Chapter 7

Clinical Features and Treatments of Yusho

7.1. Medical Aspects

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Following the onset of nonspecific symptoms such as general malaise, loss of appetite, and headache, some characteristic symptoms for Yusho gradually appeared, including increased discharge from the eyes with swelling of eyelids, dark brownish nail pigmentation, gingival pigmentation, acneiform eruptions, peripheral neuropathy, irregular menstruation in women and growth retardation in infants and children. Today nearly 25 years have past since the outbreak of Yusho, and almost all of these symptoms have improved. However, some patients still complain of dermatological changes and other subjective symptoms. Furthermore, a detectable amount of PCBs and related compounds, which caused Yusho, including those with carcinogenic potential, still remains in severely affected patients.

In this section, the medical features of Yusho seen over the past 25 years are described. These features are classified and discussed under the following headings: general clinical features, neurologic disorders, endocrine disorders, hematologic disorders, hepatic disorders, blood pressure disorders and types of treatment.

7.1.1. General Clinical Features of Yusho

1. Observation of Typical Patients at the Outbreak of This Disease

1) General Examination

Twenty-seven patients in 6 families, who presented with the typical symptoms and signs of Yusho, were each admitted to Kyushu University Hospital for 7 days, from October-November 1968. In this section, the clinical observations of the 18 adult patients, all over 15 years old, out of 27 patients are presented (Okumura and Katsuki, 1969). According to the clinical grading of Yusho, mainly based on the dermatological changes as described in section 7.2, nine of the patients were categorized into grade IV, 6 into grade III and 3 into grade II.

The general findings on admission are shown in the Table 7.1.1. The principal symptoms and signs at the beginning of the disease were general malaise, a loss of appetite, an increased discharge from the Meibomian glands with edematous eyelids, and visual disturbance. Following these signs, characteristic dermatological changes such as comedo or acne like eruptions beginning in either the perioral or zygomatic areas and dark brownish pigmentation of the nails appeared. In these 18 patients,2 female patients (Cases 10 and 12) complained of numbness of the lower limbs and 3 male patients (Cases 1, 4, and 17) complained of a bad taste when drinking sake (rice wine).

Table 7.1.1. Clinical Findings of Eighteen Adult Patients with Yusho

Case	Sex	Age	Name of family	Clinical grade	Initial complaints	Elapsed after onset (mo.)	Pyrexia (°C)	Weight loss (kg)	Remarks
1	M	39	UJI	IV	General fatigue with edema of eyelids	6	37.5		Palpable liver
2	F	35	UJI	IV	Anorexia	6	37.6		Dysmenorrhea
3	M	15	UJI	IV	General fatigue	5			
4	M	37	MIN	IV	General fatigue	6	37.3	10	
5	F	33	MIN	IV	Edema of eyelids	7	38.0	2	Abortion (affected fetus, 7th M) on July 7, 1968.
6	F	24	KAM	IV	Edema of eyelids	7	37.3	3	
7	F	28	KAM	IV	Comedo formation	6	37.4	12	
8	M	18	KAW	IV	Comedo formation	5	37.4	8	
9	F	15	KAW	IV	Comedo formation	4			
10	F	39	KAM	III	Swelling of face	6			Dysmenorrhea
11	F	41	KAW	III	Swelling of face	4			Dysmenorrhea
12	F	60	MOR	III	Conjunctival discharge	4			
13	M	34	MOR	III	General fatigue	4			
14	M	27	MOR	III	Pigmented nails	5		4	
15	F	39	MOR	III	Conjunctival discharge	4			
16	F	31	MOR	II	General fatigue	4			Delivery (affected newborn, 9th M) on Oct. 13, 1968.
17	F	39	KOG	II	Comedo formation	2		11	Palpable liver
18	F	38	KOG	II	Swelling of face	4			

Weight loss, ranging from 2–12 kg during the several months before the admission was also recognized in 7 patients. A fever ranging from 37.2 to 38.0°C was detected in 7 of 9 grade IV patients. Three of 11 female patients revealed abnormalities in menstrual cycle, with the menstrual interval either being prolonged, shortened or irregular. Case 5 had a stillborn baby who demonstrated pigmented skin changes in August 1969, while Case 16 delivered a grayish dark brown colored baby one month before admission, and PCB compounds were detected from the placenta of Case 16.

At the time of the first medical inspection, the majority of these patients revealed a grayish dark brown pigmentation on the skin, particularly on the face and nails, and other dermal lesions as described in section 7.2. Secondary infections of acne-like eruption and formation of small abscess were often observed in the worst patients (grade IV). Hyperhydrosis in the palms was observed in almost all the patients. All 18 adult patients showed edema of the upper eyelids with increased discharge from the Meibomean glands. Abnormal brownish yellow but not icteric

Table 7.1.2. Laboratory Tests and Clinical Grades in Adult Patients with Yusho
(1) Blood picture[a]

Clinical grade	No. of cases	Hemoglobin (%)	RBC ($\times 10^4$/mm^3)	WBC (/mm^3)	BSR (mm, 1 hr)
IV	9	71.4 ± 8.23	382 ± 30.2[b]	10,270 ± 2,770	27 ± 27.4
III + II	9	79.9 ± 9.13	422 ± 24.2[b]	8,490 ± 5,060	10 ± 10.6

[a]: Mean ± S.D., [b]: $p < 0.05$.

pigmentation of the bulbar conjunctiva was also observed (Ikui et al., 1969).

None of the patients showed a palpable lymph node in either the cervical, axillary, or inguinal regions. A slight murmur was audible in the heart sounds of 4 patients, although they were all recognized to be functional. The blood pressure was normal. No pathological shadow was detected on the chest X-rays. A palpable and non-tender liver with slightly increased consistency was found 2 cm below the right costal margin in two patients (Cases 1 and 17). Neither the spleen nor the kidneys were palpable.

Remarkable edema in the lower limbs was found in only one patient, Case 11, who had a cystic bursa formation on the lateral side of the foot joints.

Neurologically, both paresthesia in the lower limbs in 2 patients and decreased sensory conduction velocity in 4 patients were detected.

2) Laboratory Findings

Urinalysis: Urinary protein and glucose were all negative. A positive test for urobilinogen was found in only one patient.

Feces: No occult bleeding in the feces was observed.

Blood pictures (Table 7.1.2): Eighteen patients were divided into two groups according to the classification of clinical grade, including 9 severe (grade IV) and 9 moderate (grade III and II), and the mean values in these two groups were compared. The hemoglobin concentration in the severe group was 71.4 ± 8.34% that of normal, while it was 79.9 ± 9.13% in the moderate group. Red blood cells were $382 \pm 30.2 \times 10^4$ in the severe group whereas they were $422 \pm 24.2 \times 10^4$/mm^3 in the moderate group, and the difference was statistically significant. Leucocytosis of more than 10,000/mm^3 was seen in 7 of 9 patients from the severe group, while it was seen in only 2 of 9 patients from the other group. However, the difference in the mean values of white blood cells between the two groups was not significant because of their large variations. The higher value of the blood sedimentation rate in the severe group than in the other group was observed, although the difference in two groups was not significant. No correlation between the blood sedimentation rate and the severity of Yusho was observed. The platelet count, coagulation time

Table 7.1.3. Laboratory Tests and Clinical Grades in Adult Patients with Yusho (2) Liver function tests

Clinical grade		Icterus index	BSP (%)	ALP (K.K.)	GOT (Karmen u.)	GPT
IV	N	8	8	9	9	7
	Mean	3.3	5.9	14.9	23.0	22.6
	S.D.	±0.66	±5.19	±9.26	±7.7	±11.9
III + II	N	9	8	8	8	8
	Mean	3.3	4.9	8.7	27.1	27.5
	S.D.	±0.49	±4.97	±2.29	±22.9	±20.8

and prothrombin time were all normal.

The serum enzyme levels (Table 7.1.3): The serum levels of lactate dehydrogenase (LDH), glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were normal except for one patient, who showed 87 Karmen units in GOT and 77 u. in GPT. The mean values of the serum GOT and GPT were normal. A slight elevation of the serum alkaline phosphatase (ALP) level was observed in more than half of the adult patients. The serum ALP level of 39.6 K.K. units, 3 times as high as the normal limit, was detected in a 15 year-old male patient (Case 3 in Table 7.1.1). His ALP isoenzyme examination on agarose gel electrophoresis revealed that the majority of the enzyme was derived from the liver while the remnant was osteogenic. In the other patients, the main band of the ALP isoenzyme demonstrated a hepatic origin.

Palpable liver and liver function test: An abnormal retention rate, more than 10%, at 45 minutes in the bromsuphalein (BSP) test was observed in 3 of 18 patients. In 2 patients (Cases 3 and 8) both an elevated retention of BSP test and an elevated level of ALP with decreased serum albumin were observed. Two other patients (Cases 1 and 17) had a palpable liver below the costal margin, although they also showed normal liver function tests. The palpable liver in these patients was not considered to have any particular clinical meaning.

The serum protein pattern (Table 7.1.4): None of the Yusho patients had serum protein levels lower than 6.0 g/100 ml. However, patients of severe clinical grade, grade IV, showed significantly increased α_2-globulin levels. On the other hand, no patient revealed increased γ-globulin, while an unexpectedly low level, less than 12% of the γ-globulin fraction, was observed in 2 patients.

Serum lipids: Described in the next section.

Serum electrolytes: No remarkable change was observed in either the serum Na, K or Ca in Yusho patients (Table 7.1.5). The serum level of Cl was within the normal limits, however, the value of the mean ± S.D. in severe cases was signifi-

Table 7.1.4. Laboratory Tests and Clinical Grades in Adult Patients with Yusho
(3) Serum proteins and lipids

Clinical grade	No. of cases	Serum protein and fractionations (%)						Serum lipids (mg/100 ml)		
		Total (g/100 ml)	Al[a]	Globulin				Total	triglycerides	Cholesterol
				α_1-G[b]	α_2-G	β-G	γ-G			
IV	9	6.87 ± 0.73	56.4[c] ± 5.47	6.4 ± 0.94	12.7[d] ± 2.74	8.5 ± 1.67	15.4 ± 2.62	725.0 ± 169.5	180.6 ± 88.2	166.9 ± 46.9
III + II	9	6.91 ± 0.23	61.7[c] ± 4.42	5.8 ± 1.06	9.1[c] ± 1.23	9.0 ± 1.07	14.4 ± 1.90	763.7 ± 110.9	197.2 ± 109.2	167.7 ± 21.7

[a]: Albumin, [b]: globulin, [c]: $p < 0.05$, [d]: $p < 0.01$.

Table 7.1.5. Laboratory Tests and Clinical Grades in Adult Patients with Yusho
(4) Serum electrolytes and metals

Clinical grade		Serum electrolytes (mEq/l)					Serum metals (μg/100 ml)		
		Na	K	Ca	Cl	BUN (mg/100 ml)	Fe[a]	Cu[b]	Zn[c]
IV	N	8	8	8	8	8	6	9	8
	Mean	141.6	4.2	4.6	106.2[d]	12.0	66.0	183.9[d]	98.1
	S.D.	± 1.87	± 0.50	± 0.09	± 3.03	± 2.08	± 30.9	± 61.0	± 15.6
III + II	N	8	8	8	8	6	9	9	9
	Mean	141.6	4.3	4.6	103.0[d]	10.0	82.5	132.9[d]	111.3
	S.D.	± 2.2	± 0.33	± 0.02	± 2.54	± 1.06	± 22.4	± 21.2	± 18.6

[a]: Iron, [b]: copper, [c]: zinc, [d]: $p < 0.05$

cantly elevated as compared with moderate or light cases.

Serum heavy metals: The serum iron levels lower than 70 μg/100 ml were observed in 9 of 18 patients. The mean value of the serum iron levels in severe patients was lower than that in moderate to light patients, however, the difference was not statistically significant because of the wide dispersion of the serum levels. In five of 18 patients, the serum copper level was elevated to more than 150 μg/100 ml. The mean value of the serum copper levels in severe patients was significantly elevated as compared with the levels seen in the other group. All of serum zinc levels in severe patients were lower than that in the other patients (Table 7.1.5).

Laboratory findings in juvenile patients: As shown in Table 7.1.6, six juvenile patients from 3 families were clinically examined (Okumura and Katsuki, 1969). They consisted of 1 patient in grade IV, 2 in grade III, and 3 in grade II. Case 23 had a palpable liver without an abnormal liver function test. No patient showed anemia, but 3 patients had leucocytosis of more than 10,000/mm^3. Three patients showed a more than 12% of α_2-globulin fraction in the serum protein. The eleva-

Table 7.1.6. Laboratory Findings in Six Juvenile Patients with Yusho from Three Families Shown in Table 7.1.1.

Case	Sex	Age	Family	Clinical grade	Blood picture				Serum lipids (mg/100 ml)		
					Hb (%)	RBC ($\times 10^4$)	WBC	BSR (mm/hr)	Total	Triglyc-erides	Choles-terol
19	F	7	MIN	IV	70	390	13,500	3	1,530	617	262
20	F	12	UJI	III	77	430	11,300	15	870	366	172
21	F	7	UJI	III	95	470	20,000		785	374	152
22	M	11	KOG	II	83	420	9,200		1,040	376	190
23	F	9	KOG	II	76	388	8,200	2	1,110	408	237
24	M	8	KOG	II	80	398	8,700	2	990	324	178

tion of the total serum lipids particularly tyiglycerides was more prominent than those observed in adult patients as shown in the next section.

2. Disorders in Lipid Metabolism

Hyperlipidemia in the early stage:

The disorders in the lipid metabolism were characteristic of Yusho patients as shown in Table 7.1.7. The concentration of the serum lipid classes was determined in 24 hospitalized patients from 7 families, consisting of 14 females and 10 males, and ranging from 7 to 60 years of age (Tables 7.1.1, 7.1.6 and 7.1.7). Abnormally elevated serum triglycerides ranging from 200 to 600 mg/100 ml were observed in 12 of 24 patients. The mean value of the serum triglycerides was 188.5 mg/100 ml in 18 adult patients, while 432.2 mg/100 ml in 6 juvenile patients, in contrast to normal adults whose mean ± S.D. was estimated to be 74 ± 29 mg/100 ml in 1969 (Berry et al., 1969). Furthermore, Case 21, a 7 year-old girl shown in Table 7.1.6, was known to have had milky turbid serum when examined at a hospital on July 24, 1968.

Agarose gel electrophoresis of serum from the several patients revealed faint α, dense pre-β, no tailing behind and no chylomicron at origin, which indicated that the elevated triglycerides was of an endogenous origin (Uzawa et al., 1969). In contrast to the triglycerides, the serum total cholesterol remained unchanged while the serum phospholipids tended to be somewhat lowered.

The fatty acid composition in fractions of serum lipids was analyzed in patients who had remarkably elevated triglycerides, more than 400 mg/100 ml (Uzawa et al., 1969). No abnormal peak of fatty acids was detected in this analysis, but an increase in oleic acid (18 = 1) and a decrease in palmitic acid (16 = 0), both of which were observed in the fractionation of free fatty acids, seemed to be noteworthy. These changes suggested a stimulated release of free fatty acids from the fat

Table 7.1.7. Concentrations of Serum Lipids in the Yusho Patients in the Early Stage

Date of examination	Family	Sex	Age	TC[a]	PL[b]	TG[c]
Oct. 19, 1968	UJI	M	39	189	168	356
	UJI	F	35	171	118	151
	UJI	M	15	160	152	127
	UJI	F	12	186	172	400
	UJI	F	7	198	172	366
Oct. 25, 1968	MIN	M	37	226	128	284
	MIN	F	33	260	152	233
	MIN	F	7	264	171	617
Oct. 30, 1968	KAM	F	49	125	103	148
	KAM	F	24	120	142	117
	KAM	F	28	131	94	188
Nov. 7, 1968	KAW	F	15	145	112	100
	KAW	M	18	140	114	69
	KAW	M	41	173	119	116
Nov. 15, 1968	MOR	M	27	228	163	85
	MOR	F	31	184	142	105
	MOR	F	24	177	144	318
	MOR	M	34	205	140	116
	MOR	F	60	227	159	426
Nov. 20, 1968	KOG	F	9	237	160	408
	KOG	M	11	202	151	376
	KOG	F	38	233	144	196
	KOG	M	39	192	139	258
	KOG	M	8	211	160	424

[a]: Total cholesterol (normal range 157–229), [b]: phospholipids (normal range 156–219), [c]: triglycerides (normal range 60–107). (Uzawa et al., 1969.)

tissue.

Follow-up studies of hypertriglyceridemia:

Since the main characteristic findings of the laboratory examination was the remarkable degree of hypertriglyceridemia, serum triglycerides (TG) concentrations in 82 Yusho patients, consisting of 39 males and 43 females, were determined for three consecutive years from the onset of Yusho until 1971 (Uzawa et al., 1972). The determination of the serum TG was carried out under the supervision of the cooperative triglycerides standardization program, Atlanta, Georgia, U.S.A. In 23 of these patients, the serum TG was determined once a year while the remaining cases were done more than once a year. The total number of determinations were 275 in 1969, 165 in 1970 and 235 in 1971, respectively (Table 7.1.8). A slight decrease in the serum TG was noted during this time period, however, the difference in the mean values between 1969 and 1971 were not statistically significant.

Table 7.1.8. A Three-Year Follow-up Study for Serum Triglyceride Concentrations in 82 Patients with Yusho

Year	Number of patients	Number of determinations	Serum triglycerides (mg/100 ml)		
			Mean	S.D.[a]	S.E.M.[b]
1969	82	275	151	74	8
1970	82	165	140	56	6
1971	82	235	136	53	6

[a]: Standard deviations, [b]: standard errors of means. (Uzawa et al., 1972.)

Table 7.1.9. A Consecutive 6-Year Follow-up Study on Serum Triglyceride Levels in 40 Patients with Yusho

Sex	Age[a]	Triglycerides (mg/100 ml)						
		Year	1969	1970	1971	1972	1973	1974
Males (N = 14)	42.0	Mean ± S.D.	159 ± 57	166 ± 55	168 ± 60	174 ± 69	164 ± 68	160 ± 118
Females (N = 26)	33.4	Mean ± S.D.	155 ± 75	161 ± 70	155 ± 80	153 ± 63	129[b] ± 50	111[b] ± 56

[a]: The mean age. [b]: Mean values of the serum triglyceride levels in female patients from 1973 and 1974 are significantly lower ($p < 0.05$) than those from 1969, 1970, 1971, and 1972.

The hypertriglyceridemia seen in Yusho patients was thus maintained at least for several years at the early stage of poisoning.

The serum TG concentrations in 40 patients (14 males and 26 females) with Yusho were measured at least once a year for six successive years from the original exposure in 1968 (Okumura et al., 1975). The total number of determinations of the serum TG was 219 in 1969, 166 in 70, 206 in 71, 135 in 72, 75 in 73, and 46 in 74, respectively. As shown in Table 7.1.9, hardly any change was noted in the male patients during this period of time. On the other hand, in female patients, a decreasing trend of serum TG was seen, as shown by the statistically significant differences in the mean values noted between 1973 or 1974 and the preceding years (Table 7.1.9). These observations thus suggested that the serum TG in female patients started to decrease about 5 years after the initial poisoning, though it remained unchanged at elevated levels in males. Meanwhile, the elevated serum concentrations of TG ranging from 156 to 555 mg/100 ml as measured by Auto Chemist (AGA, Sweden) were noted in 26 (9 males and 17 females) (23.6%) out of 110 patients with Yusho who were examined during the annual examination for Yusho in 1979 (Akagi et al., 1981). It is thus evident that some patients maintain a high serum TG level for a quite long perid of time.

Table 7.1.10. The Blood PCB Concentrations and Triglyceride Level[a] in 42 Patients with Yusho Classified into Two Groups Based on the Gaschromatography Pattern

Subjects	PCB pattern	Number of cases	Age	PCB concentration (ppb)	TG[b] (mg/100 ml)
Yusho	A	26	31.9	8.6 ± 5.2[d]	134 ± 60.0[e]
	B, C	14, 2	21.4	3.8 ± 2.2	91 ± 39.8
Normal	C	37	34.5	2.8 ± 1.6	[c]

[a]: Mean values ± S.D. [b]: Triglycerides. [c]: TG level of normal controls in this series was omitted.
[d]: $p < 0.005$. [e]: $p < 0.05$.

3. Blood PCB and Serum Triglycerides

The routine measurements of blood PCB concentrations and the analysis of PCB patterns by gaschromatography in Yusho patients first became possible and was started only in 1973 (Takamatsu et al., 1974; Masuda et al., 1974). At that time, both significantly elevated blood PCB concentrations and peculiar gaschromatographic patterns of PCBs still remaining in Yusho patients were disclosed (see Chapter 4).

The blood PCBs and serum TG levels in 42 Yusho patients, 18 males and 24 females at the average age of 26.7, were studied for the first time in 1973 (Okumura et al., 1974). A significant positive correlation ($r = 0.485$) between the blood PCB concentrations and the serum TG levels was obtained in these 42 patients as shown in Figure 7.1.1. According to the peak patterns of PCBs on a gaschromatogram, 42 patients were classified into 26 type A cases (62%), 14 type B cases (33%), and 2 type C cases (5%). As shown in Table 7.1.10, patients with type A had a strikingly high concentration of PCBs (8.6 ± 5.2 ppb) in contrast to those either with type B and C (3.8 ± 2.2 ppb) or in normal persons (2.8 ± 1.6 ppb). The highest mean levels of serum TG, 134 ± 60.0 mg/100 ml, were also observed in patients with type A.

In 1988 and 1989, twenty years after the outbreak, the association of blood PCB concentrations with serum TG levels was again studied in Yusho patients using the information obtained from the annual medical examination program (Hirota and Hirohata, 1993). A clear positive association between the blood PCBs and serum TG was still observed even twenty years after the PCB exposure (see Chapter 8).

7.1.2. Neurologic Disorders

1. Early Stage

From one third to half of the patients with Yusho complained of some neurologi-

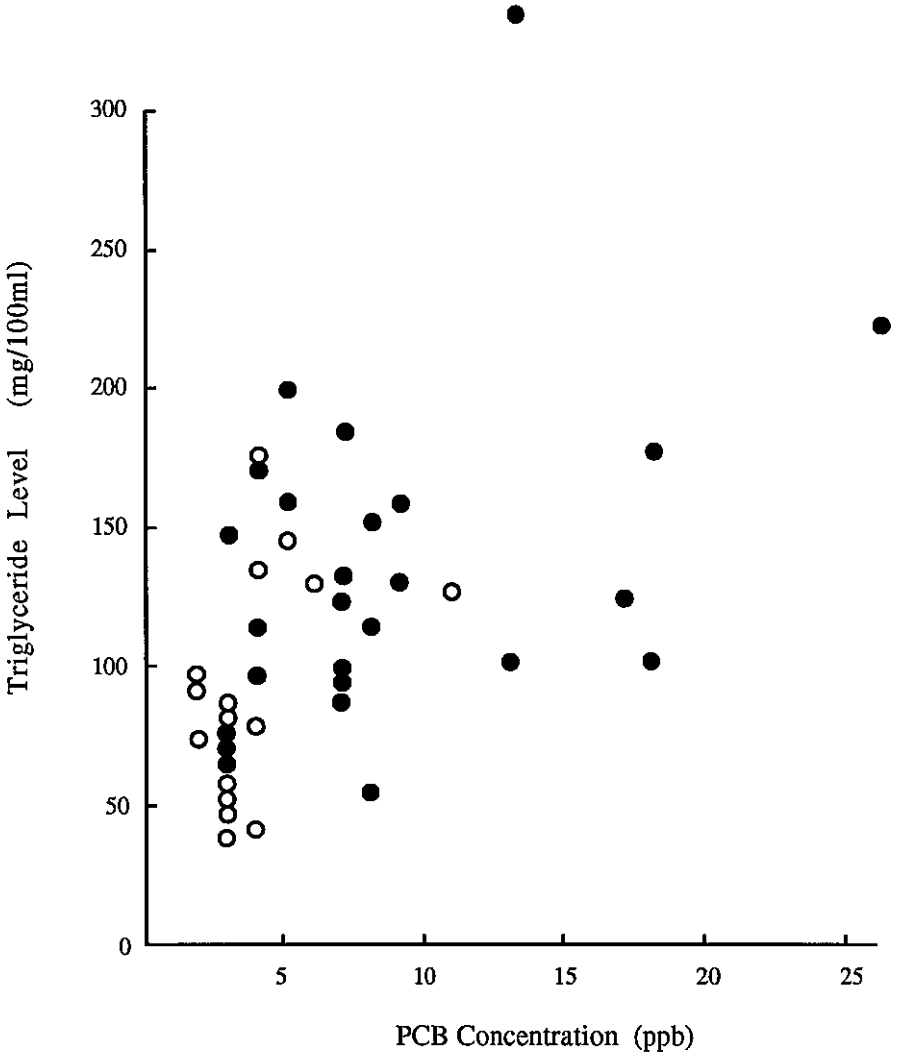

Fig. 7.1.1. The Correlation between the Blood PCB Concentrations and the Serum Triglyceride Levels in 42 Patients with Yusho
r = 0.485, ● = patients of "A" pattern on gaschromatograph, ○ = "B" or "C" pattern.

cal symptoms such as headache, visual disturbance, numbness or paresthesia in the distal extremities (Kuroiwa et al., 1969).

