoplasia of the bone marrow, and gastrointestinal catarrh. These pathological changes in organs were slight. In the cases of chronic Minamata disease, death usually resulted from other diseases and the disturbances in organs by methylmercury were difficult to find. No common pathological changes in organs caused by methylmercury was observed in the chronic cases. No specific change was found in the kidney. Among the pathological changes in the chronic cases, the disturbances of islands of Langerhans in the pancreas were found. The B cells in the islands of Langerhans are usually rich with zinc and are related to metal metabolism. The cells in the islands of Langerhans were affected by methylmercury poisoning and lead to the degenerative disintegration, resulting in a decrease in the number of cells in the islands of Langerhans and bringing about their compensatory reorganization. In remarkable cases, damage was found similar to the pathological changes observed in diabetes mellitus. The change is an important finding since it was found commonly in all autopsy cases of Minamata disease in adults, in infants, and in fetuses. During the reorganization and compensation, the islands of Langerhans functioned to the extent that diabetes mellitus could not be found by clinical examination. However, severely diseased patients revealed typical diabetic symptoms and with increased sugar content in the blood. Therefore, mercury pollution could cause diabetic disease.

432

9. Metabolic pathway of methylmercury in a living body and fundamental
    studies on the prevention and clinical treatment of Minamata disease.

The difficulty of treatment of Minamata disease has been pointed out.
However, from the study at this time, a promising treatment was found for
the external squint of the eye (strabismus) in congenital Minamata disease.
At the present time, there has been mercury pollution in some areas, and it
is supposed that mercury poisoning is going to occur again. In such
circumstances, we have to provide methods to prevent mercury accumulation
in the brain and to promote excretion of mercury from the body. For that
purpose, the study of the metabolism of methylmercury in the body is very
important.

1) Metabolism in the living body.

    Experiments showed that Methylmercury changed into methylmercuric chloride
in the stomach and combined with aminoacids (expecially cysteine) digested
in the intestine. The methylmercuric cysteine was absorbed into the
intestinal mucosa, stayed there for some time, and then entered directly
into the liver without entering into the lymph by passing through the portal
vein. Some of the methylmercury compounds in the liver were excreted with
bile and the remainder circulated in the blood stream. Methylmercuric
chloride administered by other than the oral route was mostly incorporated
into circulating erythrocytes, but some of it became bound to cysteine and,
as methylmercuric cysteine, entered into the bile. Methylmercuric cysteine
continued circulating between the intestine and the liver for a long period.
It was found that methylmercuric acetylcysteine existed in the bile. Methyl-
mercuric chloride and methylmercuric cysteine in the blood are not excreted
into urine. However, quite a bit of methylmercuric acetylcysteine is excreted
into urine through the kidney. This fact suggests that methylmercury combina-
tion with closely related aminoacids have different metabolic pathways in the body.

The amino base of methylmercury combined with the acetylradical is difficult to reabsorb in the kidney and easy to excrete with urine. This methylmercury mercaptan seemed to behave as a mercaptan complex for the detoxication mechanism. The mechanism may be applicable in the future to promoting methylmercury excretion in urine and to establish a method of clinical diagnosis for methylmercury poisoning. On the other hand, a small amount of methylmercuric cysteine in the blood can pass through the blood-brain barrier and thus into the brain. It is reasonable to assume that methylmercury can directly enter into the brain since methylmercury is soluble in lipid substances. Methylmercury cannot pass through the blood barrier of the brain when methylmercury is chemically bound with hemoglobin of erythrocytes. Methylmercury bound with hemoglobin will be able to pass only when it dissociates from hemoglobin. Methylmercury dissociated from hemoglobin seemed to be bound with cysteine in the cysteine pool in the body and entered into the serum of the blood and then transferred into the brain. It is more suggestive, therefore, that a small amount of methylmercuric cysteine enters into the blood and accumulates in the brain during methylmercury action on the nerve cells. These mechanisms and metabolic pathways of methylmercury are also suggested by our recent experiments.