Neurological Symptoms: To elucidate neurological manifestations, careful neurological examinations were carried out in 21 patients who were admitted to Kyushu University Hospital in the autumn of 1968, soon after the outbreak of Yusho (Murai and Kuroiwa, 1971). No symptoms or signs were found suggesting the involvement of either the cerebrum, cerebellum, spinal cord, or cranial nerves. Spinal nerve symptoms and signs, however, were present. Of these 21 patients, 7 (33%) complained of numbness, pain or both in the distal part of limbs. Hypoesthesia, hypalgesia, and thermohypoesthesia were found in the lower limbs in 5 cases (24%). Deep reflexes were absent in 3 cases, while they were sluggish in one. In 10 cases (48%), one or more of the neuropathic symptoms of numbness, pain, hypoesthesia, and areflexia were found. None of the patients had muscular weakness or atrophy.

Electrophysiologic Examination: The nerve conduction velocity of these 21 patients was studied. In all patients, the motor nerve conduction velocity (MCV) in the ulnar nerve was within the normal range. The MCV in the tibial nerve was also normal, except in one patient in which the deep reflexes were absent in all four limbs. On the sensory nerve conduction velocity in the radial and the sural nerves, slowing was observed in 8 (38%) and 7 cases (33%) respectively. In 10 cases (48%), the sensory nerve conduction velocity was lower than normal in the radial nerves, sural nerves or both. These findings suggested that sensory nerves were thus predominantly involved in Yusho patients at the early stage.

Electroencephalography (EEG): Most of the headaches described by Yusho patients were transient, however, some were chronic, persistent or recurrent over a period of months or years. In the early stage (1969), an EEG was performed on 20 randomly selected patients with Yusho (Nagamatsu and Kuroiwa, 1971). Nine of the 20 patients (45%) complained of various headaches, but their EEG records were recognized to all be within the normal limits. In only one of 20 patients, a slightly abnormal record with low voltage 6–7 c/s theta wave of frontal dominant was revealed. However, this was not considered to be a specific finding for Yusho in view of other neurologic or dermatologic manifestations, and in addition, the patient in question actually complained of no headache. Normal cerebrospinal fluid pressure was found in two of the patients who complained of headaches. These findings thus suggested that most of the headaches arose from either a functional disorder or emotional stress.

2. Follow-up Studies

Complaints about headache and paresthesia in the distal lower limbs were most

Table 7.1.11. A Follow-up Study on the Peripheral Nerve Conduction Velocity (m/sec) during 12 Years in a 51-year-old Male Patient

	Nerve	1968	1980	Normal
MCV[a]	r. ulnar nerve	46.2	60.3	47 <
	r. tibial nerve	41.5	53.0	41 <
SCV[b]	r. radial nerve	50.9	53.6	49 <
	r. gastrocnemial nerve	53.9	peak latency 2.9 m/sec	< 4.0 m/sec

[a]: Motor nerve conduction velocity, [b]: sensory nerve conduction velocity. (Shibazaki, 1981.)

common in the patients as stated above. Consequently, the correlation between headache and paresthesia of the limbs and blood PCB levels in Yusho patients was analyzed during the Annual Health Examination in 1973 (Iwashita et al., 1977). The frequency of headaches and paresthesia in a total of 109 patients in 1973 was still high, 66.1% and 52.3%, respectively. A tendentious relationship ($p < 0.09$) was also observed between the rate of headaches and the rate of paresthesia. However, no difference was detected in the mean value of the blood PCB levels between the patients with headaches and those without them.

The headache observed in Yusho patients from 1969–1973 was usually of a dull nature, over the entire scalp, continuous, non-pulsating, non-paroximal and tended not to be relieved by analgesics, sedatives or muscle relaxants. These agents were required in larger doses, because they were metabolized more rapidly through the drug metabolizing enzyme induction caused by PCBs. These neurological findings that appeared to be unrelated to PCB levels may thus suggest that most of the headaches arose from emotional stress.

The latest neurological study of Yusho patients was made at the Annual Health Examination in 1980 (Shibazaki, 1981). Fifty-eight percent of the patients still complained of some headaches, and 12% reported severe headaches. Forty-six percent of the patients complained of paresthesia in the limbs, and these rates were almost the same as those reported from 1969 to 1971.

The ankle jerk was absent in 15% and decreased in 19% of the patients, and this ankle jerk abnormality was more frequently seen in the patients with paresthesia than in those without. The higher rate of lost or decreased ankle jerk in patients in the 1981 series than that seen in 1969 report, however, might also be due, in some degree, to the aging factor of the patient population over the 12-year period. The presence or absence of headache, limb paresthesia or ankle jerk correlated with neither the blood PCB level nor the gaschromatographic pattern of PCBs. The motor and conduction velocities were determined in a 51 year-old male patient who had paresthesia with a decreased ankle jerk. He showed a slightly decreased

MCV in the right ulnar nerve in 1968, while a repeated study made with the same technique in 1980 revealed a normal MCV. This finding shows an improvement in peripheral neuropathy which occurred over the 12-year period (Table 7.1.11) (Shibazaki, 1981).

7.1.3. Endocrine Disorders

Anterior Pituitary Function:

The anterior pituitary lobe function in Yusho patients that was examined in connection with gonadotropin was found to not be deteriorated. The irregular menstrual cycles or disturbed sexual functions, that were observed in some patients, might be attributed to disorders in the hypothalamus or the metabolism of the peripheral steroids (Kusuda et al., 1975).

Thyoid Function:

No remarkable change in the thyroid function tests was seen in two patients who were examined at the early stage of the poisoning (Watanabe et al., 1971). In 1984, sixteen years after exposure, the thyroid function was studied in 124 adult patients with Yusho (Murai et al., 1985). The blood levels of triiodothyronine (T_3) and thyroxine (T_4) were all whithin the normal limits, but the mean values of T_3 and T_4 were significantly higher than those of the controls. On the other hand, the TSH level of the patients was lower than that of the controls, but the difference was not significant. No correlation between the blood PCB concentrations and the levels of T_3 and T_4 or TSH was confirmed. In addition no difference in the frequencies of positive thyroid antibodies between the patients and controls was observed. The mechanism of relative hyperthyroxinemia (T_3 and T_4) observed in Yusho patients, however, still remains to be elucidated.

Adrenal Function:

At the First Annual Yusho Health Examination in Fukuoka Prefecture carried out in August 1970, one hundred and thirteen patients out of 432 were advised to have an endocrine examination because of their complaint of general fatigue or a brownish pigmentation of the skin (Watanabe et al., 1971). The rapid ACTH test was performed on 86 of these 113 patients. The results of the test revealed no evidence of severe abnormalities in adrenocortical function, apart from 2 patients with a low response.

One to two years after the onset of Yusho, an analysis of urinary 17-ketosteroids (17-KS) was performed in 50 male and 45 female patients at the Yusho outpatient clinic (Nagai et al., 1971). Forty-two percent of them revealed an elevated excretion of steroids, 17-KS and 17-hydroxycorticosteroids (17-OHCS) as well. Each major 17-KS, thioandrosterone, eticholanolone and dehydrosterone, was found to be increased in the male patients while they were decreased in the female patients,

although wide variations were observed.

Two hospitalized patients (a 17- and a 38-year-old male), who had typical symptoms and signs for Yusho, and had been classified as clinical grade IV, underwent endocrine function tests for adrenal, thyroid, gonad and growth hormones (Watanabe et al., 1971). As a result, no abnormal result was obtained for the thyroid, gonad and growth hormones in these patients. For the 38-year-old patient, the tests revealed a slightly decreased adrenocortical response to ACTH and methopyrone, a low urinary luteinizing hormone (LH) concentration and a slightly reduced response of plasma growth hormone concentration to insulin administration.

These findings on endocrine abnormality in the early stage, may thus suggest the existence of subclinical endocrine disorders in certain Yusho patients at that time.

7.1.4. Hematologic Disorders

In 22 patients with Yusho, sampled randomly from the patients visiting the outpatient clinic for Yusho in Kyushu University Hospital, hematologic examinations were performed in March 1969 (Kozuru et al., 1971). These patients were composed of 10 males and 12 females with a mean age of 38. One patient was classified into clinical grade I, 2 into II, 6 into III, and 7 into IV grade, respectively.

Complete blood cell count (CBC):

The hemoglobin level, red blood cell count and hematocrit were all within the normal limits irrespective of the clinical grades, sex or age. An increase in white blood cell (WBC) count, particularly neutrophiles along with the severity of the clinical grades, was observed. A small amount of atypical lymphocytes, less than 3% of the WBC count, was noticed in about a half of 22 patients, however, no other abnormal cells were found. Moderate leucocytosis was more frequently encountered in the patients who were over 40 than in the younger patients.

Platelet: Neither hemorrhagic diathesis nor a decreased platelet count was observed in Yusho patients.

Bone marrow pictures:

In 7 of 22 patients, bone marrow aspiration was made (Table 7.1.12). Slightly increased reticular cells and plasma cells and hyperplasia in the erythroids were observed in some patients. These hematologic findings may also correspond to those seen in the acute or chronic inflammatory disorders often noted in severe cases of Yusho.

Peripheral blood lymphocytes:

The total lymphocyte counts and the percentage of B cells were normal, while the percentage of T cells and active T cells were found to be decreased in Yu-Cheng patients (Lü and Wong, 1984). They noted that the percentage of helper T

Table 7.1.12. Bone Marrow Picture in Yusho Patients

Patients	U. T.	T. K.	K. Y.	K. Y.	M. Y.	U. K.	U. T.
Yusho grades	II	IV	IV	IV	IV	IV	IV
NCC ($\times 10^4$)	21.1	17.6	5.0	11.3	14.8	7.2	24.7
Myeloblast (%)	0.8	0.8	3.2	0.4	0	0	5.6
Neutro:							
Promyelo	5.6	9.2	8.8	2.0	12.8	9.6	4.4
Myelo	3.2	4.4	1.2	4.0	8.0	12.4	7.6
Metamyelo	6.8	3.6	4.8	4.8	14.4	11.6	5.6
Band	14.4	16.4	11.6	14.4	22.4	12.8	12.4
Seg	7.2	16.0	14.8	20.0	9.2	18.4	6.4
Eosino:							
Promyelo	0	0.8	0	0.4	0.8	0.8	0
Myelo	0.8	0.4	0	0	0.8	0.4	0.4
Metamyelo	0	0.8	0.8	0.8	0	0	0.4
Band	0.4	0.4	0	0.8	0.4	0	0.4
Seg	0.4	1.2	1.6	1.2	0.4	0.8	0.8
Baso	0	0	0.8	0	0	0	0
Mitosis (M)	0	0	0	0	0	0.4	0.4
Subtotal	39.6	54.0	47.6	48.8	69.2	67.2	44.4
Mono	5.2	6.4	1.2	7.6	2.0	4.4	2.4
Lympho	14.4	9.2	13.2	16.0	4.4	9.6	8.4
Plasma	1.2	1.2	0.8	3.2	2.0	0	7.6
Reticulo	0.8	6.4	1.6	2.0	1.2	0.8	7.2
Histiobaso	0	0	0	0	0	0	0
Megakaryo	0	0.4	0.8	0	0	0	0.8
Subtotal	21.6	23.6	17.6	28.8	9.6	14.8	26.4
Erythroblast:							
Macro: Baso	5.6	0.8	1.2	0	0.8	2.8	0.4
Poly	0.4	0	1.6	0	1.6	0	0
Normo	0	0	0	0	0	0	0
Normo: Baso	5.6	0.8	3.2	2.4	4.0	3.2	2.4
Poly	25.2	14.4	26.0	14.0	12.8	6.4	21.2
Normo	1.2	6.4	2.8	5.6	2.0	4.8	4.8
Mitosis (E)	0.8	0	0	0.4	0	0.8	0.4
Subtotal	38.8	22.4	34.8	22.4	21.2	18.0	29.2

(Kozuru et al., 1971.)

Table 7.1.13. The Serum Immunoglobulin Level (mg/100 ml)[a] in 38 Adult Yusho Patients in 1970

	Yusho	Control	p
	n = 38	n = 57	
IgG	1,538.9 ± 452.7	1,242.8 ± 329.0	< 0.01
IgM	138.4 ± 71.2	170.1 ± 53.6	< 0.02
IgA	147.9 ± 89.1	206.9 ± 95.5	< 0.01

[a]: Mean values ± S.D. (Shigematsu et al., 1971.)

cells decreased significantly but not that of suppresser T cells. These findings elucidate that the chronic infections frequently observed in Yusho patients in the early stage may thus be the result of some immunological deficit.

Immunoglobulin:

The levels of serum immunoglobulins, especially IgA and IgM generally decreased (Table 7.1.13), which thus suggested a B cell dysfunction (Shigematsu et al., 1971). As shown in Table 7.1.4, the amount of α_2-globulin in the serum of Yusho patients markedly increased, while the γ-globulin level mildly decreased in severe patients. Similar results have also been reported for Yu-Cheng patients in Taiwan (Lü and Wong, 1984). Respiratory distress was often exacerbated by viral or bacterial infections persisting among more than a half of the Yusho patients examined. The IgA and IgM levels in the serum decreased considerably within the first two years after the onset of the disease and a definite decrease in the IgA levels seemed to correlate well with bacterial infections (Shigematsu et al., 1978). In the follow-up observation, however, it was difficult to determine whether or not PCBs affected the defense mechanism including the immune mechanism. For example, the prevalence of hepatitis B antigen (Hirayama et al., 1972) or hepatitis C antibody among Yusho patients did not different from that in the general population.

7.1.5. Hepatic Disorders

Several reports on the systemic intoxication caused by accidentally ingested chlorinated hydrocarbons first appeared in the late 1930s. In these cases, hepatic damage including a complete loss of liver cells , hemorrhage of the central zone of lobules, and acute yellow atrophy of the liver were described (Drinker et al., 1937; Greenburg et al., 1939). Therefore, careful studies on possible hepatic disorders which might be seen among Yusho patients appears to be quite important.

1. Early Stage

Laboratory findings:

The initial laboratory tests of Yusho patients, unexpectedly revealed no abnor-

mal values in the liver function tests, such as the serum bilirubin, GOT, GPT, LDH and BSP retention, except for a slightly elevated level of alkaline phosphatase. The serum protein fraction showed a slightly decreased albumin level, elevated α_2-globulin level, and a decreased γ-globulin level in severe cases according to the dermatological severity criteria.

Elevated values in the thymol turbidity tests (TTT) and increased M fraction of electrophoretic isoenzyme analysis of serum lactate dehydrogenese (LDH_5) were also observed in severe patients. These changes were likely to be due to the sustained hyperlipidemia and increased LDH_5 fraction to the enzymes derived from skin lesions, respectively, though the role played by the possible association of liver damage could not be denied. A slightly elevated serum alkaline phosphatase level seen in a few cases revealed a single active band of hepatic origin, which demonstrated a normal pattern in an electrophoretic isoenzyme analysis (Okumura and Katsuki, 1969; Okumura, 1972).

Morphological findings of the liver:

Studies on liver biopsy specimens obtained from a 37-year-old patient, Case 4 in Table 7.1.1, who was one of the most severely ill ones (grade IV), revealed no particular changes based on the light microscopic findings, but definite changes were recognized by electron microscopy as shown in Figure 7.1.2.

A reduction of the rough-surfaced endplasmic reticulum and a marked proliferation of the smooth-surfaced endoplasmic reticulum were evident. The mitochondria in some lobules showed morphological heterogeneity, as well as variations in size and form. Inclusion bodies were recognized both in the mitochondria matrix and within the cavity of the cristae. Lysosomes and microbodies were present in increased numbers. The size of the microbodies, located close to the smooth-surfaced endoplasmic reticulum was also increased and contained no crystalline structures (Hirayama et al., 1969). These fine structures observed in hepatic cells were considered to be typical morphological features closely related to the enzyme induction caused by PCBs intoxication (Yamamoto et al., 1971). These findings were compatible with the observations by others, in which the pretreatment of experimental animals with PCBs resulted in an enhancement of the drug metabolizing enzyme system in liver microsomes (Fujita et al., 1971).

The hepatitis B virus (HBs-antigen or HBV) carrier rate in Yusho patients was 2.4%, whereas it was 2.25% in healthy blood donors (Hirayama, et al., 1972). The prevalence of positive HCV antibody was also similar to that for the general population (Okumura, unpublished observations).

2. Follow-up Studies (Hepatic Carcinoma in Yusho)

The liver tumorgenic potency of PCBs in experimental animals has been empha-

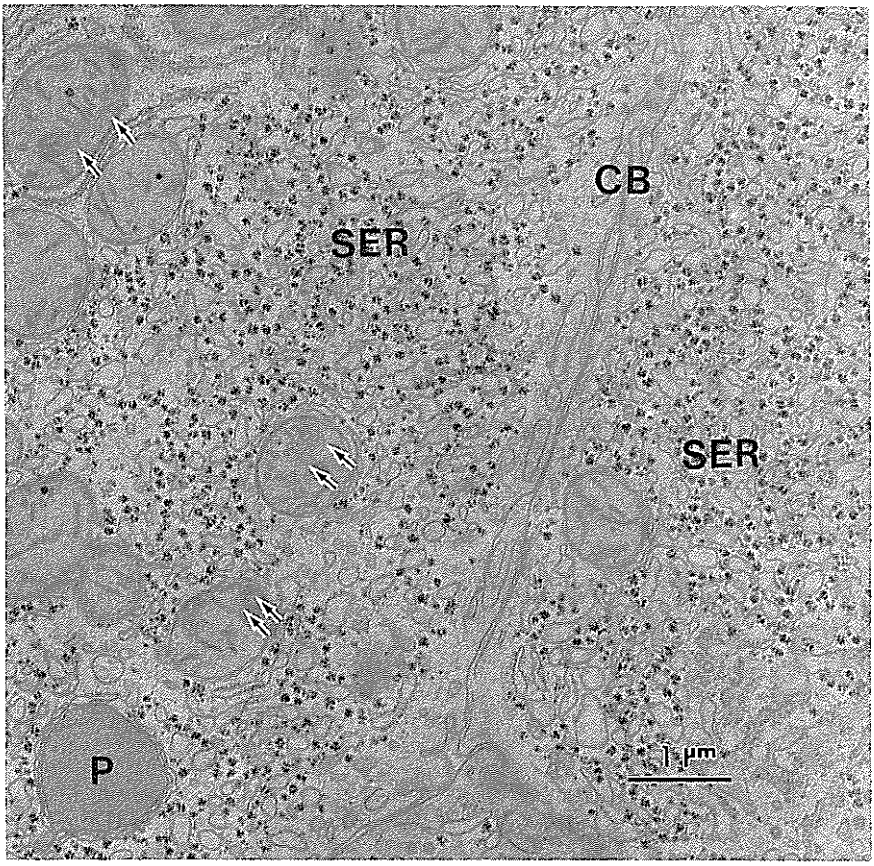

Fig. 7.1.2. An Electron Micrograph of Liver Biopsy Specimens Obtained from Case 4, a 37-Year-old Male Patient

The characteristic finding demonstrated a wide proliferation of the smooth endoplasmic reticulm (SER), which is considered to indicate the induction of microsomal drug metabolizing enzymes by PCB intoxication. Filamentous inclusions (arrows) are also noted in some mitochondria. CB: cell boundary, P = peroxisome, × 18,000. Courtesy of Professor T. Yamamoto, Nakamura Gakuen University, Fukuoka.

sized (Kimura, 1973; Ito, 1974; Kimmbrough, 1975). A noticeable cohort study on the mortality of Yusho patients, as yet a preliminary report, presented a significantly elevated standarized mortality ratio on cancer of the liver for males, though not for females (Ikeda et al., 1987). According to the autopsy findings of 12 patients with Yusho (Kikuchi, 1984), carcinomas were found in 5 of 12 cases. Two of them, including a 48-year-old female and a 69-year-old male, were reported to demonstrate hepato-cellular carcinoma with or without cirrhosis and the direct causes of death were a rupture of the liver carcinoma. These two patients showed

no particular symptoms of liver lesions in the early stage of PCBs poisoning, and revealed a gaschromatograpic "C" pattern which is the pattern of healthy persons. Namely, these liver carcinomas hardly seemed to be associated with PCB poisoning.

To evaluate the relationship between PCB poisoning and the incidence of hepatocellular carcinoma, tumor markers and the prevalence rate of HBsAg (HBV) in patients with Yusho were examined, while abdominal ultrasonography was also performed. As shown in Table 7.1.14 and 15, however, no significant difference was noticed between the Yusho patients and the normal controls (Okumura and Sakaguchi, 1985). The mean values of the serum GOT, GPT, ALP and γ-glutamyl transpeptidase levels of 157 Yusho patients, who averaged 47 years of age, did not differ from those of 36 control subjects, who averaged 49 years of age (Tsuji et al., 1987). An elevated serum AFP was observed in only one patient, a 55-year-old male, who showed a serum PCB concentration of 7 ppb with a gaschromatographic "C" pattern. He had already been diagnosed as having hepatocellular carcinoma originating from liver cirrhosis of type non B at that time. These above results all seem to suggest that there is no evidence to support an increased risk of hepatocellular carcinoma in Yusho patients.

7.1.6. Yusho and Blood Pressure

The Yusho patients examined at the early stage showed no abnormal blood pressure (Okumura and Katsuki, 1969). However, Kreiss et al. (1981) reported an association of the serum PCB level and the measured blood pressure as observed in a community wide study in Triana, Ala, U.S.A. According to their suggestion, the PCB-blood pressure association, which was independent of age, sex, the body mass index, and social class, must first be confirmed in other exposed populations.

In 1981, fifteen years after the initial exposure to PCBs we studied the correlation of the blood PCB levels or patterns and blood pressure in 59 patients with Yusho older than 40 years of age (Akagi et al., 1983). The obtained data are shown in Table 7.1.16. In 52.5% of the cases, the blood PCB concentrations were still higher than 5.0 ppb, the highest value found in the general population. The mean levels ± S.D. in the blood PCBs were 5.1 ± 2.3 ppb in males and 6.4 ± 5.3 ppb in females. No association was recognized between the blood PCB levels and the age or sex compositions. The frequency of hypertension in Yusho patients was found to be 16.9%, which was similar to that expected on the basis of the rate for the general population of the same age and sex compositions. No association between the blood pressure and either the PCB levels or PCB patterns was confirmed in 20 patients whose blood PCB levels were persistently higher than 5.0 ppb for 9 years. On the other hand, age, obesity, and habitual alcohol intake, which are common

Table 7.1.14. HBV and Tumor Markers in 79 Yusho Patients (1983)

		Yusho	Control
No. of patients		79	39
Male : female		32 : 47	19 : 20
Age (mean)		51.2	40.6
HBsAg	positive	2 (2.5%)	1 (2.5%)
Anti-HBs	positive	23 (29.1%)	14 (35.9%)
Anti-HBc	positive	30 (37.9%)	13 (33.3%)
AFP[a]	20 ng/ml <	0	0
CEA[b]	5 ng/ml <	0	0
Ferritin			
Male	140 ng/ml <	4 (12.5%)	2 (10.5%)
Female	85 ng/ml <	6 (12.7%)	2 (10.0%)

[a]: α-fetoprotein, [b]: carcinoembryonic antigen.

Table 7.1.15. Ultrasonic Examination in 124 Yusho Patients (1984)

Diagnosis	Male	Female	Total
Hepatology:			
Liver cyst	0	4	4
Fatty liver[a]	5	5	10
Deformity[b]	6	10	16
Splenomegaly with chronic liver disease	1	2	3
Cirrhosis, suspected	0	1	1
Normal study	38	52	90
Total	50	74	124
Biliary system:			
Gallstone	4	4	8
Polyp of gall-bladder	1	1	2
Common bile duct stone	0	1	1
Dilatation of common bile duct	1	1	2
Normal study	44	67	111
Total	50	74	124
Kidneys:			
Cyst	2	1	3
Hydronephrosis	0	1	1
Normal study	48	72	120
Total	50	74	124

[a]: May includes mild (in about 10%) fatty infiltration.

[b]: Means change in the diameter ratio of the right and left lobes of the liver.

Table 7.1.16. Clinical Data of Patients with Yusho and Hypertension

Sex	No. of cases	Age[a] (y.o.)	Abnormal[b] PCB level	Obesity[c]	Alcoholics	Hypertension[d]
Male	25	57 ± 10	52.0%	20.0%	40.0%	20.0%
Female	34	56 ± 10	52.9%	20.6%	0.0%	14.7%

[a]: Mean ± S.D. [b]: Having higher levels than 5.0 ppb. [c]: Obesity was defined as a Quetelet index of 25.4 or higher. [d]: Hypertension was defined as a systolic measurement of 160 mmHg or higher and/or a diastolic measurement of 95 mmHg or higher.

factors known to influence hypertension, tended to be positively associated with the measured blood pressure of Yusho patients.

7.1.7. Treatment

So far, no satisfactory treatment for Yusho has been developed. Balnerotherapy or fasting cure was recommended in the early stage of Yusho as well as Yu-Cheng patients, however, the therapeutic results have been, at best, transitional (Nakamizo and Saruta, 1971; Imamura and Tung, 1984). Trials with cholestyramine (Murai et al., 1991) or rice bran fiber with cholestyramine administration was attempted to increase the excretion rate of PCBs through the feces, but no favorable effect has yet been obtained (Murai et al., 1993). Therefore, only palliative treatments and supportive therapies are presently used for the nonspecific symptoms of Yusho patients.

References

Akagi, K., Murai, K., Shikata, T. (1981) Laboratory examination in PCBs poisoning patients with special reference to lipoprotein. Fukuoka Acta Med. 72, 245–248 (in Japanese).

Akagi, K., Tsuji, H., Kajiwara, E., et al. (1983) Association of blood pressure and PCB levels in patients with polychlorinated biphenyl (PCB) poisoning. Fukuoka Acta Med. 74, 272–275 (in Japanese).

Berry, J. E., Uzawa, H., Fujimi, S. (1969) Serum lipid profiles. In: Geriatrics. Mineapolis, Lancet Publications, 126–140

Drinker, C. K., Warren, M. F., Benett, G. A. (1937) The problem of possible systemic effects from certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 19, 283–299.

Fujita, S., Tsuji, H., Kato, K., et al. (1971) Effect of biphenyl chloride on rat liver microsomes. Fukuoka Acta Med. 62, 30–34 (in Japanese).

Greenburg, L., Mayers, M. R., Smith, A. R. (1939) The systemic effects resulting from exposure to certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 21, 29–38.

Hirayama, C., Irisa, T., Yamamoto, T. (1969) Fine structural changes of the liver in a patient with chlorobiphenyls intoxication. Fukuoka Acta Med. 60, 455–461 (in Japanese).

Hirayama, C., Nakamura, M., Yoshinari, M. (1972) Australia antigen in patients with PCB poisoning. Fukuoka Acta Med. 63, 405–407 (in Japanese).

Hirota, Y., Hirohata, T. (1993) Laboratory findings in the medical examination of chronic "Yusho" (PCB poisoning) patients: with special reference to blood PCB and serum triglyceride. Fukuoka Acta Med. 84, 287–293.

Ikeda, M., Kuratsune, M., Nakamura, Y., et al. (1987) A cohort study on mortality of Yusho patients—A preliminary report. Fukuoka Acta Med. 78, 297–300.

Ikui, H., Sugi, K., Uga, S. (1969) Ocular signs of chronic chlorobiphenys poisoning ("Yusho"). Fukuoka Acta Med. 60, 432–435 (in Japanese).

Imamura, M., Tung, T. (1984) A trial of fasting cure for PCB-poisoned patients in Taiwan. Am. J. Ind. Med. 5, 147–153.

Ito, N., Nagasaki, H., Makiura, S., et al. (1974) Histopathological studies on liver tumorigenesis in rats treated with polychlorinated biphenyls. Gann 65, 545–549.

Iwashita, H., Shida, K., Masuda, Y. (1977) Headache, paresthesia of the limbs and blood polychlorinated biphenyls (PCB) concentration in chronic PCB poisoning. Fukuoka Acta Med. 68, 139–144 (in Japanese).

Kikuchi, M. (1984) Autopsy of patients with Yusho. Am. J. Ind. Med. 5, 19–30.

Kimbrough, R. D., Squire, R. A., Linder, R. E., et al. (1975) Induction of liver tumors in Sherman strain female rats by polychlorinated biphenyl Aroclor 1260. J. Natl. Cancer Inst. 55, 1453–1459.

Kimura, N.T., Baba, T. (1973) Neoplastic changes in the rat liver induced by PCB. Gann 64, 105–108.

Kozuru, M., Motomura, S., Sakai, K., et al. (1971) Hematological observation on the patients with chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 163–166 (in Japanese).

Kreiss, K., Zack, M. M., Kimbrough, R. D., et al. (1981) Association of blood pressure and polychlorinated biphenyl levels. J.A.M.A. 245, 2505–2509.

Kuroiwa, Y., Murai, Y., Santa, T. (1969) Neurological and nerve conduction velocity studies on 23 patients with chlorobiphenyls poisoning. Fukuoka Acta Med. 60, 462–463 (in Japanese).

Kusuda, M., Nagata, Y., Nakamura, M. (1975) Anterior pituitary function of "Yusho" patients (polychlorinated biphenyls poisoning). Fukuoka Acta Med. 66, 635–639 (in Japanese).

Lü, Y., Wong, P. (1984) Dermatological, medical, and laboratory findings of patients in Taiwan and their treatments. PCB Poisoning in Japan and Taiwan. Am. J. Ind. Med. 5, 81–115.

Masuda, Y., Kagawa, R., Shimamura, K., et al. Polychlorinated biphenyls in the blood of Yusho patients and ordinary persons. Fukuoka Acta Med. 65, 25–27 (in Japanese).

Murai, K., Tsuji, H., Fujishima, M. (1991) Treatment of Yusho patients with cholestyramine. Fukuoka Acta Med. 82, 326–329 (in Japanese).

Murai, K., Tsuji, H., Kajiwara, E., et al. (1985) Thyroid function in patients with PCB poisoning. Fukuoka Acta Med. 76, 233–238 (in Japanese).

Murai, Y., Kuroiwa, Y. (1971) Peripheral neuropathy in chlorobiphenyl poisoning. Neurology 21, 1173–1176.

Nagai, J., Furukawa, M., Tojo, A., et al. (1971) Colorimetric and gas-chromatographic determinations of urinary 17-Ketosteroids. Fukuoka Acta Med. 62, 51–65 (in Japanese).

Nagamatsu, K., Kuroiwa, Y. (1971) Electroencephalographical studies on 20 patients with chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 157–158 (in Japanese).

Nakamizo, Y., Saruta, T. (1971) The effects of balneotherapy of Yusho. Fukuoka Acta Med. 62, 176–181 (in Japanese).

Okumura, M. (1972) Course of serum enzyme change in PCB poisoning. Fukuoka Acta Med. 63, 396–400 (in Japanese).

Okumura, M., Katsuki, S. (1969) Clinical observation on Yusho (Chlorobiphenyls poisoning). Fukuoka Acta Med. 60, 440–448 (in Japanese).

Okumura, M., Masuda, Y., Nakamuta, S. (1974) Correlation between blood PCB and serum triglyceride levels in patients with PCB poisoning. Fukuoka Acta Med. 65, 84–87 (in Japanese).

Okumura, M., Sakaguchi, S. (1985) Hepatic cell carcinoma and the patients with Yusho. Fukuoka Acta Med. 76, 229–232 (in Jaoanese).

Okumura, M., Yamanaka, M., Nakamuta, S., et al. (1975) Consecutive six year follow up study on serum triglyceride levels in patients with Yusho. Fukuoka Acta Med. 66, 620–623 (in Japanese).

Shibasaki, H. (1981) Neurological studies of patients with rice oil disease 12 years after the onset. Fukuoka Acta Med. 72 , 230–234 (in Japanese).

Shigematsu, N., Ishimaru, S., Saito, E., et al. (1978) Respiratory involvement in polychlorinated biphenyls poisoning. Environ. Res. 16, 92–100.

Shigematsu, N., Norimatsu, Y., Ishibashi, T., et al. (1971) Clinical and experimental studies on respiratory involvement in chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 150–156 (in Japanese).

Takamatsu, M., Inoue, Y., Abe, S. (1974) Diagnostic meaning of the blood PCB. Fukuoka Acta Med. 65, 28–31 (in Japanese).

Tsuji, H., Akagi, K., Murai, K., et al. (1987) Liver damage and hepatocellular carcinoma in patients with Yusho. Fukuoka Acta Med. 78, 343–348 (in Japanese).

Tsuji, H., Ikeda, K., Nomiyama, K., et al. (1993) Effects of treatment with rice bran fiber and cholestyramine on clinical and laboratory findings in Yusho patients. Fukuoka Acta Med. 84, 282–286 (in Japanese).

Uzawa, H., Ito, Y., Notomi, A., et al. (1969) Hyperglyceridemia resulting from intake of rice oil contaminated with chlorinated biphenyls. Fukuoka Acta Med. 60, 449–454 (in japanese).

Uzawa, H., Notomi, A., Nakamuta, S., et al. (1972) Consecutive three year follow up study of serum triglyceride concentrations of 82 subjects with PCB poisoning. Fukuoka Acta Med. 63, 401–404 (in Japanese).

Watanabe, A., Irie, S., Nakajima, et al. (1971) Endocrinological studies on chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 159–162 (in Japanese)

Yamamoto, T., Hirayama, C., Irisa, T. (1971) Some observations on the fine structure of the mitochondria in hepatic cells from a patient with chlorobiphenyls intoxication. Fukuoka Acta Med. 62, 85–88 (in Japanese).

7.2. The Clinical Course of Dermatological Symptoms in Yusho Patients over the Past 25 Years

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A 3-year-old girl visited the dermatological out-patient clinic of Kyushu University Hospital on June 7, 1968, complaining of an acneiform eruption. Thereafter, 13 patients from 4 families with the same skin symptoms visited the clinic. These patients were the first reported cases of Yusho, an incidence of massive polychlorinated biphenyl (PCB) poisoning. The number of such patients steadily increased since then. The affected families had all used Kanemi rice oil/rice bran oil contaminated with PCBs.

Most patients initially noticed an increase in the amount of eye discharge as the first sign of PCB poisoning. Thereafter, various skin symptoms appeared. Systemic symptoms such as general fatigue, loss of appetite, headache, nausea, vomiting, etc., also occurred after the skin symptoms had emerged. The skin symptoms were the most striking and peculiar features of Yusho in the initial stage of the outbreak. Chloracne was first suspected by dermatologists because of the clinical skin manifestations of the patients. At that time, the diagnosis of Yusho was officially made based mainly on the skin and mucous membrane symptoms, since this was before a method to accurately quantify the PCB levels in the blood of the patients had been developed.

Almost 25 years have passed since this wide-spread incident of PCB poisoning occurred in the northern Kyushu district. The skin eruptions have changed over the past 25 years along with a decrease in the blood PCB levels of the patients through the spontaneous excretion of PCBs. In this article, the skin symptoms in the initial acute intoxication period, the changes in the skin symptoms over the 25-year period, the relationship between the skin severity index and the peripheral blood PCBs, and the treatment of such skin eruptions will be described.

7.2.1. Skin Symptoms in the Initial Period of Intoxication

The initial skin symptoms of Yusho have been described by Goto and Higuchi in detail (Goto and Higuchi, 1969). They consisted of acneiform eruptions, a marked enlargement and elevation of follicular openings, pigmentation of the skin, lips, gingiva, and mucous membrane of the oral cavity, the formation of hyperkeratotic plaque in the soles and palms, hyperidrosis, hypertrichosis, swelling of the Montgomery gland of the breast, cyst formation in the sebaceous gland of the genital region, dry skin and localized follicular keratosis in the case of children. The skin

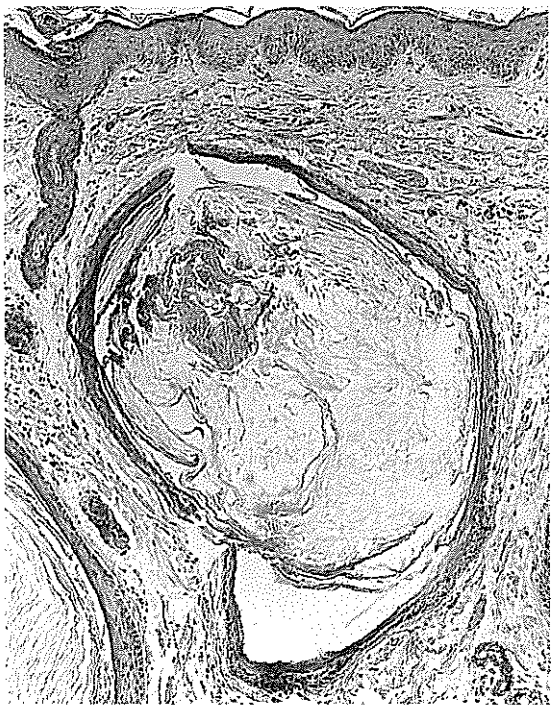


Fig. 7.2.3. Histopathology of an Acneiform Eruption Exibiting Large Cysts in the Dermis Containing Keratinous Material. × 100

symptoms normally appeared 2 to 3 months after such eye symptoms as an increased discharge from the eye, a swelling of the eyelids, and weakened eyesight had emerged.

i) Acneiform eruption

The acneiform eruptions of Yusho patients were basically Chloracne, and they were based on hyperkeratinization associated with an abnormal lipid metabolism (Goto and Higuchi, 1969). The acneiform eruptions were pale or straw-colored cysts and were about the size of a pin head or a pea. They seemed to be a type of big white comedo (Fig. 7.2.1; see color frontispiece). If secondary infections occurred, the acneiform eruptions with inflammation showed a clinical appearance similar to that of infected atheromas or cutaneous abscesses. The eruptions persisted for a long time without a cure. The predilection sites of these eruptions were the cheeks, auricles and retroauricular areas, trunk, inguinal regions and external genitalia. Aggregating black comedones on the cheeks of children with Yusho were extremely characteristic (Fig. 7.2.2; see color frontispiece).

Histopathologically, these acneiform eruptions formed a big cyst which then

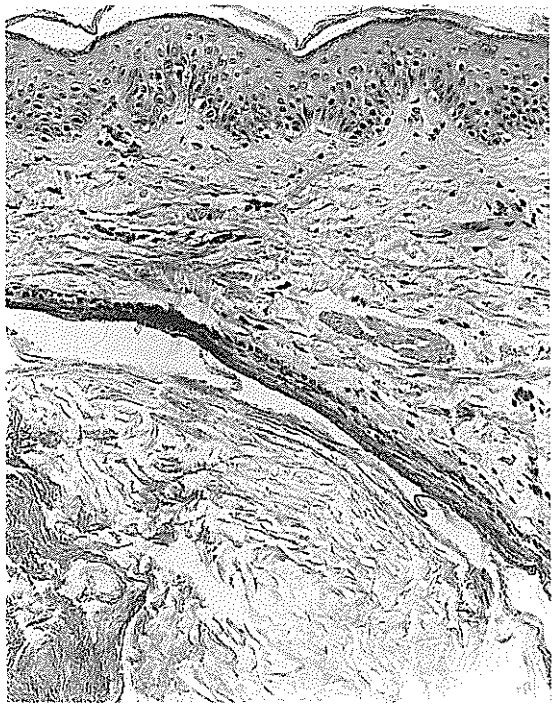


Fig. 7.2.4. Melanin Pigmentation Seen in the Basal Cells of the Epidermis in an Acneiform Skin Lesion. × 200

opened to the skin surface and was filled with keratinous material (Fig. 7.2.3). A part of the cyst wall showed destruction which led to the formation of foreign body granuloma with an infiltration of inflammatory cells. An increase of melanin pigmentation was also seen in the basal cells of the epidermis adjacent to the follicular orifice (Fig. 7.2.4).

ii) Enlargement and elevation of the follicular opening

A marked enlargement of the follicular opening was one of the most characteristic skin symptoms of Yusho. The eruption appeared as tiny papules of follicular dots (Fig. 7.2.5; see color frontispiece). Black keratotic material plugged the dilated follicles. These skin lesions were mainly observed in the axillae, groin, cubital fossa, and popliteal space. The trunk and extremities were two other sites where these eruptions were seen.

Histopathologically, a follicular dot showed an enlargement of the follicular opening and keratinous material filling the orifice (Fig. 7.2.6). The surrounding epidermis became flat without any prominent rete ridges. Inflammatory infiltrates were also seen in the dermis (Fig. 7.2.7).

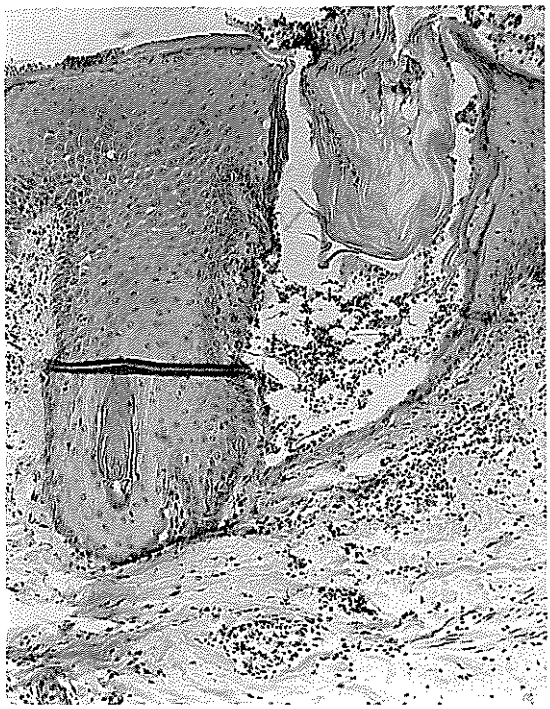


Fig. 7.2.6. Histopathology of a Follicular Dot Showing an Enlargement of a Follicular Opening Filled with Keratinous Material. × 100

iii) Pigmentation

In Yusho, a characteristic pigmentation was seen but the pigmented lesions showed a localized distribution rather than a generalized one. The finger- and toenails were the most frequently pigmented (Fig. 7.2.8; see color frontispiece). The pigmentation of the face, especially the ala nasi, cornea, palpebral conjunctiva, gingiva, and lips was also frequently seen. Generally, the entire nail would be diffusely pigmented with longitudinal linear striae. A flattening of the nails was sometimes accompanied by this pigmentation. The gingiva characteristically exhibited a band-like brown pigmentation along the gingival edge . The pigmentation of the trunk and extremities occurred mainly around the hair orifices, but some diffuse pigmentation of the skin was also observed. A stillborn fetus delivered by a mother with Yusho also showed diffuse and systemic pigmentation (the so-called "black baby") (Fig. 7.2.9; see color frontispiece).

iv) Skin symptoms in children with Yusho—dry skin, and acneiform eruptions

Dry skin was found mainly in children with Yusho. The dry skin in children was frequently associated with eczema with follicular papules and scaling. Histopa-

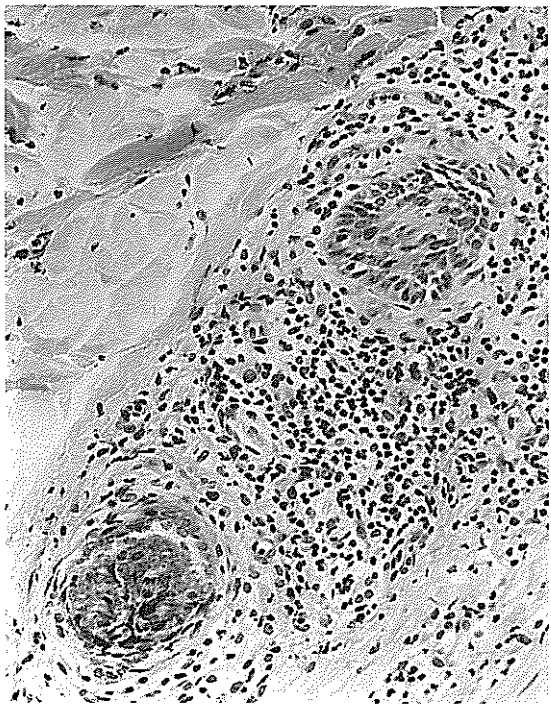


Fig. 7.2.7. Histopathology of Inflammatory Infiltrates around the Hair Follicle in the Dermis. × 200

thologically, the lesions showed both hyperkeratosis and obstruction of the follicular orifice by keratinous material. The acneiform eruptions in the children were characterized by comedo formation. Such eruptions emerged primarily due to abnormal follicular keratinization.

v) Other skin symptoms

Dysidrosis (generally hyperidrosis) was seen on the palms and soles, and in the auricular fossa. Hyperkeratosis of the palms and soles was conspicuous, in addition to the presence of hyperidrosis, in the most severe cases.

The nails with pigmentation sometimes showed deformities. In particular, a flattening of the thumb nail was characteristic.

Dome-shaped tumors with fluctuations containing yellow translucent fluid were seen on the cubital, popliteal, and foot auricular areas. The tumors were thought to be mucous cysts.

7.2.2. The Clinical Course of the Skin Symptoms of Yusho over the Past 25 Years

Twenty-five years have passed since the accidental poisoning by PCBs in the

Table 7.2.1. Severity Grading of Skin Symptoms of Yusho

Grades	Criteria 1 (Goto and Higuchi, 1969)	Criteria 2 (Toshitani and Kitamura, 1971)
0	—	No skin eruptions
I	Increased cheese-like discharge from the Meibom gland, pigmentation of nails	Circumscribed pigmentation
II	Grade I plus comedon	Black comedon
III	Grade II plus an acneiform eruption, cyst formation in the genital sebaceous gland, and a follicular opening in the neck and chest	Acneiform eruptions
IV	Enlarged and elevated follicular openings all over the body and an extensive distribution of acneiform erutions	An extensive distribution of acneiform eruptions

northern part of Kyushu island occurred. The severe and characteristic skin symptoms, such as acneiform eruptions, an enlargement of follicular openings, black dots, pigmentation, etc., have all gradually healed with time. A physical examination, including that of skin symptoms, has been carried out by various specialists from Kyushu University Hospital every year. In terms of evaluating the severity of skin symptoms and analyzing their changes over time, the severity grading of skin symptoms was first established by Goto (Goto and Higuchi, 1969), and then was later modified with a slight simplification by Toshitani (Toshitani and Kitamura, 1971) (Table 7.2.1).

i) Changes in the skin symptoms during the first 5 years after the outbreak of Yusho

One-hundred thirty-eight patients with Yusho had their skin symptoms examined in the out-patient clinic of Kyushu University Hospital in 1968 as the first general examination. Patients with various degrees of skin symptoms were divided into 4 groups according to Goto's criteria. In the initial period, patients recorded as grades III or IV constituted more than 50% of the total. The relationship between skin severity and the age of the patients was obvious. Thus, patients 20–30 years old showed the most severe skin symptoms because of their active sebum secretion into the skin. The symptoms of adolescent or middle-aged patients (30–40 years old) were also relatively severe. On the other hand, the symptoms of children (less than 10 years old) or older patients (more than 40 years old) were comparatively less severe (Goto and Higuchi, 1969).

Table 7.2.2 shows the time course of the distribution of the patients classified

Table 7.2.2. Time Course of the Severity Grading for Skin Symptoms from 1969 to 1972

Grades	1969	1970	1971	1972
0	41 (11%)	32 (17%)	4 (3%)	2 (3%)
I	86 (25%)	77 (37%)	49 (38%)	41 (46%)
II	90 (26%)	59 (29%)	32 (25%)	20 (22%)
III	87 (25%)	27 (13%)	31 (24%)	16 (18%)
IV	47 (13%)	10 (4%)	13 (10%)	10 (11%)
Total	352	205	129	89

(Koda et al., 1974)

according to Toshitani's severity grading. Classification was determined in the annual general examinations held from 1969 to 1972. The patients classified as grade IV decreased dramatically in 1970, from 13% in 1969 to 4% in 1970. However, the patients showing severe skin symptoms (grades III or IV) did not decrease thereafter. The distinctive pigmentation of the face (especially around the nose), of the nails, and of the eyelids was still observed. None of the large subcutaneous cysts were cured at all. The cysts were usually secondarily infected and formed abscesses. Even when the comedones or cysts were cured, a considerable degree of scar formation remained.