2) Fundamental findings for clinical and preventive treatment---development of drugs for treatment.

i. The oxidized forms of pyridoxine-4-thiol and pyridoxine-5-thiol, are similar in structure to vitamin B6 and have -SH radicals which can react easily with methylmercury. When these chemicals were administered by oral, subcutaneous, and intravenous routes, the mercury content in the organs, i.e. brain, liver, was decreased and mercury compounds were excreted into urine and feces. The strength of the effects decreased in the order of pyridoxine-4-thiol, pyridoxine-4-thiol oxidized form,

434

and pyridoxine -5- thiol. When pyridoxine -5- thiol was oxidized in the body, it changed to pyrithioxine which had the effect of activating metabolism in the brain. The pyridoxine -5- thiol had little effect on the excretion of mercury, but would have very good effects as a remedy for mercury poisoning. Therefore, pyridoxine -5- thiol will be studied further. If it should be acceptable for the treatment of humans poisoned with mercury, it could decrease the mercury content accumulated in the brain and could be useful in the prevention of and as a remedy for the disease.

ii. Methylmercury is excreted into bile over a long period, and thus remains in circulation between the intestine and the liver. Therefore, if there were some method to prevent reabsorption into the portal vessel from the intestine, methylmercuric cysteine could be stopped from entering the blood stream. To prevent methylmercury excretion into bile, chemicals with strong binding affinities for methylmercury have been studied for their effects on methylmercury excretion into bile. When a resin having -SH radical was administered, it was found that the mercury content in the liver was decreased but not the mercury content in the brain and in the kidney. Therefore, we are looking for some other synthesized resin with -SH radicals. Up to the present time, the effect of the synthesized resin with the -SH radical was not better than the effect of powdered hair. The hair powder will be more useful for the treatment to mercury poisoning.

iii. For possible use of the drug in diagnosis, we are studying the drug's effect on methylmercury excretion into urine. During the course of the study, we have found that pyridoxine-4-thiol has a strong effect on excretion of methylmercury into urine and also has the effect of decreasing the methylmercury content in the brain. Therefore, the chemical looks promising for use in the diagnosis of small amounts of methylmercury contamination in the body.

The results and the new findings mentioned above are still being investigated in experimental animals. However, there is no doubt that the findings have fundamental importance for treatment with the drug.

10. Methylation of inorganic mercury

It is well known that methylmercury is synthesized from inorganic mercury in some microorganisms or in some fungi. It has also been reported that methylmercury is possibly synthesized in the liver of some fish. However, in the natural field, it was not completely understood how to change the inorganic mercury in the mud from the Minamata bay into methylmercury. The results obtained at this time showed that inorganic mercury can react with active methyl radicals in the municipal sewage or in the drainage from the factory. In this case, if the inorganic mercury is mercury sulfide, methylmercury cannot be synthesized. However, under aerobic conditions, the mercury sulfide can be changed into an active form of inorganic mercury and can be synthesized to methylmercury. Actually, a great amount of inorganic mercury was found in the mud from the Minamata bay. Most of the inorganic mercury compounds cannot change into organic mercury compounds, but it is possible that the inorganic mercury can be changed into methylmercury under aerobic conditions or in the presence of ultraviolet light. Therefore, in the Minamata bay areas, polluted with mercury, methylmercury poisoning will be prevented only by eliminating the above condition or by removing the inorganic mercury in the mud.

VII. Closing Remark

During the past two years, we made a great effort to study and survey mercury pollution in Minamata district and the surrounding areas and also the progress of Minamata diseased patients. The results obtained were separately reported in the first year and in the second year, and were completely described in this report. From the study, it is suggested that the inorganic mercury in the mud from the Minamata bay will be chemically changeable into methylmercury compounds under some conditions. At the present time, it must be considered that many problems are presented in the prevention of methylmercury intoxication in these areas and in the medical treatment for Minamata disease. However. some problems remained to examine or study in future work. The results and findings obtained will be greatly useful in preventing the occurrence of Minamata disease in detecting the subclinical patients, and in treating residents having health effects due to mercury poisoning. We hope that the area of Shiranui Sea and Ariake Sea will be kept in a good and healthy natural environmental condition.

VIII ACKNOWLEDGEMENT