On the other hand, an increase in the number of patients classified as grade I was clearly observed during these periods. The slight acneiform eruptions markedly decreased within 3 or 4 years of the outbreak, although a small number of cysts remained in some cases. Black comedones usually disappeared within 5 or 6 years. Follicular dots usually disappeared within 4 years. Pigmentation also diminished with time. The flattening of the nails improved within 2 or 3 years. These results indicated that in severe cases of Yusho, skin symptoms hardly improved within 5 years after the outbreak, but the slight cases demonstrating only a slight degree of symptoms gradually improved during these periods.

ii) Changes in the skin symptoms 5 to 10 years after the outbreak of Yusho

In the severe cases, the skin symptoms showed almost no change during the first 5 years after the outbreak of Yusho. A clear improvement in skin symptoms was first recorded in 1974 when compared with the severity gradings of 1973. When more than 5 years had passed after the outbreak, however, the previous severity grading of skin symptoms of Yusho patients proposed by Toshitani could not be effectively applied because of the general decrease in the severity of skin symptoms. For example, the patients who showed even a few instances of acne were classified as grade III in Toshitani's grading, although the severity of the skin

Table 7.2.3. Time Course of Skin Severity Grading and Scoring from 1969 to 1974

Case	1967	1968	1969	1970	1972	1973	1974
64 y.o. M	—	III[a]	III	III	II	II	II
	—	5[b]	5	3	2	1	1
56 y.o. F	III	III	II	II	II	II	II
	7	7	5	4	4	7	4
43 y.o. M	—	IV	IV	III–IV	III	III	II
	—	15	13	7	6	7	3
39 y.o. F	IV	IV	IV	IV	IV	IV	IV
	18	18	18	18	16	16	16
12 y.o. F	—	IV	IV	III–IV	III–IV	III	II
	—	16	13	10	9	7	5
9 y.o. F	IV	IV	IV	IV	IV	III	III
	11	13	12	13	12	9	9
24 y.o. M	IV	IV	III	III	—	III	III
	16	17	11	10	—	5	4
48 y.o. F	IV	IV	III	III	III	III	III
	16	17	16	15	14	14	13
20 y.o. F	IV	IV	III	III	III	III	III
	16	17	11	8	8	8	8
56 y.o. M	—	II	III	III	0	I	0
	—	2	1	2	0	1	1
50 y.o. F	III	IV	III	III	III	III	III
	11	12	8	8	7	7	7
22 y.o. F	III	III	II	I	I	III	III
	9	9	3	2	2	3	3
40 y.o. M	—	II	II	II	I–II	II	II
	—	5	3	2	3	3	3

y.o.: years old, M: male, F: female, [a]: severity grade (Toshitani and Kitamura, 1971), [b]: severity score (Asahi et al., 1975).

symptoms had improved a great deal.

A new standard for the severity grading of the skin symptoms of Yusho was thus proposed by Asahi (Asahi et al., 1975). The new standard was characterized by its quantitative nature (point count system). Table 7.2.3 shows the time course of the symptoms, using both the skin severity grading by Toshitani's criteria and Asahi's standard, of 13 cases examined from 1968 to 1974. Both sets of data were quite consistent in showing the improvement in the severity of skin symptoms during this period.

When the severity of skin symptoms in 1972 and 1975 in the same patients was

Table 7.2.4. Comparison of the Skin Severity Grading of 1972 and 1975

No change			Improvement			Exacerbation		
No.	1972	1975	No.	1972	1975	No.	1972	1975
4	I	I	4	I	0, I	1	I	I, II
1	III	III	4	I	0	1	I	II
2	IV	IV	1	II	I, II	1	II	III
			1	II	I	1	III	IV
			2	II	0			
			2	II, III	II			
			1	III	II, III			
			1	III	II			
			1	III, IV	III			
			2	III, IV	I, II			
			1	IV	III			
Total 7 cases (23%)			Total 20 cases (64%)			Total 4 cases (13%)		

No.: number of patients (Toshitani et al., 1977).

compared, it was obvious that the severity had definitely declined (Table 7.2.4). A total of 64% of the patients showed improvement, 23% had no change, and 13% exhibited an exacerbation of symptoms during the period.

iii) Changes in the skin symptoms between 10 and 25 years after the outbreak of Yusho

In the initial period of poisoning, skin symptoms were the major manifestations of Yusho and other physical examinations or laboratory data could not identify the definite abnormalities of PCB poisoning at that time. However, various kinds of physical symptoms appeared and increased in spite of the fact that the skin eruptions improved during the chronic intoxication periods (5–10 years after the outbreak). These phenomena suggested that the severity of Yusho intoxication should be evaluated based on the total severity of symptoms, including physical, ophthalmological, dental as well as dermatological grading, when more than 5 years had passed after the initial PCB poisoning.

Table 7.2.5 shows the changes from 1976 to 1980 in the populations of patients as classified by a severity grading of skin symptoms, according to both Toshitani's grading and Asahi's standard. Both data showed the tendency of a gradual improvement of skin symptoms during this period. The patients with a score of 0 or 1 increased every year, while those with a score of 6 or 7 decreased dramatically in 1980. The most severe cases, with a score of more than 8, did not apparently seem to change.

Table 7.2.5. Changes in the Number of Yusho Patients Classified by the Severity Grading and the Scoring of Skin Symptoms

Severity grades	1976	1977	1978	1979	1980
0	25	30	42	38	56
0, I	12	10	17	9	15
I	15	13	16	12	7
I, II	4	7	3	5	1
II	10	12	9	15	13
II, III	14	11	15	10	6
III	6	14	20	17	7
III, IV	1	3	0	2	1
IV	3	2	1	2	1
Total	90	102	123	110	107

Severity scores	1976	1977	1978	1979	1980
0-1	32	32	43	44	63
2-3	24	35	43	29	25
4-5	20	22	13	15	14
6-7	10	7	14	13	2
8-9	3	5	7	6	2
10–13	1	0	2	1	1
14–	1	2	1	2	0
Total	91	103	124	110	107

(Asahi et al., 1981)

Table 7.2.6 shows the changes from 1981 to 1992 in the populations of patients, also classified by the severity grading of skin symptoms. The skin symptoms improved remarkably during the period (Fig. 7.2.10; see color frontispiece). The most severe cases also dramatically improved during the period. The population of the patients with a skin symptom severity score of less than 3 points was 83.4% in 1985, and eventually surpassed 90% by 1992.

7.2.3. Relationship between Skin Symptom Grading and Blood PCBs of Yusho Patients

The analysis of PCBs in the sera of Yusho patients has been carried out at the facilities of a collaborative Yusho research team organized by Kyushu University since 1973. Masuda (Masuda et al., 1974) initially developed the method for detecting and quantifying PCBs in the blood of Yusho patients by gas chromatography and found that there were some characteristic patterns of gas chromatograms in the Yusho patients. They classified the patterns into three types labeled: A, B,

Table 7.2.6. Changes in the Severity Scoring of Skin Symptoms from 1981 to 1992

Score	1981 No. (%)	1983 No. (%)	1985 No. (%)	1987 No. (%)	1990 No. (%)	1992 No. (%)
0-1	52 (48.6)	63 (52.5)	88 (56.4)	62 (63.3)	66 (72.5)	44 (53.0)
2-3	33 (30.8)	27 (22.5)	42 (26.9)	28 (28.6)	20 (22.0)	32 (38.6)
4-5	12 (11.2)	11 (9.2)	20 (12.8)	4 (4.1)	4 (4.4)	6 (7.2)
6-7	7 (6.5)	10 (8.3)	5 (3.2)	2 (2.0)	0	0
8-9	2 (1.9)	4 (3.3)	1 (0.6)	0	1 (1.1)	1 (1.2)
10–13	0	5 (4.2)	0	2 (2.0)	0	0
14–	1 (0.9)	0	0	0	0	0
Total	107	120	156	89	91	83
Mean	2.15	2.36	1.58	1.37	0.96	1.47

No.: number of cases (Nakayama et al., 1993).

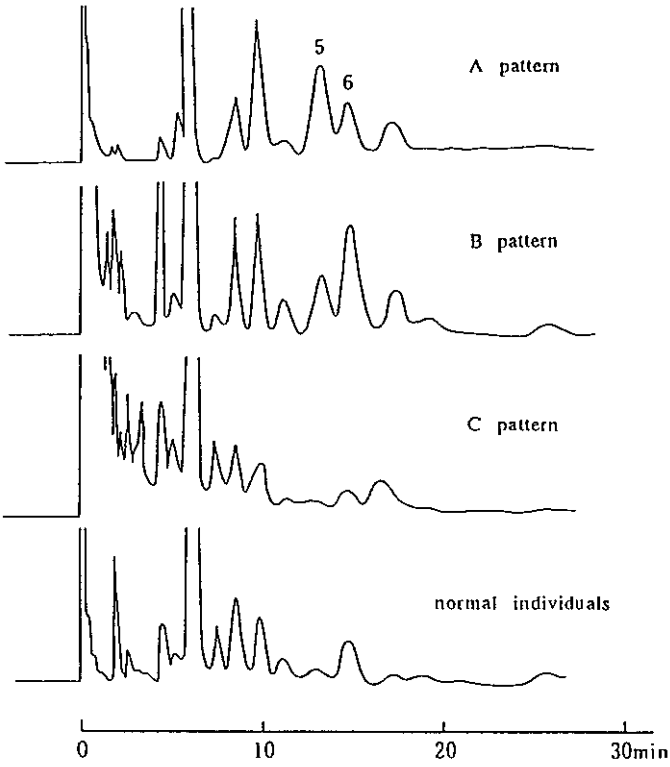


Fig. 7.2.11. Three PCB Patterns of Gas Chromatogram for the Blood of Patients with Yusho and Normal Individuals. A pattern: the typical Yusho pattern; B pattern: an intermediate pattern of A and C; C pattern: a non-Yusho pattern.

Table 7.2.7. PCB Patterns and Concentrations for Yusho Patients and Others in 1973

Group	Pattern	PCB concentrations (ppb)															Total number (%)	Average conc. (ppb)
		1	2	3	4	5	6	7	8	9	11	12	13	17	18	26		
Yusho patients	A		1	7	8	2	5	6	5	2	1		2	1	2	1	43 (59.7)	7.2 ± 4.9
	B	1	5	9	5	1	1	1			1	1	1				26 (36.1)	4.3 ± 3.1
	C	1	2														3 (4.2)	1.7 ± 0.2
	A + B + C	2	8	16	13	3	6	7	5	2	2	1	3	1	2	1	72 (100)	5.9 ± 4.5
Others	C	2	4	3													9	2.1 ± 0.8

(Koda and Masuda, 1975)

Table 7.2.8. Changes in the PCB Patterns and Concentrations from 1973 to 1978

Pattern			Number of cases	Pattern	PCB concentration	
1973		1978			1973	1978
A	→	A	26	A	(ppb) 10.7	(ppb) 7.6
	→	B	1			
	→	C	0			
B	→	A	10	B	6.4	2.6
	→	B	2			
	→	C	2			
C	→	A	1	C	4.9	2.7
	→	B	3			
	→	C	8			

(Asahi et al., 1979)

and C (Fig. 7.2.11). A is the typical Yusho pattern, C is a non-Yusho pattern which can be obtained from normal individuals, and B is an intermediate pattern between A and C.

Koda and Masuda (Koda and Masuda, 1975) analyzed the relationship between the blood PCB patterns or concentrations and the severity grading of skin symptoms and reported it in 1973 (5 years after the outbreak of Yusho) (Table 7.2.7). The results showed that the mean blood PCB level of 72 patients was 5.9 ± 4.5 S.D. ppb. The mean PCB level of the type A group (43 patients) was 7.2 ± 4.9 S.D. ppb, while that of the type B group (26 patients) was 4.3 ± 3.1 S.D. ppb, and that of the type C group (3 patients) was 1.7 ± 0.2 S.D. ppb. Clinically, patients with severe skin symptoms mostly belonged to the type A group. The patients who belonged to type C hardly showed any skin symptoms. General symptoms such as fatigue and headache were noticed in the patients with either type A or B blood PCB patterns. The same type of PCB pattern was observed in all family members for 15 families out of 21.

The blood PCB pattern in each patient remained stable when the data from 1973 to 1978 for the same patient were compared. Thus, the PCB patterns hardly changed from A to B or C even when the skin symptoms clearly improved during those periods. In contrast, the blood PCB concentrations were found to decrease with time in all three PCB patterns (Table 7.2.8). The severity grading of skin symptoms showed a gradual improvement from 1969 to 1973, and thereafter demonstrated a rapid improvement from 1973 to 1978, although the blood PCB patterns did not change in the individual patients during those periods. The above results indicate that the severely intoxicated patients (grades III or IV) showed mostly the A pattern, and the number of patients belonging to the A pattern did not change over time, even though there was considerable improvement in the skin symptoms.

The severity grading of skin symptoms according to Toshitani's criteria and Asahi's standard, and blood PCB patterns and concentrations in the sera of 20 patients are shown in Table 7.2.9. The mean PCB concentration of patients in 1977–1978 was 5.76 ppb, and thereafter decreased to 4.94 ppb in 1986–1987, and to 4.72 ppb in 1991–1992. These results indicate that the blood PCBs were excreted gradually but the excretion rate was extremely low when the patients were in the chronic intoxication periods. The severity of skin symptoms clearly demonstrated an improvement in all patients 25 years after the outbreak, although active acneiform eruptions or atheroma-like cysts were still seen in a very small number of patients. The mean scores for the severity of skin symptoms in these 20 patients were 3.00 in 1977–1978, 2.66 in 1981–1982, 1.00 in 1986–1987, 1.12 in 1989–1990, and 1.08 in 1991–1992. Thus, it was found that a remarkable decrease in the scores was seen after 1986–1987 (Fig.7.2.12).

Treatment

The essential therapeutic modality for Yusho patients is the effective excretion of PCBs, but so far no effective treatment modality has yet been established. The skin symptoms have improved naturally and gradually but some surgical treatment was necessary in the initial period after the outbreak of Yusho. In the early period, glutathione, xyritol, vitamin B_2 and E, and estrogen, etc., were administered but they did not prove to be satisfactory. The topical application of vitamin A acid dissolved in 95% ethyl alcohol and propyrene glycol was reported to have some beneficial effect on acne-like eruptions, although it also showed some irritation to the skin (Toshitani and Kitamura, 1971). The various surgical treatments included a resection of the infected cysts or abscesses, planing of the facial scar formations, nail dissection of ingrown nails, or the scraping of hyperkeratotic clavus or tylosis. Finally, treatment with either vitamin C or glutathione did not induce any improve-

Table 7.2.9. Clinical Course of Dermatological Symptoms from 1977 to 1992

Case	1977–1978		1981–1982		1986–1987		1989–1990		1991–1992	
1	III[a]·A8[b]·1[c]	III·A10·1	—	III·A7·2	—	III·A10·3	0·A6.2·0	—	II·A6.4·1	0·A6.5·0
2	—	I·A16·4	—	III·A9·4	0, I·A7·1	—	—	I, II·A7.9·2	II·A9.2·2	I, III·A6.1·4
3	III·A15·6	III·A9·5	II, III·A17·8	III·A16·9	0·A14·0	III·A11·2	III·A15.7·3	II, III·A11.3·4	II, III·A11.3·2	I·A12.4·2
4	II, III·C3·3	II, III·C2·4	III·C3·6	II, III·C4·4	I, II·C4·3	I·C3·2	II·C4.3·2	III·C4.3·2	0·C4.5·1	0·C3.5·0
5	0, I·B3·3	—	III·C3·2	II·C4·1	I, II·C3·1	0·B2·0	0·C3.1·1	0·C2.4·0	—	II, III·C2.0·2
6	I·A7·2	I·A6·2	I·A7·2	0, I·B5·1	0, I·B7·1	0, I·A6·1	0, I·B7.4·1	0·B6.4·0	0·A7.2·1	0, I·A7.3·2
7	0·C2·1	0·C5·1	0·B5·0	—	0·C2·0	0·C2.7·0	0·C1.9·0	0, I·C1.5·1	0·C1.7·0	0·C1.7·0
8	—	0, I·A3·2	0·B5·0	—	—	0·A4.1·0	0·A3.1·0	0·A2.5·0	II·A2.8·1	0·B2.7·1
9	I, II·A2·4	0·BC1·3	III·C1·5	—	II·C1·3	I·C1·2	II, III·C1.8·2	—	0·C1.7·0	I·C1.4·3
10	0, I·A6·2	—	I·A9·2	0·A5·1	—	0·A7·0	II·A6.0·1	0·A3.3·0	0·A3.5·0	I·A3.7·1
11	0, I·A5·2	I·A5·2	I·A4·2	0, I·B7·1	0·A3·0	0·A4·0	0·B6.4·0	0·B7.4·0	0·B5.7·0	0·A2.4·1
12	III·A13·9	III·A18·6	III·A10·7	—	III·A7·4	III·A7·3	II, III·A12.6·4	III·A14.4·4	II, III·A13.4·1	I, II·A10.1·2
13	I·B3·2	0, I·B3·1	—	0·B3·0	0·B4·0	II·B3·2	0, I·B2.9·1	II·B3.1·2	—	0·B1.6·1
14	I·A6·6	0, I·A6·2	I·A6·2	0, I·A4·4	I·A5·2	0, I·A6.7·1	—	I, II·A3.0·2	I·A2.9·1	0·A3.1·1
15	0·BC4·1	—	0, I·BC3·1	—	0·C4·0	I·B3·1	0·B1.8·0	0·C2.2·0	I, II·BC1.8·2	—
16	—	II·C3·1	—	0·C2·0	II·C1.7·2	—	II·C1.0·1	—	II·C2.3·1	0·C1.2·2
17	I·C3·2	II, III·C3·5	III·C3·5	—	0, I·C3·1	0·C3.8·0	I, II·C2.9·3	0·C1.9·0	0·C3.4·0	0·C3.9·0
18	0, I·A5·3	I·A4·2	I·A8·3	I·A7·2	0·A5·0	0·A6.5·0	0·A6.9·0	0·A4.0·0	0·A4.6·0	0·A9.2·1
19	III, IV·C2·5	II, III·C2·2	—	0·C2·2	0·C2.5·0	0·C3.0·0	—	II, III·C1.5·2	I, II·C2.9·2	II, III·C2.1·3
20	I, II·A8·2	0·A5·0	0·A5·0	0·A5·1	0·A5·0	0·A11·0	0·A6.9·0	0·B10.6·0	0·A6.6·0	0·A6.7·0

[a]: Skin severity grading.

[b]: PCB pattern·concentration (ppb).

[c]: Skin severity scoring.

(Nakayama et al., 1993)

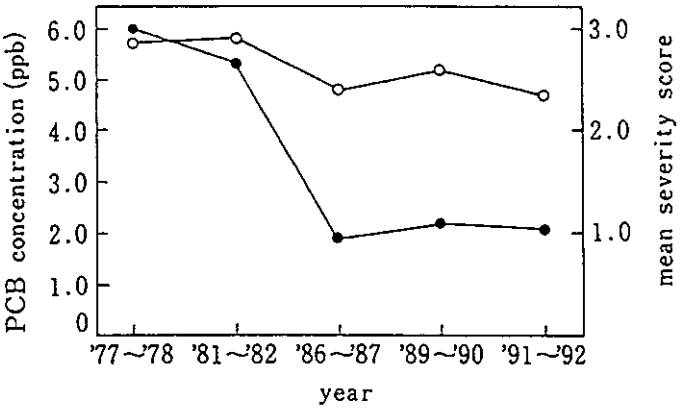


Fig. 7.2.12. Changes in the PCB Concentrations in the Sera and the Mean Severity Scoring of Skin Symptoms of Yusho Patients Observed over a Long Time Period (1977–1992).
○——○: PCB concentrations (ppb);●——● : the mean severity score of skin symptoms.

ment in the pigmented skin lesions.

References

Asahi, M., Koda, H., Toshitani, S. (1975) Alteration in skin severity grading of Yusho in the general examination in 1973 and 1974, and presentation of a new standard for the skin severity of Yusho by point count system. Fukuoka Acta Med. 66, 629–634 (in Japanese).

Asahi, M., Koda, H., Urabe, H., et al. (1979) Dermatological symptoms of Yusho. Alterations in this decade. Fukuoka Acta Med. 70, 172–180 (in Japanese).

Asahi, M., Toshitani, S., Hino, Y., et al. (1981) Dermatological findings and their analyses in the general examination of Yusho in 1976–1981. 72, 223–229 (in Japanese).

Goto, M., Higuchi, K. (1969) The symptomatology of Yusho (chlorobiphenyls poisoning) in dermatology. Fukuoka Acta Med. 60, 409–431 (in Japanese).

Koda, H., Asahi, M., Toshitani, S. (1974) Dermatological findings of the patients with Yusho (PCB poisoning) in a general examination in 1972. Fukuoka Acta Med. 65, 81–83 (in Japanese).

Koda, H., Masuda, Y. (1975) Relation between PCB level in the blood and clinical symptoms of Yusho patients. Fukuoka Acta Med. 66, 624–628 (in Japanese).

Masuda, Y., Kagawa, N., Shimamura, K., et al. (1974) Polychlorinated biphenyls in the blood of Yusho patients and ordinary persons. Fukuoka Acta Med. 65, 25–27 (in Japanese).

Nakayama, J., Hori, Y., Toshitani, S., et al. (1993) Dermatological findings in the annual examination of patients with Yusho in 1991–1992. Fukuoka Acta Med. 70, 172–180 (in Japanese).

Toshitani, S., Asahi, M., Koda, H. (1977) Alteration in dermatological findings of patients with Yusho (PCB poisoning) in the general examination in 1975. Fukuoka Acta Med. 68, 152–155 (in Japanese).

Toshitani, S., Kitamura, K. (1971) Clinical observation on Yusho (Chlorobiphenyl poisoning), especially further study of its dermatological findings. Fukuoka Acta Med. 62, 132–138 (in Japanese).

7.3. Respiratory and Immunologic Aspects of Yusho

Yoichi Nakanishi

One of the target organs of PCBs and their related compounds is the respiratory system including bronchiolar Clara cells. These chemicals have been detected in the respiratory system of patients with Yusho, and their administration to experimental animals is known to induce both pathologic and functional changes in the respiratory system (Bergman et al., 1979; Lund et al., 1985; Brandt et al., 1985).

Clinical observations of the symptoms and time course of Yusho suggest that the respiratory symptom is pathognomonic for Yusho for a long period of time (Hirota et al., 1991). The major clinical findings of respiratory involvement in Yusho patients include airway symptoms such as coughing, the production of sputum, and wheezing, and secondary airway infections are not rare (Shigematsu et al., 1978). The severity of the respiratory symptoms and the frequency of airway infections correlate with the concentration of PCBs in the patient's blood (Shigematsu et al., 1977). Immunologic disorders are also thought to be related to such poisoning. We herein describe both the clinical and experimental aspects of the respiratory and immunologic involvement in patients with Yusho.

7.3.1. Respiratory Symptoms in Yusho Patients

The first detailed surveillance of respiratory symptoms in Yusho patients began in September 1969, 1 year after an awareness of clinical manifestations other than those involving the respiratory system had developed. Initially coughing, expectoration and wheezing were the major identifiable symptoms. The former two appeared with skin eruptions, while the latter ensued several months later (Shigematsu et al., 1978). Thirty-eight percent of 203 patients medically examined complained of various amounts of sputum expectoration and clinical symptoms that were similar to those observed in the patients with chronic bronchitis (Shigematsu et al., 1971). In 1977, Shigematsu et al. reported additional data on sputum expectoration among 289 patients with Yusho, which was seen in 76 (68%) of 112 smoking patients and in 43 (24%) of 177 non-smoking patients. Although clinical backgrounds regarding age, gender, and accompanying diseases were not considered in the analysis, it was noteworthy that the observed respiratory symptoms persisted in both smokers and non-smokers for a long time. A follow-up study of 79 patients with Yusho revealed that the respiratory symptoms improved gradually for the first 10 years but thereafter changed little over the next 5 years (10 to 15 years following onset) in most cases (Nakanishi et al., 1985a).

Table 7.3.1. Results of Pulmonary Function Tests in 12 Non-smoking Patients[a] with Reticulolinear Shadows Approximately 1 Year Following the Onset of Respiratory Distress and Thereafter

Measurement		1970	1973–1974	1983
VC/predicted VC (%)	≧ 100	5	6	
	> 90	1	5	
	> 80		1	
FEV_1/FVC (%)	≧ 80	3	9	
	> 75	3	3	
$\dot{V}_{max\,50}$ (L/sec)	≧ 4.0		6	2
	> 2.0		6	4
$\dot{V}_{max\,25}$ (L/sec)	≧ 1.5		5	2
	≧ 1.0		5	3
	≧ 0.7		2	1
PaO_2 (mmHg)	≧ 85	3	4	5
	> 70	2	7	1
	> 60	1	1	0

[a]: Ages of patients ranged from 30 to 49 years at the first medical examination.

The sputum samples expectorated by these Yusho patients were described as *starch-like*, white, mucous, and in some cases muco-purulent. Shigematsu et al. (1971) observed increased neutrophils in the sputa of nearly 40% of the patients, which suggested a high incidence of airway infections.

Wheezing was another prominent respiratory symptom. Shigematsu et al. (1978) reported that mild wheezing was audible in 2% of 401 patients with Yusho. Hirota et al. (1991) analyzed the data from a nation-wide medical examination of patients performed in 1988 (20 years following the outbreak of Yusho), and reported that abnormal breath sounds which could still be ausculated were significantly positively correlated with the serum PCB concentration.

In Taiwan, a similar poisoning by PCBs and related compounds (Yu-Cheng) occurred (Lü et al., 1985) in which a cough was observed in 50 (14.2%) of 358 patients, although no expectoration of sputum was reported.

7.3.2. Chest Roentgenogram and Laboratory Findings

On chest roentgenograms, 35% of the Yusho patients revealed reticulo-nodular densities, upon which acinar, patchy, or atelectatic shadows were superimposed in about 10%. However, these roentgenographic findings did not correlate well with the severity of the dermal eruptions (Shigematsu et al., 1974). The results of pulmonary function tests in 12 non-smoking patients are presented in Table 7.3.1. The vital capacity and the FEV_1/FVC ratio were almost normal, but the arterial oxygen tension (P_{O_2}) decreased in eight patients. The maximal expiratory flow at 50% and

25% of vital capacity ($V_{max\ 50}$ and $V_{max\ 25}$) demonstrated a mild decrease in about half of these patients; in the latter, both inspiratory and expiratory rhonchi were audible at all times from 1970 to 1974 (Shigematsu et al., 1978). The same tests were repeated in 1983, noting a slight improvement with no audible rhonchi in these patients (Nakanishi et al., 1985b).

The respiratory effects of occupational exposures to PCBs containing no PCDFs have been reported in US capacitor workers (Warshaw et al., 1979) and transformer repair workers (Emmett et al., 1988a, 1988b). Although wheezing, coughing and respiratory dysfunction were noted in these workers, the collective evidence demonstrated that chronic PCB exposure was not associated with either pulmonary dysfunction or respiratory diseases (James et al., 1993).

7.3.3. PCBs in Sputum and Their Distribution in the Respiratory System

Kojima (1971) first reported the concentration of PCBs in the sputa of patients with Yusho. Shigematsu et al. (1978) serially measured the concentrations of PCBs in sputum samples collected from December 1969 to July 1970. Definite PCB peaks were always detected in the sputa collected before May 1970, but they were lower in those samples obtained thereafter. The concentrations of PCBs in sputa collected after 1975 were similar to those collected after June 1970, and ranged from one tenth to one third of those in blood. Haraguchi et al. (1984) identified more than 60 different methylsulfonyl-PCBs in the lungs of Yusho patients. These sulfur-containing PCB metabolites were also identified in animal studies, which indicated that they arise during the enterohepatic circulation of PCB-glutathione conjugates (Mio et al., 1976; Jansen and Jansson, 1976; Bakke et al., 1982; Bakke and Gustafsson, 1984).

In rats, the distribution of KC-400 administered orally was the highest in the skin, adipose tissue, and liver, followed by the plasma, but it was relatively low in the lung (Yoshimura, 1971). The distribution of PCBs in animals has been extensively studied by Brandt et al. (1975, 1976, 1985), and PCBs were found to accumulate in the bronchial mucosa of mice, thus demonstrating a dose-dependence and structural requirements.

Radioactively labeled PCB methyl sulfone has been shown to accumulate in the cytoplasm of nonciliated, bronchiolar (Clara) cells and in the airway lumens of rat and mouse lungs (Lund et al., 1985; Brandt et al., 1985). This selective *in vivo* deposition corresponds to the localization of a 13-kDa secretory protein of Clara cell origin which binds methylsulfonyl-PCBs with high affinity (Lund et al., 1985, 1988a). This protein has been purified from the rat lung (Lund et al., 1988b), and a physicochemically similar protein has also been characterized in human bronchoalveolar lavage fluid (Lund et al., 1986). The cDNA of the PCB-binding protein

has been cloned and it was revealed that it shares 53% of the positions of amino acids with uteroglobin, a progesteron-binding protein found in the rabbit uterus and lung (Nordlund-Moller et al., 1990).

The fact that 1) PCBs are detected in sputa of Yusho patients, 2) they accumulate in the airway, including the bronchiolar Clara cells, and 3) the PCB-binding protein is localized in Clara cells, is thus considered to provide additional evidence that the respiratory system is one of the major target organs of PCBs.

7.3.4. Pathologic Changes in the Respiratory System

The pathologic findings in the respiratory systems of seven autopsied patients with Yusho consisted primarily of macrophage infiltration and focal atelectasis in the alveolar spaces (Shigematsu et al., 1974). Peribronchiolar changes were also seen which may have been due to either PCB poisoning or associated infections (Shigematsu et al., 1978). Focal hemorrhages and/or pulmonary edema, and pleural and pericardial effusions or adhesions were observed in three patients who died within 4 to 12 months following the onset of poisoning (Kikuchi et al., 1971; Kikuchi, 1972). Marked hyperemia, atelectasis, and alveolar hemorrhages were also noted in a stillborn baby (Kikuchi et al., 1969).

Peribronchial and peribronchiolar cell infiltrations were observed in the lungs of rats administered PCBs (Shigematsu et al., 1978). Electron microscopic studies have revealed large lipid vacuoles and altered lamellar bodies or lysosomes in both type II alveolar cells and alveolar macrophages (Shigematsu et al., 1978). Necrosis of the Clara cells, mild pulmonary edema, and vascular congestion were seen in the lungs of rats administered PCDFs, which are recognized as being the major causative agents of Yusho (Fig. 7.3.1; Nakanishi et al., 1985b). These changes were more severe in the lungs of rats administered PCDFs than in those given a ten times larger dose of PCBs (Nakanishi et al., 1985a). A furan derivative (4-ipomeanol) produced a characteristic lesion in several species of laboratory animals which was identified as being necrosis of the non-ciliated bronchiolar (Clara) cells. Relatively late occurring manifestations of lung toxicity following the administration of comparatively large doses of furan derivatives include pulmonary edema, pleural effusions, vascular congestion and hemorrhages (Boyd, 1982). There are some similarities between the pathologic changes seen in the lungs of Yusho patients and those observed in experimental animal models, although the lesions of Clara cells were more marked in animals.

7.3.5. Respiratory Tract Infections and Immunologic Disorders in Yusho

As mentioned above, some patients expectorated mucopurulent sputa with an increased number of neutrophils. A serial bacteriologic examination of the sputa in

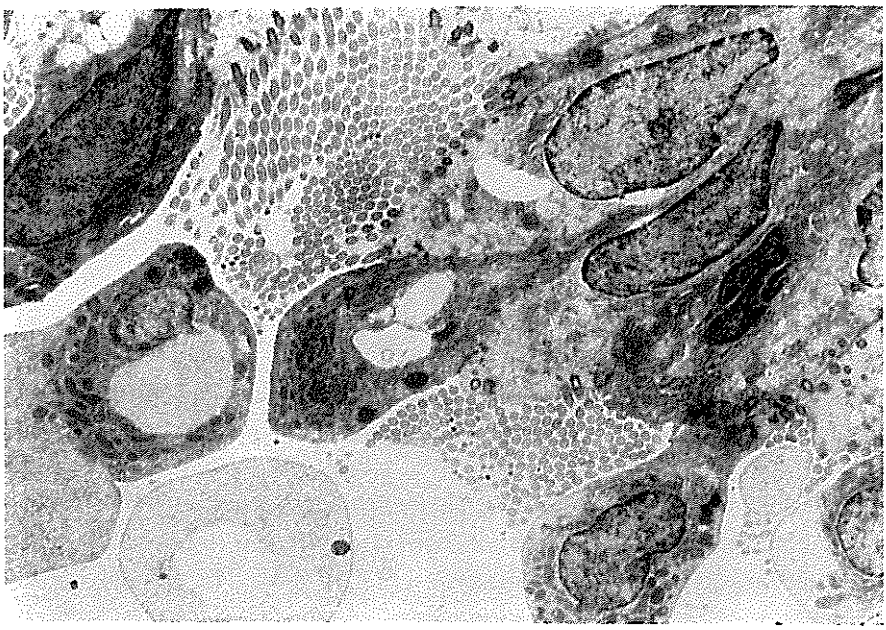


Fig. 7.3.1. An Ultrastructural View of the Rat Lungs That Received PCDFs for 2 Weeks.
Both degeneration and necrosis of the Clara cells can be seen (× 2600).

12 non-smoking patients with respiratory distress for longer than 2 years revealed the presence of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas*, and/or *Hemophilus*. In patients with high concentrations of PCBs in their sputa, these bacteria were found persistently (Shigematsu et al., 1971). Chronic airway infections were present in approximately half of the patients with respiratory distress and often were exacerbated by either viral or bacterial infections. Respiratory tract infections are also known to be frequently seen in Taiwanese Yu-Cheng patients (Lü et al., 1985).

Alterations in the defense mechanisms of the systemic immune system has also been suggested. When the immunoglobulin concentrations in Yusho patients were first determined in 1970, 2 years following the outbreak of Yusho, the serum IgA and IgM concentrations had decreased, while the IgG concentrations increased. The concentrations returned to normal by 1972, except for three patients whose IgA concentrations remained low. Although no statistically significant relationship between the IgA concentrations and clinical symptoms was detected, IgA concentrations of less than 100 mg/ml were found in five of 29 patients with respiratory symptoms, but in none of 24 asymptomatic patients. The IgM concentrations significantly decreased in patients with severe dermatologic symptoms, while the IgA

Table 7.3.2. Serial Serum Immunoglobulin Concentrations in Adult Yusho Patients

Date of Examinations		Case	IgG[a]	IgM[a]	IgA[a]
March–June	1970	28	1,655 ± 414*	127 ± 57⁺	151 ± 77⁺
July–October	1970	27	1,843 ± 628*	186 ± 92	286 ± 100
January–March	1971	9	1,516 ± 471*	225 ± 103*	253 ± 149
June–August	1971	13	1,571 ± 341*	185 ± 65	203 ± 64
October–December	1971	24	1,586 ± 604*	172 ± 88	232 ± 70
January–March	1972	29	1,340 ± 447	166 ± 91	206 ± 97
November–December	1980	15	1,307 ± 239	153 ± 61*	199 ± 72
Control		57	1,243 ± 329	170 ± 54	207 ± 96

[a]: Each value represents Mean ± SD (mg/dl).

*Significant increase ($p < 0.01$) compared to the control.

⁺Significant decrease ($p < 0.01$) compared to the control.

concentrations in the sputum were normal (Shigematsu et al., 1978). The immunoglobulin concentrations were examined again in 1980, and were all found to be in the normal range (Table 7.3.2; Nakanishi et al., 1985a). The changes in the humoral immune response in Japanese and Taiwanese patients were almost the same; that is, the serum concentrations of IgA and IgM decreased in the patients of both countries approximately 2 years following disease onset, but returned to normal after 3 years (Lü et al., 1985).

In terms of the cellular immune system, a low CD4+/8+ cell ratio (i.e. a low helper/suppressor T-cell ratio) and an enhanced responsiveness to a non-specific mitogen-PHA were reported in Taiwanese patients suffering from PCB poisoning 3 years following its onset (Lü et al., 1985). On the other hand, a high CD4+/8+ cell ratio and a lowered responsiveness to PHA were reported in Japanese Yusho patients 14 years following disease onset (Nakanishi et al., 1985a). A suppression of the delayed type of skin response to streptokinase and streptodornase was also reported in the Taiwanese patients (Chang et al., 1982).

The immunologic disorders observed in these patients are supported by some animal experiments (Vos et al., 1972; Street et al., 1975; Loose et al., 1977; Kanegae et al, 1987), which demonstrated both humoral and cellular immunity impairment caused by exposure to PCBs and/or PCDFs. Although these studies may explain the frequent episodes of airway infections seen in Yusho patients, no definitive conclusion can yet be made in this regard, because no detailed epidemiologic data regarding their chronic airway infections are yet available. In addition, it has recently been suggested that immune disorders in the target organs of these chemicals do not always reflect the systemic immune status from the analysis of T-cell surface markers in bronchoalveolar lavage (Nakanishi et al., 1995). Further studies are thus still needed to clarify the relationship between air-

way infection and the immune status of the respiratory system caused by these chemicals.

7.3.6. Lung Carcinogenesis and Yusho

Kuratsune et al. performed a cohort study on the mortality of Yusho patients (1987). In an analysis of 120 deaths seen among 1,761 patients, a significantly increased mortality from cancer at all sites was observed in males. Mortality from cancer of the lung as well as the liver significantly increased in males but not in females. Such increased mortality may be associated with this poisoning, but according to the authors, it is still too early to draw any conclusions because of the lack of adequate epidemiologic data necessary for a more detailed analysis.

In animal experimental models, PCBs have been reported to have tumor-promoting effects on N-nitrosodimethylamine-initiated lung and liver tumors (Anderson et al., 1986). The PCBs may act as tumor promoters by activating cytochrome P450 IA1 in the lung (Anderson et al., 1991). Boyd et al. (1984) described that a furan compound, 4-ipomeanol, may be a factor critical to the progenitor role of the Clara cells in chemically-induced bronchogenic carcinoma. Although PCDFs themselves do not initiate carcinogenesis, they do act as tumor promoters in 20-methyl-cholanthrene-induced skin tumors (Hirose et al., 1989). However, the effects of PCDFs on lung carcinogenesis have not yet been elucidated.

Thus, the roles of PCBs and PCDFs in human lung carcinogenesis remain unclear. However, a potential causal relationship should not be overlooked in view of both the high risk of lung and liver cancer seen in male Yusho patients as well as the potency of these compounds as a tumor-promoter in animal models.

References

Anderson, L. M., Ward, J. M., Fox, S. D., et al. (1986) Effects of a single dose of polychlorinated biphenyls to infant mice on N-nitrosodiethylamine-initiated lung and liver tumors. Int. J. Cancer 38, 109–116.

Anderson, L. M., Beebe, L. E., Fox, S. D., et al. (1991) Promotion of mouse lung tumors by bioaccumulated polychlorinated aromatic hydrocarbons. Exp. Lung Res. 17, 455–471.

Bakke, J., Gustafsson, J. -A. (1984) Mercapturic acid pathway metabolites of xenobiotics. Generation of potentially toxic metabolites during enterohepatic circulation. Trends Pharmacol. Sci. 5, 517–521.

Bakke, J. E., Bergman, A. L., Larsen, G. L. (1982) Metabolites of 2,4,5′-trichlorobiphenyl by the mercapturic acid pathway. Science 217, 645–647.

Bergman, A., Brandt, I., Darnerud, P. O., et al. (1982) Metabolism of 2,2′,5,5′-tetrachlorobiphenyl: formation of mono- and bis-methyl sulfone metabolites with a selective affinity for the lung and kidney tissues in mice. Xenobiotica 12, 1–7.

Boyd, M. R. (1982) Metabolic activation of pulmonary toxins. In: Witschi, H. and Nettesheim, P. eds., Mechanisms in Respiratory Toxicology, vol. II. CRC Press, Cleveland, 85–112.

Boyd, M. R., Reznik-Schuller, H. M. (1984) Metabolic basis for the pulmonary Clara cell as a target for pulmonary carcinogenesis. Toxicol. Pathol. 12, 56–61.

Brandt, I. (1975) The distribution of 2,2′,3,4,4′,6′ and 2,3,4,4′,5,6-hexachloro-biphenyl in mice studied by autoradiography. Toxicology 4, 275–287.

Brandt, I., Bergman, A., Wachtmeister, A. (1976) Distribution of poly-chlorinated biphenyls: Structural requirements for accumulation in the mouse bronchial mucosa. Experimentia 32, 497–498.

Brandt, I., Lund, J., Bergman, A., et al. (1985) Target cells for the polychlorinated biphenyl metabolite 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl in lung and kidney. Drug Metab. Dispos. 13, 490–496.

Chang, K. J., Hsieh, K. H., Tang, S. Y., et al. (1982) Immunologic evaluation of patients with polychlorinated biphenyl poisoning: evaluation of delayed-type skin hypersensitive response and its relation to clinical studies. J. Toxicol. Environ. Health 9, 217–223.

Emmett, E. A., Maroni, M., Schmith, J. M., et al. (1988a) Studies of transformer repair workers exposed to PCBs. I. Study design, PCB contaminations, questionnaire, and clinical examination results. Am. J. Ind. Med. 13, 415–427

Emmett, E. A., Maroni, M., Schmith, J. M., et al. (1988b) Studies of transformer repair workers exposed to PCBs. II. Results of clinical laboratory investigations. Am. J. Ind. Med. 14, 47–62.

Haraguchi, H., Kuroki, K., Masuda, Y., et al. (1984) Determination of methylthio and methylsulphone polychlorinated biphenyls in tissues of patients with "Yusho." Food Chem. Toxicol. 22, 283–288.

Hirose, R., Hori, M., Toyoshima, H., et al. (1989) Influences of polychlorinated dibenzofuran on experimental carcinogenesis in mice. Fukuoka Acta Med., 80, 246–254 (in Japanese).

Hirota, Y., Hirohata, T., Kataoka, K., et al. (1991) Associations between blood PCB level and symptoms of Yusho patients, twenty years after outbreak. Fukuoka Acta Med. 82, 335–341 (in Japanese).

James, R. C., Busch, H., Tamburro, C. H., et al., (1993) Polychlorinated biphenyl exposure and human disease. J. Occup. Med. 35, 136–148.

Jensen, S., and Jansson, B. (1976) Anthropogenic substances in seal from the Baltic. Methyl sulfone metabolites of PCB and DDE. Ambio 3, 257–260.

Kanegae, H., Sham, L., Kurita, Y., et al. (1987) Experimental studies on long-term influence of polychlorinated dibenzofurans to respiratory and immune status (preliminary study). Fukuoka Acta Med., 78, 219–222 (in Japanese).

Kikuchi, M., Mikagi, Y., Hashimoto, M., et al. (1971) Two autopsy cases of chronic chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 89–103 (in Japanese).

Kikuchi, M. (1972) An autopsy case of PCB poisoning with liver cirrhosis and liver cell carcinoma. Fukuoka Acta. Med. 63, 387–391 (in Japanese).

Kikuchi, M., Hashimoto, M., Hozumi, M., et al. (1969) An autopsy case of stillborn of chlorobiphenyls poisoning. Fukuoka Acta. Med. 60, 489–495 (in Japanese).

Kojima, T. (1971) Chrolobiphenyls in the sputa and tissues. Fukuoka Acta Med. 62, 25–29 (in Japanese).

Kuratsune, M., Nakamura, Y., Ikeda, M., et al. (1987) Analysis of deaths seen among patients with Yusho—A preliminary Report. Chemisphere 16, 2085–2088.

Kuratsune, M., Ikeda, M., Nakamura, Y., et al. (1988) A cohort study on mortality of "Yusho" patients: A preliminary report. In: Miller, R. W., Watanabe, S., Fraumeni, J. F., et al., eds., Unusual Occurrences as Clues to Cancer Etiology. Japan Sci. Soc. Press, Tokyo/Taylor & Francis, Ltd., London and Philadelphia, 61–66.

Loose, L. D., Pittman, K. A., Benitz, K. F., et al. (1977) Polychlorinated biphenyl and hexachlorobenzene humoral immunosuppression. J. Reticuloendothel. Soc. 22, 253–271.

Lü, Y.C. (1985) Clinical findings and immunological abnormalities in Yu-Cheng patients. Environ. Health Perspect. 59, 17–29.

Lund, J., Brandt, I., Poellinger, L., et al. (1985) Target cells for the polychlorinated-biphenyl metabolite 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlobiphenyl: characterization of high affinity binding in rat and mouse lung cytosol. Mol. Pharmacol. 27, 314–323.

Lund, J., Andersson, O., Ripe, E. (1986) Characterization of a binding protein for the PCB metabolite 4,4′-bis(methylsulfonyl)-2,2′,5,5′-tetrachlorobiphenyl present in bronchoalveolar lavage from healthy smokers and non-smokers. Toxicol. Appl. Pharmacol. 83, 486–493.

Lund, J., Devareux, T., Glaumann, H., et al. (1988a) Cellular and subcellular localization of a binding protein for polychlorinated biphenyls in rat lung. Drug Metab. Dispos. 16, 590–599.

Lund, J., Nordlund, L., Gustafsson, J. -A. (1988b) Partial purification of a binding protein for polychlorinated biphenyls from rat lung cytosol: physicochemical and immunochemical characterization. Biochemistry 27, 7895–7901.

Mio, T., Sumono, T., and Mizutani, T. (1976) Sulfur-containing metabolites of 2,2′,5,5′-tetrachlorobiphenyl, a major component of commercial PCBs. Chem. Pharm. Bull. 24, 1958–1960.

Nakanishi, Y., Shigematsu, N., Kurita, Y., et al. (1985a) Respiratory involvement and immune status in Yusho patients. Environ. Health Perspect. 59, 31–36.

Nakanishi, Y., Kurita, Y., Kanegae, H., et al. (1985b) Respiratory involvement and immune status in polychlorinated biphenyls and polychlorinated dibenzofurans poisoning. Fukuoka Acta Med. 76, 196–203 (in Japanese).

Nakanishi, Y., Nomoto, Y., Matsuki, A., et al. (1995) Effect of polychlorinated biphenyls and polychlorinated dibenzofurans on leukocyte in peripheral blood and bronchoalveolar lavage fluid. Fukuoka Acta Med. 86, 261–266.

Nordlund-Muller, L., Andersson, O., Ahlgren, R., et al. (1990) Cloning, structure, and expression of a rat binding protein for polychlorinated biphenyls. J. Biol. Chem. 265,12690–12693.

Shigematsu, N., Norimatsu, K., Ishibashi, T., et al. (1971) Clinical and experimental studies on respiratory involvement in chlorobiphenyls poisoning. Fukuoka Acta Med. 62,150–156 (in Japanese).

Shigematsu, N., Ishimaru, S., Hirose, T., et al. (1974) Clinical and experimental studies on respiratory involvement in PCB poisoning (2). Fukuoka Acta Med. 65, 88–95 (in Japanese).

Shigematsu, N., Ishimaru, S., Ikeda, T., et al. (1977) Further studies on respiratory disorders in polychlorinated biphenyls (PCB) poisoning—Relationship between respiratory disorders and PCB concentrations in blood and sputum. Fukuoka Acta Med. 68, 133–138 (in Japanese).

Shigematsu, N., Ishimaru, S., Saito, R. (1978) Respiratory involvement in polychlorinated biphenyls poisoning. Environ. Res. 16, 92–100.

Street, J. C., Sharma, R. P. (1975) Alteration of induced cellular and humoral immune responses by pesticides and chemicals of environmental concern. Toxicol. Appl. Pharmacol. 32, 587–602.

Vos, J. G., Dejoij, T. H. (1972) Immunosuppressive activity of a polychlorinated biphenyl preparation on the humoral immune response in guinea pigs. Toxicol. Appl. Pharmacol. 21, 549–555.

Warshaw, R., Fishbein, A., Thornton, J., et al. (1979) Decrease in vital capacity in PCB-exposed workers in a capacitor manufacturing facility. Ann. N. Y. Acad. Sci. 320, 277–283.

Yoshimura, H. (1971) Group of studies on the tissue distribution of PCB: Studies on the tissue distribution and the urinary and fetal excretion of ^{3}H-Kanechlor (chlorobiphenyls) in rats. Fukuoka Acta Med. 62, 12–19 (in Japanese).

7.4. Ophthalmological Aspects of Yusho

YOSHITAKA OHNISHI and *TOSHIHIKO* KOHNO

7.4.1. Ocular Findings in Yusho Patients

Ocular symptoms in Yusho were manifested as an increase in conjunctival discharge, edema of the upper eyelid, visual disturbances and ocular pain. The earliest symptoms in patients appeared at least one month after exposure to the compounds.

The ocular signs of the disease within one year after an accumulated ingestion included the hypersecretion of the meibomian gland (52.6%), an abnormal pigmentation of the bulbal conjunctiva (48.2%), an unusual pigmentation of the limbal conjunctiva (45.2%), a pigmentation of the tarsal conjunctiva (29.4%) and edema of the eyelid (15.8%) (Ikui et al., 1969). These ocular signs were closely related to the concentrations of PCBs and the gas-chromatographic patterns of PCBs in the patient's blood (Ohnishi and Yoshimura, 1977)

These ocular findings gradually decreased over the years. However, almost all patients continued to complain of an abnormal discharge from the eyes. Sixteen years after the onset of Yusho, 107 out of the 122 patients (88%) seen at the outpatient Yusho clinic in Kyushu University complained of such eye discharge. There was no edema of the upper eyelid in these patients. An abnormal pigmentation of the conjunctiva, cyst formation of the meibomian gland, and the secretion of a white, cheesy material from the ducts of the meibomian glands (Fig. 7.4.1., see the color frontispiece) were observed in 12%, 36% and 20% of the patients, respectively. The discharged materials from 22 patients were analyzed by gas chromatography, and PCBs were found to be present in the meibomian gland contents of 15 patients (Kohno et al., 1985).

The eyelid of one 25-year-old man who had demonstrated symptoms of Yusho for one year was examined at autopsy. The meibomian gland showed a dilation of the duct with an accumulation of keratin and atrophy of the alveoli with squamous metaplasia (Fig. 7.4.2). Pathological changes of the meibomian glands in Yusho patients were regarded as a process of keratinous cyst formation of the duct. These findings also corresponded to the clinical signs of hypersecretion of the meibomian gland (Yoshihara et al., 1979).

A similar incident of PCB poisoning occurred in Taiwan 11 years after the onset in Japan (Fu, 1983). Between October 1979 and March 1980, 117 patients were examined. The most common complaint was an increase in eye discharge, and this

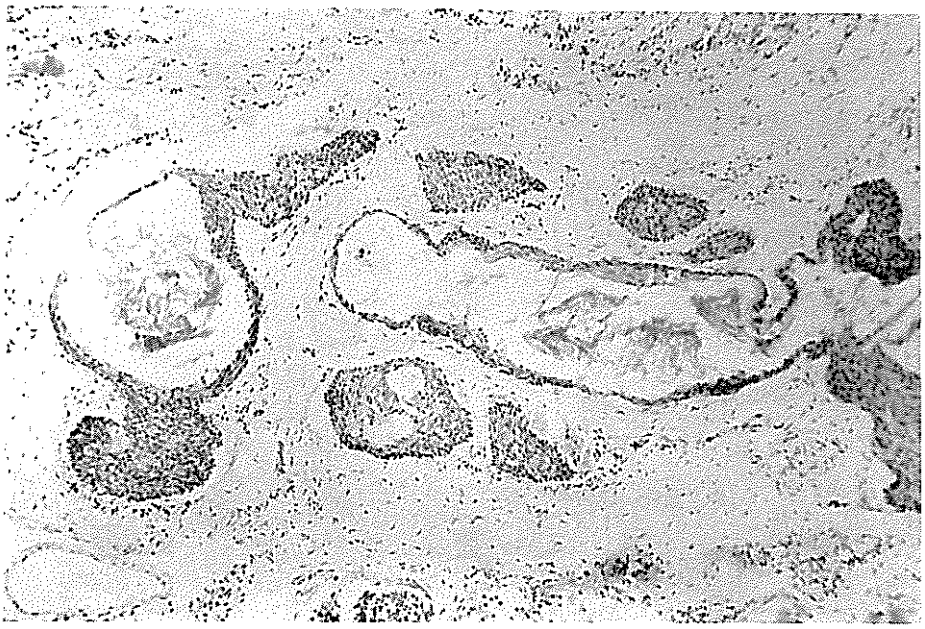


Fig. 7.4.2. A Light Micrograph of the Meibomian Gland in a 25-year-old Man
The acini of the meibomian glands are atrophic. HE staining. Original magnification × 25.

symptom was identified in 81% of the patients. Common eye findings included edematous swelling of the eyelids (59%), conjunctival pigmentation (67%) and the hypersecretion of the meibomian glands (70%). The frequency and severity of these findings were also closely related to PCB concentrations in the blood.

Pathological changes in the meibomian gland were clinically observed in chronic blepharitis. A dysfunction of the meibomian gland was diagnosed based on meibomian gland expression, meibography, tear osmolarity and Schirmer's test (Mathers et al., 1991).

7.4.2. Animal Studies

Allen and Norback (1973) reported that PCBs induced hyperplasia of the gastric mucosa with an extension of the gastric glands into the submucosa while forming submucosal cysts and squamous metaplasia with keratin cyst formation of the sebaceous glands in the skin of monkeys. Swollen eyelids and a purulent discharge from the eyes of the monkeys were apparent. Numerous experiments on small animals using PCBs have been done, but little attention has been directed to PCB toxicity of the eye.

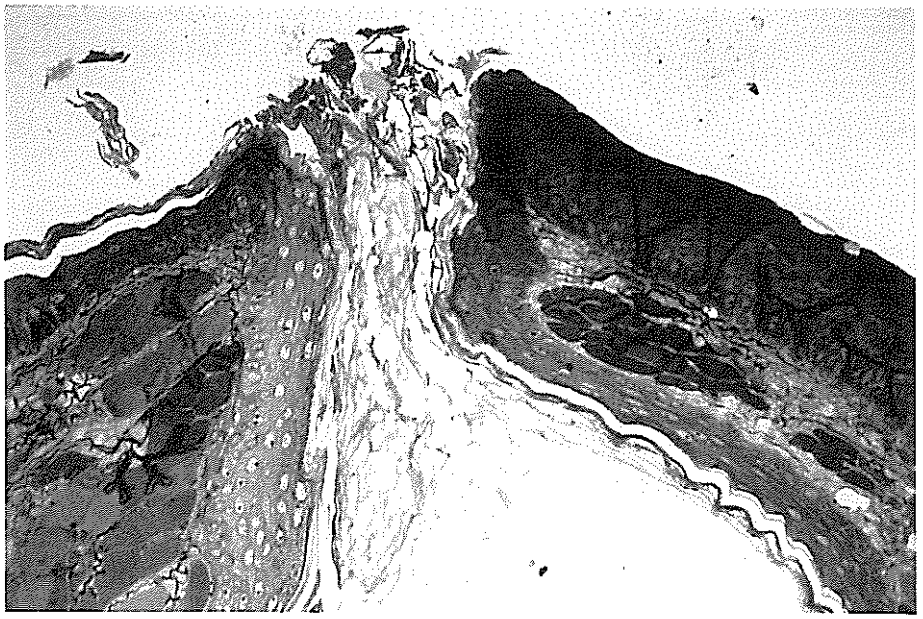


Fig. 7.4.5. A Light Micrograph of the Orfice of the Meibomian Gland
The orifice in a PCB-intoxicated monkey is filled with abnormal materials. Azur II staining. Original magnification × 64.

Ten years after the onset of PCB poisoning in Japan, the Yusho Study Group planned experiments on monkeys using Kanechlor 400, a commercial PCB, and polychlorinated dibenzofurans (PCDF) (Yoshihara et al., 1979; Ohnishi and Kohno,1979). One month after the ingestion of PCBs, a 17.3% reduction in body weight was observed in these monkeys, and within 3 months, 3 monkeys demonstrated hair loss on the head and upper extremities.

The same ocular signs seen in Yusho patients were also present in these animals (Fig. 7.4.3, see the color frontispiece). One month after PCB ingestion, there was little spontaneous discharge from the eyes, but when pressure was applied to the eyelids, white cheesy material was excreted. Histological studies showed the meibomian glands to be compressed by a keratin cyst while they had atrophied (Fig. 7.4.4, see the color frontispiece). Squamous metaplasia and the hyposecretion of the glands were also present. At this time the acini in the glands were absent. On the other hand, the epithelial cells of the ducts of meibomian glands were hyperplastic. In normal ducts there are 4 to 5 layers of stratified squamous epithelial cells, but in the duct of the experimental animals 7 to 10 cell layers were present. In the basal layer of the epithelium, there were numerous mitotic cells, while in the superficial layer irregular shaped keratohyaline granules were present

in the cytoplasm. The lumen of the duct was enlarged and filled with keratinized cells. The orifice of the duct was also enlarged (Fig. 7.4.5). Within several months after the oral administration of PCB, either with or without PCDFs, typical swelling of meibomian glands and edema of eyelids became apparent. The retina and choroid were normal. The meibomian glands in transparent specimens were macroscopically visible after treatment with KOH and Sudan III (Fig. 7.4.6., see the color frontispiece). A partial disappearance of the glands and an enlargement of the ducts were both evident in the PCB-intoxicated animals (Ohnishi et al., 1983) (Fig. 7.4.7., see the color frontispiece).

References

Allen, J. R., Norback, D. H. (1973) Polychlorinated biphenyl- and Triphenyl-induced gastric mucosal hyperplasia in primates. Science 179, 498.

Fu, Y. A. (1983) Ocular manifestation of polychlorinated biphenyl (PCB) intoxication. Arch. Ophthalmol. 101, 379–381.

Higuchi, K. (1976) PCB poisoning and pollution. Kodansha Ltd., Tokyo and Academic Press, New York.

Ikui, H., Sugi, K., Uga, S. (1969) Ocular signs of chronic chlorobiphenyls poisoning ("Yusho"). Fukuoka Acta Med. 60, 432–439 (in Japanese).

Jensen, S., Johnels, A. G., Olsson, M., et al. (1969) DDT and PCB in marine animals from Swedish waters. Nature 224, 247–250.

Kohno, T., Ohnishi, Y., Hironaka, H. (1985) Ocular manifestations and polychlorinated biphenyls in the tarsal gland contents of Yusho patients. Fukuoka Acta Med. 76, 244–247 (in Japanese).

Mathers, W. D., Shields, W. J., Sachdev, M. S., et al. (1991) Meibomian gland dysfunction in chronic blepharitis. Cornea 10, 277–285.

Ohnishi, Y., Yoshimura, T. (1977) Relationship between PCB concentrations or patterns in blood and ocular signs among people examined for "Yusho." Fukuoka Acta Med. 68, 123–127 (in Japanese).

Ohnishi, Y., Kohno, T. (1979) Polychlorinated biphenyls poisoning in monkey eye. Invest. Ophthalmol. Visual Sci. 18, 981–984.

Ohnishi, Y., Kohno, T., Ishibashi, T., et al. (1983) Macroscopic observation of monkey meibomian glands treated with polychlorinated biphenyls. Fukuoka Acta Med. 74, 240–245 (in Japanese).

Risebrough, R. W., Rieche, P., Peakall, D. B., et al. (1968) Polychlorinated biphenyls in the global ecosystem. Nature 220, 1098–1102.

Yoshihara, S., Ozawa, N., Yoshimura, H., et al. (1979) Preliminary studies on the experimental PCB poisoning in rhesus monkeys. Fukuoka Acta Med. 70, 135–171 (in Japanese).

7.5. Gynecologic and Obstetric Aspects of Yusho: Pregnancy, the Fetus, Newborn Babies and Problems in Breast Feeding

TEIJI HAMADA

Yusho involves risk to the general populace, especially to pregnant women, the fetus, and newborn babies. The babies born to contaminated mothers have been termed either as "black babies" or "Cola babies," because of the dark brown pigmentation of the skin. Such babies exhibited peculiar clinical manifestations which were defined as a new clinical entity designated as either PCB induced fetopathy or Yusho fetopathy (Funatsu et al., 1971). In this chapter, the discussion is directed towards the effects of PCB contamination during the course of pregnancy and its outcomes on the basis of surveys carried out at Kyushu University and Kurume University Hospitals.

7.5.1. Effect of Yusho on Pregnancy, the Fetus and the Newborn Babies

In pregnant women PCBs act the same as in any other individuals and the fetus may be highly sensitive to their effects. Since the placenta is permeable to essentially everything taken into the maternal compartment, the fetus is thus exposed to almost anything despite its apparent sequestered locus within the uterus.

7.5.1.1. Pregnancy

Sixteen pregnant mothers complicated by PCB contamination were analyzed with respect to the outcomes of their pregnancies based on the findings of previously published reports (Taki et al., 1969; Yamaguchi et al., 1971).

Abortion and preterm labor: Only a limited amount of data was available regarding the incidence of abortion among pregnant mothers with Yusho. In 16 pregnant women who ingested 0.3–2.6 l of cooking oil contaminated with PCBs, 2 cases resulted in stillbirths. The incidence of preterm labor in the remaining 14 cases was 14.3 percent, which was not considered to be significantly different from the percentage found in normally pregnant women.

Perinatal mortality rate: The perinatal mortality rate among babies from mothers contaminated with PCBs was 125.0 (2 cases of stillbirth out of 16 pregnancies), which was significantly higher than that of the control population (30.1 in 1965) (Maternal and Childhealth Division, Children and Families Bureau, Ministry of Health and Welfare, Japan, 1993). The late fetal death rate of 125.0 was also significantly higher as compared to the controls (21.9 in 1965).

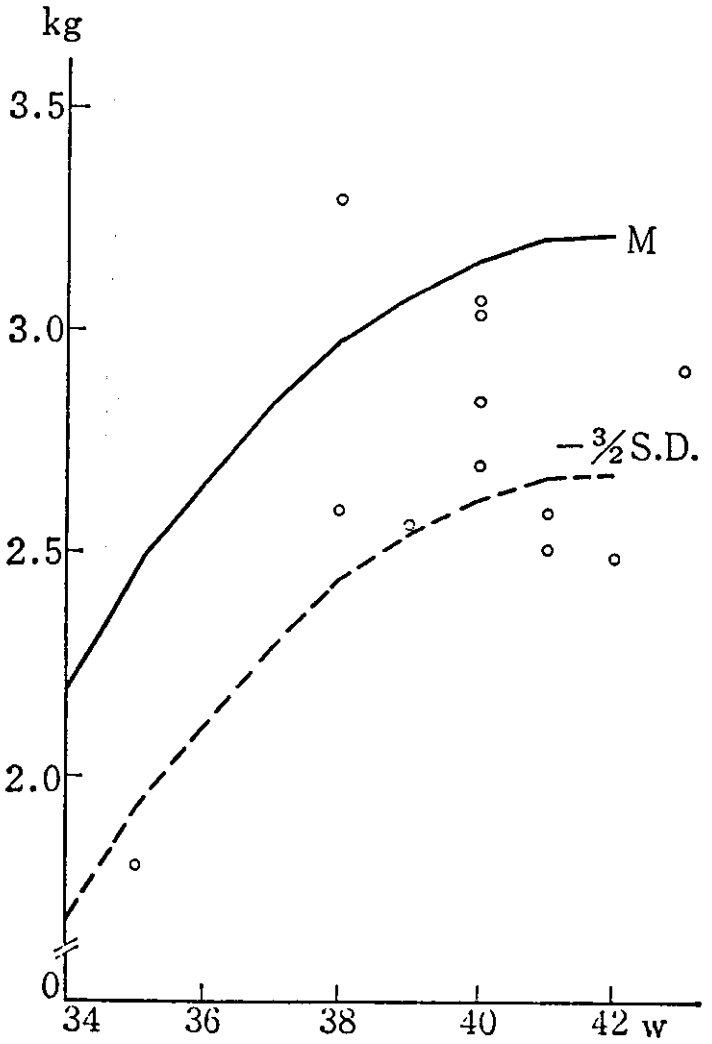


Fig. 7.5.1. Birth Weight According to the Gestational Age
The mean values and −1.5 SD of birth weights were calculated on the basis of the standard values of gestational ages as postulated by Funakawa.

Maternal complications: The ingestion of PCBs by pregnant mothers was associated with a greater incidence of preeclampsia, even though the severity of preeclampsia in all cases was classified as being mild. The incidences of edema, proteinuria and hypertension were 68.8 percent (11/16), 18.8 percent (3/16) and 43.8 percent (7/16), respectively.

As stated above, it is conceivable that an increased risk for stillbirth appears to be a prominent adverse effect of PCB intoxication on pregnancy. However, the

specific mechanisms for stillbirth have yet to be clarified. In the case of a woman who had been poisoned by PCBs during her first pregnacy and underwent another pregnancy 7 years after the original contamination, the outcomes of both pregnancies were stillbirths. The autopsy findings of each baby did not elucidate the exact etiology of stillbirths. Although the subsequent baby exhibited no clinical manifestations of Yusho fetopathy characterized by growth retardation and so on, a gas chromatographic analysis of the fat tissue from the baby revealed the typical chromatographic pattern of PCBs in Yusho. These findings indicate that the highly lipid-soluble and poorly metabolized compounds tend to accumulate in maternal fat deposits and are then transferred to fetus via the placenta, even though the woman conceived more than 7 years after the initial ingestion of PCB-contaminated rice oil (Kikuchi et al., 1977).

7.5.1.2. Intrauterine Development

The most significant clinical feature of the babies born to the poisoned mothers was that of intrauterine growth retardation. Yamaguchi et al. (1971) reported that 33 percent (4/12) of the babies were diagnosed as small-for-gestational-age (SGA) babies according to the fetal growth curve constructed by Funakawa (1968) (Fig. 7.5.1 and Fig. 7.5.2, see color frontispiece). The infant whose birthweight was 1.5 standard deviation less than the average appropriate for the stated gestational age was categorized as SGA in this study. Taki et al. (1969) experienced 9 cases of Yusho fetopathy, all of which showed birth weights of below the mean-1.5 standard deviation by gestational age (Funakawa, 1968). Such clinical evidence clearly indicated that the fetal growth of the babies born to contaminated mothers was extensively impaired. Although the threshold level of PCBs, above which there is cause for concern has not yet been elucidated, the consumption of cooking oil in two mothers with SGA babies was reported to be 0.7 and 1.1 L, respectively. No reports are available regarding the mechanism of growth supression, and whether it is attributable to placental insufficiency due to impaired uteroplacental perfusion or alterations of intrinsic factors in the fetal compartment. Since PCBs have been proven to be transferred to fetus through the placenta, it has been speculated that modifications of the fetal metabolic functions induced by PCBs may occur in the fetal tissues, predominantly in the liver, which may thus be responsible for the suppression of fetal development.

7.5.1.3. Other Clinical Features of Yusho Fetopathy

In addition to growth supression, a total of 4 babies in the Kurume series (Funatsu et al., 1971) showed other characteristic manifestations for Yusho fetopathy, as is outlined in Table 7.5.1. In these cases, the pregnant mothers con-

Table 7.5.1. Clinical Manifestations in 4 Infant Cases with Yusho Fetopathy

Symptoms and Signs	Case			
	1	2	3	4[a]
1. Dark brown pigmentation:				
skin in general, near the fingernails, hair follicles	+++	+	+++	+
genital organ, axilla	+++	+++	+++	+
2. Dark brown pigmentation on lips, gingiva, palate	+++	++	+++	?
3. Color ring on limbic cornea	yes	no	yes	no
4. Desquamation (perchment-like)	+++	++	+++	?
5. Gingival hyperplasia	+++	+?	+++	−?
6. Dentition at birth	yes	no	yes	no
(number of teeth)	(1)		(2)	
7. Calcification on skull	yes	yes	yes	no
8. Wide, open saggital suture of skull	yes	yes	yes	?
9. Larger fontanelles (frontal, occipital)	yes	yes	yes	?
10. Exophthalmic, edematous eyelid	yes	yes	yes	?
11. Rocker-bottom heel (prominent heel)	yes	yes	yes	yes
12. Secreta on conjuntival palpebra	yes	yes	yes	no
13. Hepatomegalia with increased consistency	no	no	yes	no
			(3QFB)	
14. Fever in the neonatal period	no	no	yes	no

[a]: Examinated at 3 months of age.

sumed from 0.6–10 L of cooking oil contaminated with PCBs, and the clinical manifestations observed are summarized as follows:

① Skin and mucous membranes:

All patients had dark or grayish brown skin pigmentation (Fig. 7.5.2, see color frontispiece) and demonstrated a perchment-like desquamation of the skin at birth. The pigmentation was distributed over the entire area of both the skin and mucous membranes. The sites of increased prominence on the skin were the genitalia (Fig. 7.5.3, see color frontispiece), the axilla, the fingers near the nails, and the hair follicles, while such sites on the mucous membranes were the lips, the gingiva, the palate and the corneal limbus (noted as color ring) and the conjunctiva. Skin biopsy findings revealed marked hyperkeratosis (Fig. 7.5.4).

② Head:

The frontal and occipital fontanels showed larger and wider openings and the sagital suture remained wider than usual in newborns. Spotty calcifications or platelike dense areas were also noted in the parieto-occipital area of the skull in the X-ray findings.

③ Oral cavity:

Dark brown pigmentation on the lips, the mucous membranes of the oral cavity, and the palate was noted. The gingiva was hypertrophic and associated with either

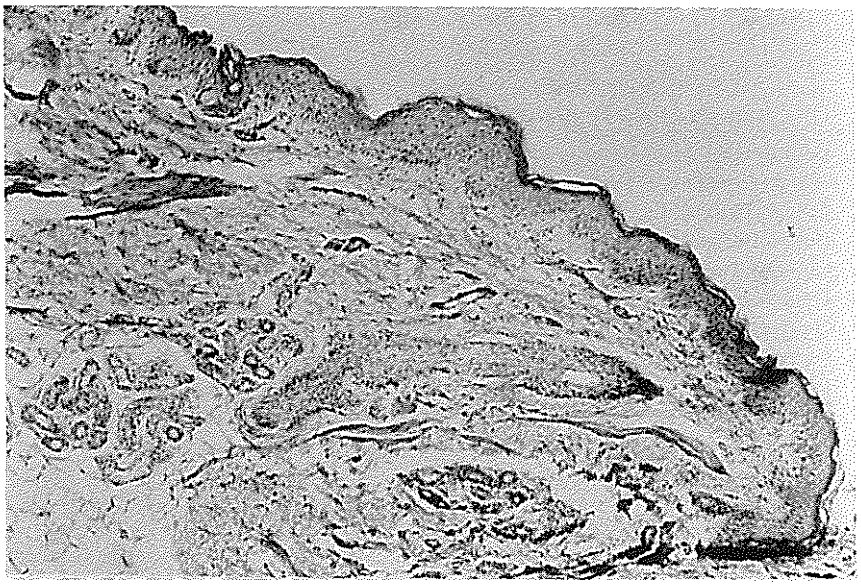


Fig. 7.5.4. A Light Micrograph of the Skin Obtained by a Biopsy from a Baby with Yusho Fetopathy
Marked hyperkeratosis is indicated.

an uneven surface or dentition at birth.

④ Eyelids:

Atypical facial appearances included edematous eyelids, exophthalmus, and a depressed nasal bridge.

⑤ Neurological signs:

No neurological abnormalities were observed.

⑥ Laboratory findings:

There were no marked alterations in the laboratory findings. A baby born to a mother who ingested 10.5 L of cooking oil during pregnancy showed a slight increase in protein concentration in cerebrospinal fluid with a maximum level of 185 mg/dl (Funatsu et al., 1971a).

⑦ Metabolic and endocrine systems:

Although transient elevations of β-lipoprotein, alkaline phosphatase and GOT were observed in some cases, liver function tests, function of the hypophyseo-adrenocortical axis by Metopiron test and other endocrine function tests were all within normal limits.

7.5.1.4. Neonatal and Infantile Periods

Follow-up health checks of the babies with Yusho fetopathy demonstrated that the pigmentation on the skin and the mucous membranes disappeared within 3–5

months of age in all patients. Regarding the subsequent growth of individual affected newborn, appropriate-for-gestational-age (AGA) babies showed growth curves parallel to the normal curves for weight gain and height increase. Growth retarded babies in utero almost always demonstrated slow growth after birth. Their weight and height at 9 months of age were below the lower limits of standards for unaffected normal babies. Eighty-two percent of the affected babies tended to show susceptibility to common colds and bronchitis (Yamaguchi et al., 1971b), however, the subsequent neurological and intellectual developments were all normal.

In 1979, a mass poisoning occurred in Taiwan from cooking oil contaminated by thermally degraded PCBs. The clinical manifestations of the poisoned patients were almost identical to those observed among patients in Japan. The children who had been exposed to PCBs by transplacental passage or by breast milk feeding, showed a delay in physical development and characteristic clinical features that were very similar to those of Japanese children. The follow-up health checks revealed no abnormal reflexes or any localizing findings in the neurological examination. However, the exposed children did show late development in comparison to same age controls when they performed such tasks as saying phrases and sentences, turning pages, carrying out requests, pointing to body parts, holding pencils, imitating drawn circles, or catching a ball (Rogan, et al., 1988). In contrast to the normal intellectual development observed in Japanese children, they always scored lower than the controls on various tests for evaluating IQ. Since the number of affected children in Taiwan was far greater than that reported in Japan, the Taiwanese data appear to be potentially more instructive in this matter.

7.5.2. Yusho and Female Sexual Function

The effects of Yusho on the female sexual function have not been well defined so far because the number of sexually mature patients was limited. A clinical survey performed by Kusuda et al. (1971) revealed that more than 50 percent of the women with Yusho showed irregularities in their menstrual cycle, quantitative and qualitative alterations of menstrual flow, and abnormal menstrual duration and interval. The degree of such abnormalities in the menstrual duration and flow tended to correlate with the severity of the Yusho disease. However, the reasons for such abnormal sexual function have not yet been clarified. An LH-RH test given to the affected women yielded normal blood levels of LH and FSH, which thus indicated that the anterior pituitary lobe function regarding gonadotroph, had not deteriorated (Kusuda et al., 1975). Animal experiments using castrated rats demonstrated that PCB pretreatment potentiated the action of estradiol to increase the uterine wet weight and uterine glycogen content (Komatsu, 1972). Hence, it was suggested

that the instances of abnormal sexual function noted in women with Yusho might thus be related to the disturbance of hypothalamic or ovarian function possibly provoked by PCBs, which subsequently resulted in the alteration of the estradiol metabolism.

7.5.3. Problems of Breast Feeding

There have been various reports regarding the breast feeding of mothers who have a history of exposure to PCBs during pregnancy or during the puerperium. Breast-fed infants from lactating mothers who were initially exposed to PCBs during their nursing periods, also exhibited characteristic features of Yusho fetopathy (Yoshimura, 1974). Thus, it is evident that breast feeding is a potential risk factor for Yusho. Subsequently polychorinated dibenzofurans (PCDFs) were detected in a commercial brand of polychlorinated biphenyls (Kanechlor-400), a PCB preparation and Kanemi Yusho Oil (Nagayama, et al., 1975). PCDFs are well known to be highly toxic and much attention has since been directed to these chemicals, and they are now considered to be the principle compounds in the pathogenesis of Yusho disease. PCDFs were reported to persist in the adipose tissue of Yusho patients over 18 years since the epidemic was noted and the oil was removed from the market. The concentrations of PCDFs in the adipose tissue of these patients were 100 times higher than those of normal controls (Iida et al., 1989). In order to evaluate the validity of breast feeding, the levels of PCDFs were measured in human breast milk obtained from 2 Yusho patients who were diagnosed as being neonatal Yusho 18 years prior to the study. As well as the PCDFs in the adipose tissue, the concentrations in the mother's milk were also significantly higher in comparison to the normal controls (Matsueda et al., 1993). These observations indicate that once PCB contamiation occurs, a very long-term excretion of PCDFs into the mother's milk is unavoidable. The daily intake of PCDFs from the breast milk was calculated to be 5–10 percent of 28 ng/kg/day, which is equivalent to the minimal dosage necessary to provoke this disease (Masuda, 1992). Therefore, attention should be paid to the possible health effects on babies due to PCDFs and PCBs in the breast milk of Yusho mothers. As a result, women should not be encouraged to breast-feed since a well documented history of exposure to PCBs has been demonstrated.

References

Funakawa, H. (1968) Gestational age and fetal growth. Acta Neonat. Jap. 4, 129–133 (in Japanese).

Funatsu, I., Yamashita, F., Yoshikane, T., et al. (1971a) A chlorobiphenyl induced fetopathy. Fukuoka Acta Med. 62, 139–149 (in Japanese).

Iida, T., Nakagawa, R., Takenaka, S., et al. (1989) Polychlorinated dibenzofurans (PCDFs) in the

subcutaneous adipose tissue of Yusho patients and normal controls. Fukuoka Acta Med. 80, 296–301 (in Japanese).

Kikuchi, M., Kurihara K., Higuchi, Y., et al. (1977) A case of pulmonary carcinoma and a stillborn from a Yusho patient. Fukuoka Acta Med. 68, 156–161 (in Japanese).

Komatsu, F. (1972) Estradiol-potentiating action of PCB. Fukuoka Acta Med. 63, 374–377 (in Japanese).

Kusuda, M. (1971) Yusho and female sexual function. Sanka to Fujinka 38, 1063–1072 (in Japanese).

Kusuda, M., Nagata, Y., Nakamura, M. (1975) Anterior pituitary function of Yusho patients. Fukuoka Acta Med. 66, 635–639 (in Japanese).

Masuda, Y. (1992) Approach to risk assessment of PCDDs and PCDFs in Yusho food poisoning. Toxic Substances J. 12, 175–180.

Matsueda T., Iida, T., Hirakawa, H., et al. (1993) Concentration of PCDDs, PCDFs and coplanar PCBs in breast milk of Yusho patients and normal subjects. Fukuoka Acta Med. 84, 263–272 (in Japanese).

Maternal and Child Health Division, Children and Families Bureau, Ministry of Health and Welfare, Japan (1993), In: Maternal and child health statistics of Japan. Tokyo, 75–83 (in Japanese).

Nagayama, J., Masuda. Y., Kuratsune M. (1975) Chlorinated dibenzofurans in kanechlors and rice oil used by patients with Yusho. Fukuoka Acta Med. 66, 593–599

Rogan, W. J., Gladen, B. C., Hung, K. L., et al. (1988) Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. Science 15, 334–336

Taki, I., Hisanaga, S., Amagase, Y. (1969) Report on Yusho (chlorobiphenyls poisoning) pregnant women and their fetuses. Fukuoka Acta Med. 60, 471–474 (in Japanese).

Yamaguchi, A., Yoshimura, T., Kuratsune, M. (1971) A survey on pregnant women having consumed rice oil contaminated with chlorobiphenyls and their babies. Fukuoka Acta Med. 62, 117–122 (in Japanese).

Yoshimura, T. (1974) Epidemiological study on Yusho babies born to mothers who had consumed oil contaminated by PCB. Fukuoka Acta Med. 65, 74–80 (in Japanese).

7.6. Growth of Affected Children

TAKESUMI YOSHIMURA

In 1968, there was an outbreak of food poisoning in Kyushu, Japan due to rice oil contaminated with PCBs and their thermal degradation products, such as PCDFs, PCQs (Kuratsune et al., 1972; Nagayama et al., 1975). Eleven years later, in 1979, a regrettable outbreak of food poisoning due to cooking oil contaminated with the same chemical substances as seen in Japan, occurred in central Taiwan (Hsu et al., 1985). Over 1,800 persons in Japan and about 2,000 persons in Taiwan thus suffered from the disease called "Yusho" in Japan and "Yucheng" in Taiwan. Based on these two episodes, the growth and development of such affected children is herein described.

At the outbreak of Yusho in Japan, 9 grayish darkbrown colored babies and fetuses born to mothers who consumed rice oil contaminated with PCBs were reported and theses babies were named neonatal Yusho babies or Fetal PCB Syndrome babies (Taki et al., 1969; Yamashita and Hayashi, 1985). All the delivered babies from February 15, 1968 to December 31, 1968 among females in Yusho families in Fukuoka Prefecture, Japan, were examined (Yamaguchi et al., 1971). It was reported that 13 females with Yusho had 11 live births and 2 still-births. Among them, 10 showed the characteristic grayish darkbrown colored skin at birth, 9 an increased eye discharge, and 5 had pigmented nails and gingiva. The majority of the babies were small-for dates babies as reported by Taki (Yamaguchi et al., 1971; Taki et al., 1969). It was suggested by these observations and later confirmed that PCBs and PCDFs were transferred through poisoned females to their fetuses via the placenta and breast milk (Tsukamoto et al., 1969; Masuda et al., 1978; Kodama and Ota, 1980).

In Taiwan, between October 1979 and February 1983, 39 hyperpigmented babies were born to affected mothers (Hsu et al., 1985). Since 68% of the female Yucheng patients ranged in age from 10 to 39 years, many births of transplacental Yucheng babies would be expected. Therefore, the potential reproductive hazard was considered to be more serious in Taiwan than in Japan.

7.6.1. Growth and Development of Transplacental Yusho or Yucheng Babies

In Japanese Yusho babies, exposed either transplacentally or through breast milk to PCBs, no definite growth delay or retardation in physical and mental activities had been observed by 9 months after birth (Yamaguchi et al., 1971; Funatsu et al., 1971). The growth of 7 Yusho babies exposed to the chemicals in infancy

showed no striking growth retardation (Hayashi and Yamashita, 1983). It was, however, reported that by 7 years after exposure the 13 children were shown to be apathetic and dull with IQs in the 1970s (Harada, 1976). No systematic follow-up studies have been reported on the growth and development of the transplacental Yusho babies in Japan.

In Taiwan, on the other hand, well designed epidemiological studies on these issues have been conducted since 1985. In 1985, a field survey of all living children who were known to have been in utero during or after the period of oil contamination was conducted (Rogan et al., 1988). One hundred seventeen children born to women with Yucheng and 108 unexposed controls were examined neurologically, dysmorphologically, dermatologically, odontologically and were also given a general physical check-up. Even 6 years after the discontinuation of the contaminated oil, the exposed children were smaller than the controls, averaging 93% of the control weight and 97% of control height, with statistical significance, after being adjusted for age and sex. In addition, the characteristic symptoms of Yucheng were found more in the exposed subjects than in the controls. It was also reported that the exposed children demonstrated a delay in the age at which they performed certain tasks. Regarding cognitive development and behavioral assessment, age-appropriate tests were carried out. The exposed children scored lower than the controls on the three tests (Bayley Scale, Stanford-Binet (IQ), and Wechsler Intelligence Scale for Children (WISC) for development and cognitive assessment), except for verbal IQ on the WISC. All of the three scales for behavioral problems of the Rutter scales were higher in the exposed children than in controls (Rogan et al., 1988).

A previous study done by Rogan was extended, and a new set of controls was used for comparison and a modified battery of tests was adopted to assess cognitive development. As a result, detectable cognitive deficits were observed up to seven years of age for the exposed babies (Chen et al., 1992). However, no dose response relationship was shown in the study. The exposed children who were smaller in size and had exhibited neonatal symptons of intoxication showed delayed development (Yu et al., 1991).

The growth of 7 transplacental Yucheng babies was followed up for 5 years, compared to growth curve of the body weight under the age of 6 years in Taiwan (Yen et al., 1989). The results showed that the Yucheng babies tended to catch up with normal children during their early childhood.

Using the Chinese Child Developmental Inventory (CCDI) for development and Rutter's Child Behavior Scale A for behavioral problems, children born 7 to 12 years after their mothers' or fathers' contamination with PCBs were examined (Guo et al., 1994). It was concluded that children born 7 to 12 years after mothers'

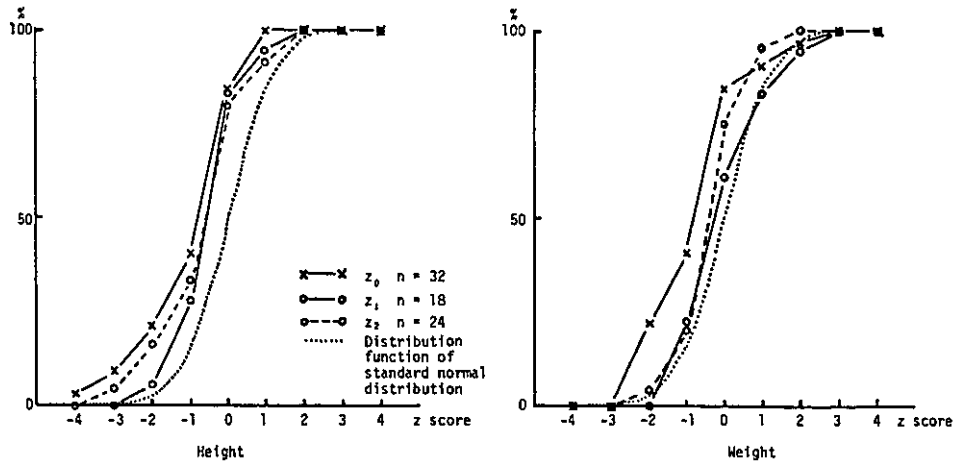


Fig. 7.6.1a. Cumulative Percentage Polygon of z Scores of Boys with Yusho

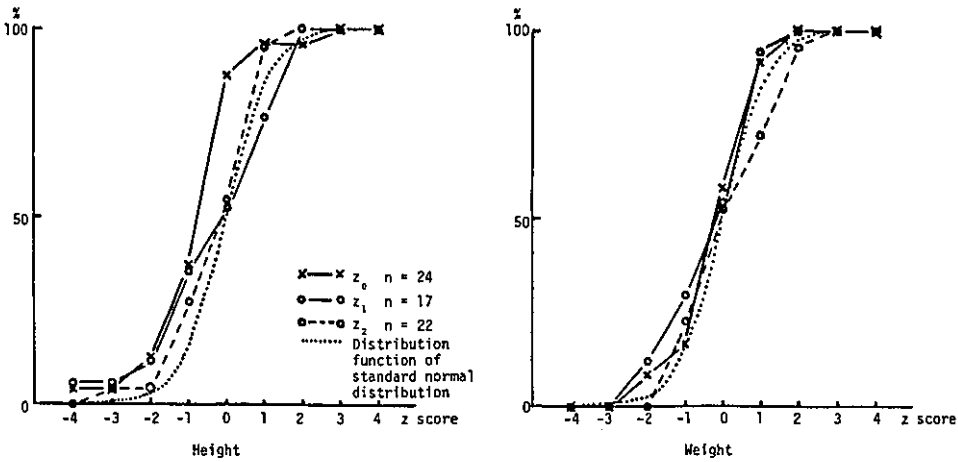
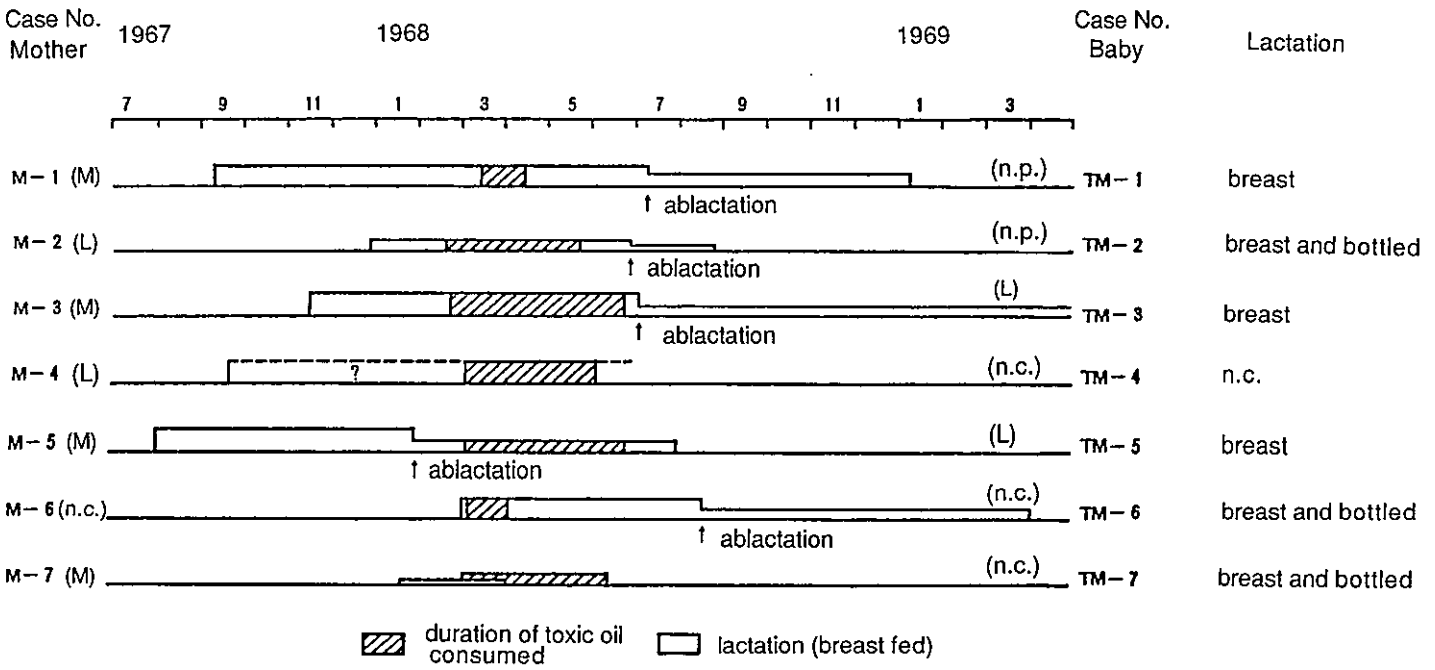


Fig. 7.6.1b. Cumulative Percentage Polygon of z Scores of Girls with Yusho

exposure to PCBs demonstrated delayed development, but they did not show any serious behavioral problems when compared with the unexposed controls.

7.6.2. *Growth of School Children Who Consumed Contaminated Rice Oil*

The height and weight gains for school children with Yusho each year (before and after the outbreak, that is, from 1967 through 1971) was assessed to clarify the effect of Yusho suffering. The "Z-score" for each patient was calculated, using the distribution of height and weight gains of classmates of the same sex and age as the



In parenthesis, Yusho grade was shown, M: mild, L: light
n.p: nothing particular, n.c.: not confirmed

Fig. 7.6.2. Possible Cases of "Trans-milk Yusho Babies"

standard distribution. The physical measurement data were obtained from the school records. As shown in Fig. 7.6.1, it was suggested that the growth of school children with Yusho was disturbed after the Yusho incidence and that their subsequent increments of height and weight tended to be close to the average level of the healthy controls (Yoshimura, 1971; Yoshimura and Ikeda, 1978). No similar reports appeared for the Yucheng incident.

7.6.3. Growth of "Trans-milk Babies"

"Trans-milk Yusho baby" was defined as a breast fed Yusho baby whose only source of the PCBs was the mothers contaminated breast milk.

In the Yusho and Yucheng series, it might be quite difficult to identify trans-milk Yusho babies, because the timing of both lactation and the mothers consumption of contaminated rice oil should have been carefully examined. Yoshimura tried to identify the trans-milk Yusho babies among the babies born to mothers who started to consume contaminated rice oil after the delivery of the baby and the babies who had no opportunity to have foods contaminated with the rice oil.

Two out of the 7 babies fed mainly by breast milk contaminated by the PCBs were diagnosed as Yusho as shown in Fig. 7.6.2 (Yoshimura, 1974). One case (TM-5) could not be definitively considered a "trans-milk Yusho baby" for sure, because the baby may have been fed not only with breast milk, but also with foods contaminated by PCBs. But another case (TM-3) was considered to be a " trans-milk Yusho baby" even without any chemical analysis data for the breast milk. However, no follow-up study has been done on these cases to assess their growth and development.

It was suggested that the quantity of PCBs transferred to infants from their mothers via lactation was much greater than that transferred placentally (Kodama and Ota, 1980). Therefore, trans-milk Yusho or Yucheng babies, if identified, should be carefully followed up.

For transplacental Yusho babies in Japan, no delayed growth or development could be observed. This, however, may in part be due to the short follow-up periods and/or due to small number of the cases observed. In Taiwan, however, even 6 years after the exposure, the height and weight of Yucheng children were smaller than the controls. It was also concluded that Yucheng children showed detectable cognitive deficits. For Yusho children who directly consumed the contaminated rice oil, a growth disturbance was observed just after the incidence, although no supportive data are available. In addition, no data have been accumulated on the growth and development of "trans-milk" Yusho or Yucheng babies.

References

Chen, Y-C, Guo, Y-L, Hsu, C-C, et al. (1992) Cognitive development of Yu-Cheng ('Oil Disease') children prenatally exposed to heat-degraded PCBs. JAMA. 268 (22), 3213–3218.

Guo, Y. L., Chen Y., Yu, M., et al. (1994) Early development of Yu-Cheng children born seven to twelve years after the Taiwan PCB outbreak. Chemosphere 29 (9–11), 2395–2404.

Funatsu, I., Yamashita, F., Yoshikane T., et al. (1971) A chlorobiphenyl induced fetopathy. Fukuoka Acta Medica. 62 (1), 139–149 (in Japanese).

Harada, M. (1976) Clinical and epidemiological studies and significance of the problem. Bull. Inst. Constitutional Med., Kumamoto Univ. 25 (Suppl.) 1–60.

Hayashi, M., Yamashita, F. (1983) The growth and sexual maturation of patients with PCB poisoning. Fukuoka Acta Medica. 74 (5), 280–283 (in Japanese).

Hsu, S-T, Ma, C-I, Hsu, K-H, et al. (1985) Discovery and epidemiology of PCB poisoning in Taiwan: a four-year followup. Environ. Health Perspect. 59, 5–10.

Kodama, H., Ota, H. (1980) Transfer of polychlorinated biphenyls to infants from their mothers. Arch. Environ. Health. 35, 95–100.

Kuratsune, M., Yoshimura, T., Matsuzaka, J., et al. (1972) Epidemiologic study on Yusho, a poisoning caused by ingestion of rice oil contaminated with a commercial brand of polychlorinated biphenyls. Environ. Health Perspect., 1, 119–128 .

Masuda, Y., Kagawa R., Kuroki, H. (1978) Transfer of polychlorinated biphenyls from mothers to foetuses and infants. Fd. Cosmet. Toxicol. 16, 543–546.

Nagayama, J., Masuda, Y., Kuratsune, M. (1975) Chlorinated dibenzofurans in Kanechlors and rice oils used by patients with Yusho. Fukuoka Acta Medica. 66 (10), 593–599 (in Japanese).

Rogan, W. J., Gladen, B. C., Hung, K. L., et al. (1988) Congenital poisoning by polycholorinated biphenyls and their contaminants in Taiwan. Science 241, 334–336.

Taki, I., Hisanaga, S., Amagase, Y. (1969) Report on Yusho (chlorobiphenyls poisoning) pregnant women and their fetuses. Fukuoka Acta Med. 60 (6), 471–474 (in Japanese).

Tsukamoto, H., Makisumi, S., Hirose, H., et al. (1969) The chemical studies on detection of toxic compounds in the rice bran oils used by the patients of Yusho. Fukuoka Acta Med. 60 (6), 496–512 (in Japanese).

Yamaguchi, A., Yoshimura, T., Kuratsune, M. (1971) A survey on pregnant women having consumed rice oil contaminated with chlorobiphenyls and their babies. Fukuoka Acta Med. 62 (1), 117–122 (in Japanese).

Yamashita, F., Hayashi M. (1985) Fetal PCB syndrome: clinical features, intrauterine growth retardation and possible alteration in calcium metabolism. Environ. Health Perspect. 59, 41–45.

Yen, Y. Y., Lan, S. J., Ko, Y. C., et al. (1989) Follow-up study of reproductive hazards of multiparous women consuming PCBs-contaminated rice oil in Taiwan. Bull. Environ. Contam. Toxicol. 43. 647–655.

Yoshimura, T. (1971) A case control study on growth of school children with "Yusho". Fukuoka Acta Med. 62 (1), 109–116 (in Japanese).

Yoshimura, T. (1974) Epidemiological study on Yusho babies born to mothers who had consumed oil contaminated by PCB. Fukuoka Acta Med. 65 (1), 74–80 (in Japanese).

Yoshimura, T., Ikeda, M. (1978) Growth of school children with polychlorinated biphenyl poisoning or Yusho. Environ. Res. 17, 416–425.

Yu, M-L, Hsu, C-C, Gladen, B. C., et al. (1991) In utero PCB/PCDF exposure: relation of develop-

mental delay to dysmorphology and dose. Neurotoxicology and Teratology 13, 195–202.

7. 7. Oral Lesions in Yusho

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7.7.1. Oral Findings in Yusho Patients

Oral lesions such as pigmentation or parakeratosis of the gingiva, anomalies of the dental root shape (Fig. 7.7.1, see color frontispiece), retarded eruptions of permanent teeth (Fig. 7.7.2) and a deficiency of tooth germs have all been observed in patients with Yusho (Fukuyama et al., 1979). Oral pigmentation is the most prominent feature of the oral lesions seen in patients with Yusho but no specific pattern of pigmentation characteristic of Yusho has yet been established. Such pigmentation tends to be mostly brownish in color and is pleomorphic in shape, while exhibiting a diffused band-like form, a rounded form, a tree-like form and so on (Fig. 7.7.3, see color frontispiece). Gingival pigmentation is observed at a much higher frequency than pigmentation of the lips or buccal mucosa, however, pigmentation of the palate or tongue is only rarely observed. In the gingiva, pigmentation is mostly observed in the anterior and buccal attached gingiva. Soon after the onset of Yusho, Aono and Okada (1969) found that the prevalence of oral pigmentation was seven times higher in Yusho patients than in clinically healthy persons as a result of the higher prevalence of pigmentation in Yusho patients of all ages. In addition, no definitive difference between sexes was observed, although the prevalence of oral pigmentation in female patients seemed to be somewhat higher than that in male patients. It was particularly noteworthy that 62.5% of the affected children (0–10 years old) suffered from oral pigmentation. Since the concentration and pattern of blood PCBs have been reported to be roughly related to the appearance of oral pigmentation (Fukuyama et al., 1977), it is thus suggested that such pigmentation is probably caused by PCB poisoning. The prevalence of oral pigmentation has decreased somewhat over the years, but it remains higher in Yusho patients than in normal subjects (Fukuyama et al., 1979; Akamine et al., 1983). It has also been reported that such oral pigmentation reappeared even after surgical removal (Fukuyama et al., 1979). Surgical elimination was performed for esthetic reasons by scraping the pigmented gingiva with a sharp curet in two female Yusho patients who suffered from oral pigmentation (Figs. 7.7.4 and 7.7.5, see color frontispieces). As time passed, however, oral pigmentation similar to that observed before surgery reappeared at the operated area of both patients (Fig. 7.7.6, see color frontispiece). Even though the blood PCB concentration gradually decreases after PCB exposure, it is still higher in Yusho patients than in normal subjects

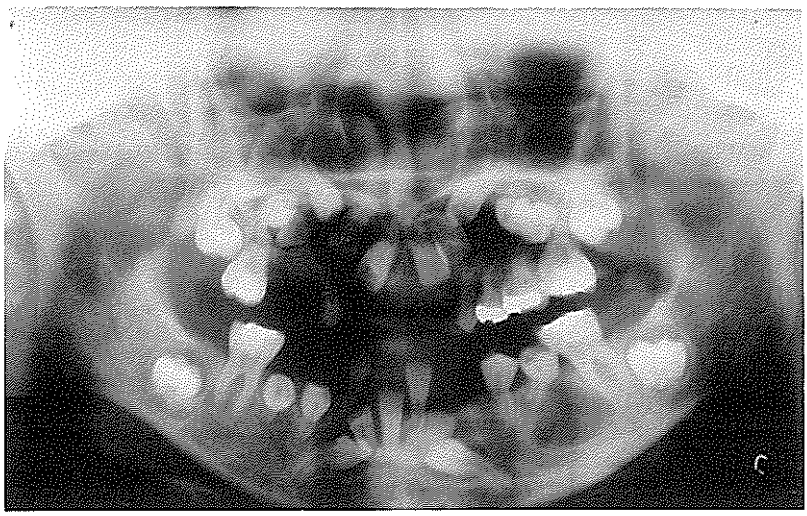

Fig. 7.7.2. A Panoramic Radiograph of a 12-Year-Old Female Yusho Patient
Multiple impacted teeth and an ectopic direction of the crowns are observed. Hypoplasia and the dilaceration of the tooth roots are also evident in some teeth.

(Honbo et al., 1991; Ohgami et al., 1991). Okumura et al. (1987) reported that the mean value of PCB concentration, PCQ concentration and the CB% ratio in the buccal mucosa biopsied from Yusho patients were 298.55 ppb, 81.65 ppb and 2.65 ppb, respectively, which were all significantly higher than those from normal subjects. In addition, the PCB concentration and PCQ concentration were found to be 36 and 91 times higher in the buccal mucosa than in the blood, respectively (Okumura et al., 1987). Taking these findings into consideration, it is thus suggested that either PCB or the PCB-related compounds still remaining in Yusho patients, especially those found in the oral mucosa, may be responsible for the higher prevalence of oral pigmentation and the reappearance of oral pigmentation after surgical elimination.

We histologically examined the biopsied gingiva with pigmentation from Yusho patients (Hashiguchi et al., 1987). In the light microscopic examination, toluidine blue staining showed a diffused deposition of light or yellowish browned particle-like materials in both the basal cell layer and in the lamina propria (Fukuyama et al., 1979; Hashiguchi et al., 1987). An electron microscopic examination revealed that numerous melanosomes and melanosome complexes were present in the keratinocytes from the basal cell layer to the suprabasal cell layer (Fig. 7.7.7), which corresponds to the histological findings of the conjunctiva from Yusho patients and from rats with experimentally induced PCB poisoning (Ikui et al., 1969; Aoki, 1975). It is thus suggested that the oral pigmentation observed in

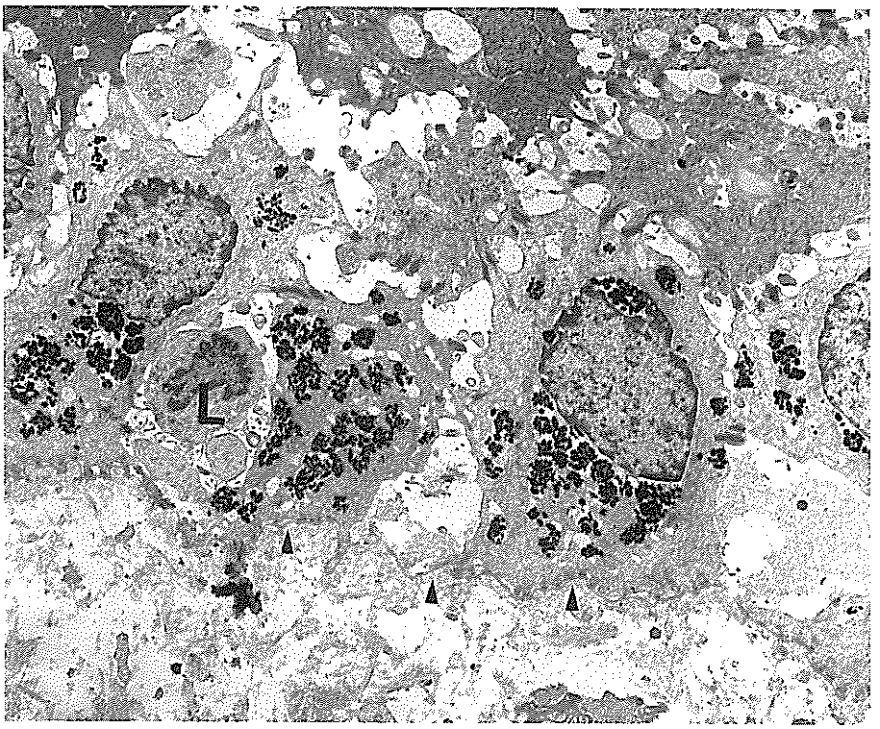

Fig. 7.7.7. Numerous melanosomes and melanosome complexes are observed in the basal cells. Degenerated keratinocytes and widened intercellular spaces between the keratinocytes are also observed. In addition, poorly developed collagen fibers and cell debris can be seen in the lamina propria. L: lymphocyte, arrowhead: basal lamina (× 7,400).

Yusho patients may have been the result of an overproduction of melanosomes. It has been reported that melanocyte stimulating hormone (MSH) and adrenocorticotropic hormone (ACTH) secreted by the pituitary body both stimulate the melanocytes directly (Bleehen and Ebling, 1979), but little information is available concerning the effects of PCBs on the pituitary body. As PCBs have been known to suppress the secretion of adrenocortical hormones which are thought to inhibit the secretion of MSH and ACTH (Inao, 1970), it is probable that PCBs stimulate melanocytes to produce melanosomes indirectly. Alternatively, it is also possible that other local factors may also be involved in the appearance of oral pigmentation. Several kinds of degeneration of keratinocytes and widened intercellular spaces were observed from the basal cell layer to the suprabasal cell layer (Fig. 7.7.7). In the lamina propria, poorly developed collagen fibers and cell debris were also observed in part (Fig. 7.7.7). It is accepted that melanosomes secreted by melanocytes are transported into the keratinocytes to be digested in the lysosomes

Table 7.7.1. Distribution of the Teeth with Periodontal Pockets Deeper than 4 mm by Age Group

Age	Number of teeth with periodontal pockets (≧4 mm)														Total
	0		1		2		3		4		5		6		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
10–19	1[a]	1	1	0	0	0	0	0	0	0	0	0	0	0	3
20–29	1	1	2	1	1	0	0	1	0	1	0	0	0	0	8
30–39	3	7	2	3	0	1	0	1	2	0	1	0	0	0	20
40–49	1	7	2	4	3	3	1	0	2	1	1	0	1	0	26
50–59	4	9	3	4	2	2	2	2	1	1	0	1	0	1	32
60–69	1	9	2	1	1	3	1	2	0	0	0	0	0	0	20
70–79	4	2	0	2	0	0	0	0	0	0	0	0	0	0	8
80–89	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Total	15	36	13	15	7	9	4	6	5	3	2	1	1	1	118

M: male, F: female. [a]: Number of patients.

(Hashimoto, 1979). In the altered keratinocytes, a suppression of phagocytic activity may occur. If so, melanosomes may be left behind in either the keratinocytes or in the intercellular spaces, which may thus contribute to the appearance of oral pigmentation. Since the mechanism concerning the appearance of oral pigmentation has not sufficiently been clarified, further studies are needed to better understand the mechanism of oral pigmentation caused by PCB poisoning.

It has been generally accepted that Yusho patients are more susceptible to bacterial infections (Saito et al., 1972). In addition, a considerable number of Yusho patients have also complained of periodontal diseases such as gingival swelling, gingival bleeding and pus discharge from periodontal pockets. Therefore, an epidemiologic examination was carried out to reveal the prevalence of periodontal diseases in 118 Yusho patients (47 males and 71 females) at the annual Yusho health examination in Fukuoka Prefecture. The periodontal pocket depth at the mesio-buccal site of six index teeth (tooth numbers 16, 21, 24, 36, 41, 44) was measured and panoramic radiographs were also taken to observe the condition of marginal bone resorption. It was determined that 149 teeth out of a total of 594 examined teeth had a periodontal pocket deeper than 4 mm while 67 patients had at least one tooth with a periodontal pocket deeper than 4 mm (Table 7.7.1). A chronological examination revealed that the patients between the ages of forty and sixty had a high prevalence of periodontal diseases. It is of particular interest that severe periodontal diseases were even found in younger patients. The prevalence of deep periodontal pockets in Yusho patients was higher than that in the clinically healthy reference population (Akamine et al., 1985). Systemic factors have not

been suggested to be a primary cause of periodontal diseases. However, since the effects of local and systemic factors are interrelated, it does seem probable that systemic factors aggravate periodontal diseases caused by local factors (Glickman, 1972). It has been demonstrated that metabolic disorders of vitamin D and calcium, which occur following PCB exposure, cause anomalies of bone ossification and also arrest development in childhood (Yoshimura, 1971, Fukuyama et al., 1979; Hirayama, 1979). In animals with experimental PCB poisoning, it has been reported that PCBs accumulated in the bone affect the metabolism of calcium (Yagi et al., 1976). PCBs may thus possibly cause a disordered calcification in the alveolar bone, resulting in the high prevalence of deep periodontal pockets. However, differences in the frequency of teeth with periodontal pockets deeper than 4 mm were observed among the six index teeth. The lower left first molar showed the highest incidence of teeth with a periodontal pocket deeper than 4 mm (38.0%) out of all six index teeth. The lower first molar is the first permanent tooth to appear in the oral cavity. It is also reported that the posterior teeth generally accumulate more dental plaque than the anterior teeth, while the lower lingual surfaces have more dental plaque than the comparable upper surfaces (Hall and Douglass, 1977). Taking these findings into consideration, it is suggested that local factors such as dental plaque may thus play a major role in the pathogenesis of periodontal disease in Yusho patients and the systemic disorder caused by PCB poisoning may intensify the periodontal tissue changes caused by local irritants.

Immune responses have been suggested to play an important role in the progression and perpetuation of marginal periodontitis (Newman et al., 1990). PCBs have also been reported to cause disorders in the immune reaction (Vos and De Roij, 1972). We examined the biopsied gingiva with marginal periodontitis from Yusho patients immunohistochemically about 22 years after PCB exposure (Hashiguchi et al., 1991). Many infiltrated inflammatory cells were observed in the oral epithelium and lamina propria. The proportions of plasma cells, B cells, T cells and macrophages in the inflammatory cells were 47.9%, 18.3%, 10.7% and 4.6%, respectively. In the plasma cells, the predominant cell type was IgG-bearing plasma cells (42.0% in the infiltrated inflammatory cell) and there were also IgA- (4.6%), IgM- (0.7%), and IgE- (0.6%) bearing plasma cells, listed by their order of frequency. The percentages of IgA- and IgM-bearing plasma cells were lower than those reported by Hara et al. (1985). It is interesting to note that the levels of IgA- and IgM-bearing plasma cells were found to decrease in the serum from the Yusho patients soon after PCB exposure (Saito et al., 1972). Three years after PCB poisoning, however, the levels of IgA- and IgM-bearing plasma cells in the serum were observed to be normal (Saito et al., 1972). In addition, different views also exist in respect to the percent ratio of the plasma cells detected in marginal

periodontitis (Seymour et al., 1979; Hara et al., 1985). Taking these findings into account, we consider that PCBs therefore probably have no close relation to the present decrease in the number of IgA- and IgM-bearing plasma cells. As for the subset of T cells, the $T^H/_I$ cells (12.8% in the infiltrated inflammatory cells) predominated over the $T^S/_C$ cells (4.5%) and the CD4/CD8 ratio was 2.84. The higher CD4/CD8 ratio may indicate that some anomalies exist in the regulation of immune response. However, we can not assert that this finding is characteristic of Yusho patients, because similar results have also been obtained in the gingiva from clinically healthy persons with marginal periodontitis (Kitamura et al., 1987). However, the analysis of T-cell subsets in the peripheral blood from Yusho patients 14 years after onset revealed that there was a slight increase in the number of helper T-cells and a slight decrease in the number of suppressor T-cells, which thus resulted in a higher CD4/CD8 ratio (Nakanishi et al., 1985). These changes were especially evident in the Yusho patients with a higher PCB concentration in the blood. In addition, SD rats given PCDFs have also shown severe atrophy and a decrease in the size of the thymus in the acute phase of PCDF poisoning (Nakanishi et al., 1985). It is not clear whether these changes are the direct effect or the indirect effect of PCBs and PCDFs, but it is suggested that PCBs and PCDFs did affect the immunoregulatory mechanism of the T-cell subsets. Since immune reactions in the local lesions are thought to be initiated following the supply of immune cells from the peripheral blood, we thus suppose that the disorder of immune responses caused by PCBs and PCDFs may be correlated with the development of periodontal diseases.

7.7.2. Oral Findings in Animals with Experimental PCB Poisoning

To analyse the effects of PCBs and PCDFs on the gingiva and dental hard tissue in detail, we examined the animals given these compounds, however, no gingival pigmentation similar to that seen in Yusho patients was observed (Yoshihara et al., 1979; Hashiguchi et al., 1983; Hashiguchi et al., 1985; Hashiguchi et al., 1989). Although the reason for such a conflicting result remains to be elucidated, differences in the species may have contributed to this discrepancy.

7.7.2.1. Histological Findings in the Gingiva

Rhesus monkeys were fed diets containing KC-400 and PCDFs and gingival biopsies were made periodically (Hashiguchi et al., 1983). Dyskeratosis in the rete pegs and keratocysts in the lamina propria were observed histologically in the biopsied gingiva (Figs. 7.7.8 and 7.7.9). A light microscopic examination on the area of dyskeratosis showed that there were few changes in the basal and suprabasal keratinocytes around the dyskeratosis but flattened keratinocytes were

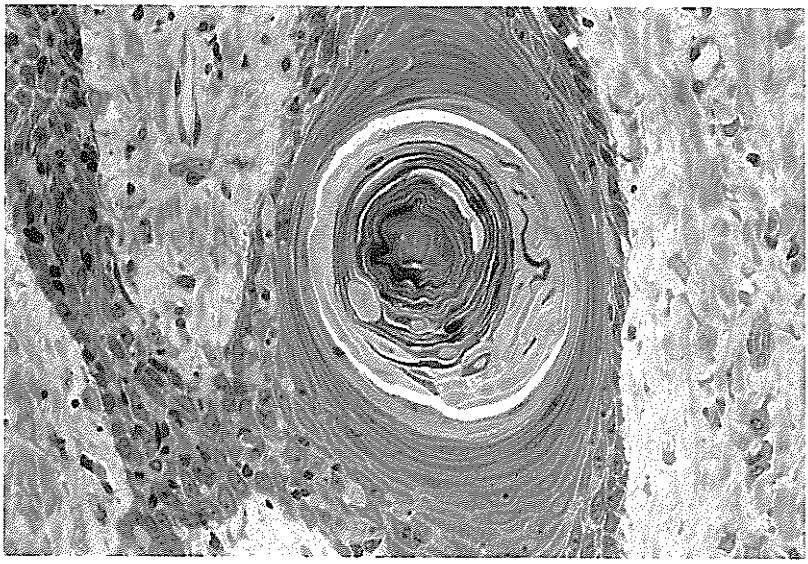

Fig. 7.7.8. Dyskeratosis in the Middle Part of the Rete Pegs
Flattened keratinocytes are observed in the areas of dyskeratosis (× 380).

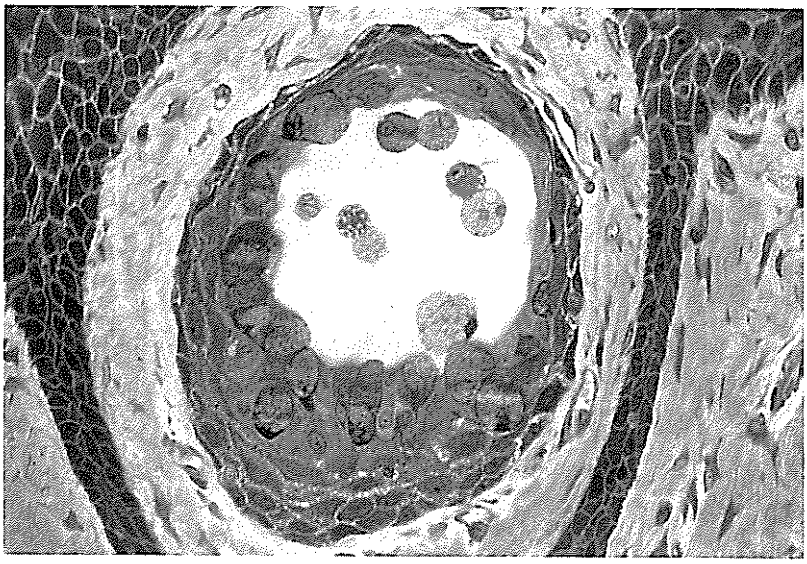

Fig. 7.7.9. A Keratocyst in the Lamina Propria
Degenerated swollen keratinocytes are seen in the keratocyst (× 380).

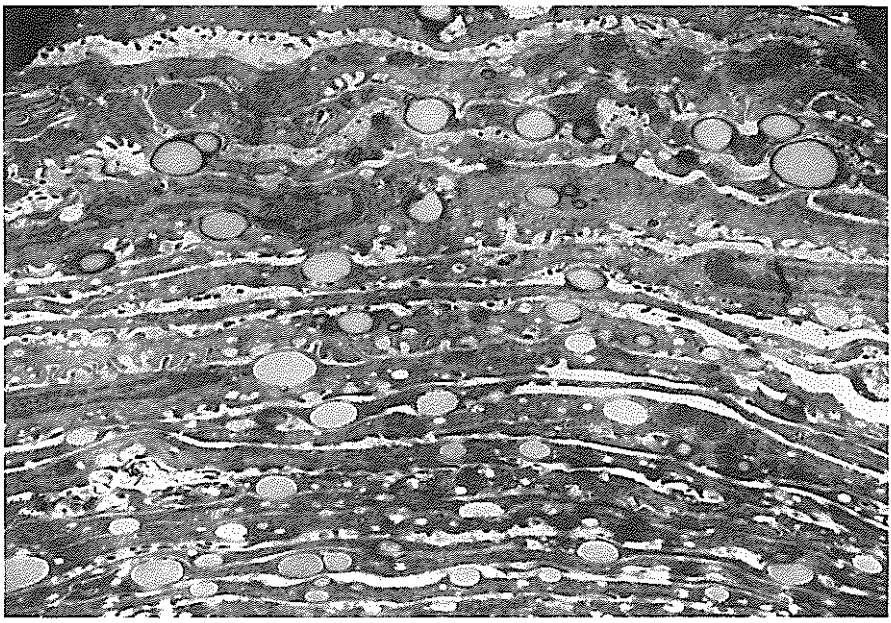

Fig. 7.7.10. A Higher Magnification of Dyskeratosis
Various sized round bodies are observed in the flattened keratinocytes
(× 10,300)

observed near the center of the dyskeratosis in which keratin-like materials were seen (Fig. 7.7.8). Under an electron microscope, tonofilaments were observed to increase in number in the flattened keratinocytes while the nuclei in some keratinocytes, near the center of the dyskeratosis, were not observed. Various sized round bodies, similar to vacuoles with high or low electron densities appeared in the flattened keratinocytes (Fig. 7.7.10). We thus consider that PCBs and PCDFs may either directly or indirectly accelerate the turnover in the keratinocytes and result in dyskeratosis. The reasons for this are as follows: First of all, we found little difference between the keratinizing organization in the dyskeratosis and that in the normal gingiva except for the appearance of the round bodies which looked like vacuoles. Secondly, the dyskeratosis was mainly observed in the rete pegs where basal cells with multipotency were thought to be more abundant as compared with the epithelium over connective tissue papillae (Löe et al., 1972). A light microscopic examination on the keratocyst showed that swollen and degenerated spinous cells were observed not only in the cystic lumen but also arround the cystic lumen (Fig. 7.7.9). In the degenerated spinous cells lining the cystic lumen, picnotic nuclei were located eccentrically on the opposite site of the cystic lumen (Hashiguchi et al., 1983). Under an electron microscope, few morphological changes were seen in the basal cells, but numerous structures similar to pinocytic

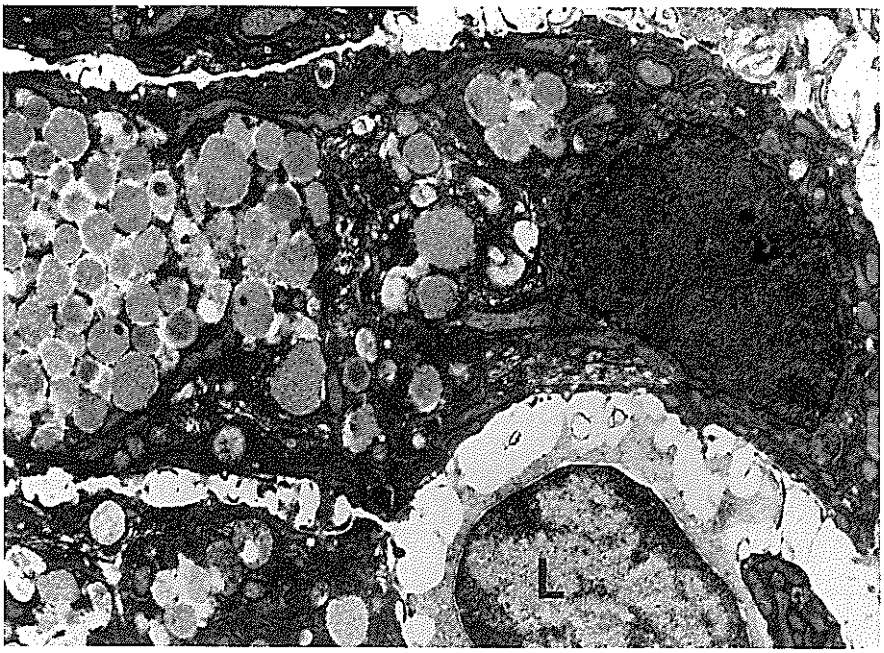

Fig. 7.7.11. A Higher Magnification of the Keratocyst
Many round bodies are observed in the degenerated spinous cells. Some round bodies are observed to fuse with one another and form large vacuoles. L: lymphocyte (× 11,600).

vesicles appeared in some suprabasal cells (Hashiguchi et al., 1983). In the degenerated spinous cells with high electron density lining the cystic lumen, many round bodies similar to the vacuoles containing amorphous materials were observed fusing to one another and forming large vacuoles along with a decrease in the number of cytoplasmic organelles (Fig. 7.7.11). The degenerated spinous cells in the keratocyst were positive for PAS staining even after amylase digestion (Fig. 7.7.12, see color frontispiece). Reaction products were slightly observed with Sudan black B and Sudan III staining. In view of these results, we consider that PCBs and PCDFs are thus likely to cause the excessive accumulation of glucose or lipid. It is also suggested that the precursors of the two substances increase in amount, because they cannot enter the normal metabolic map due to PCBs and PCDFs. We suppose that the keratocysts may result from these metabolic disorders occuring in the keratinocytes. It is not clear why the serious morphological changes were observed only in the epithelium. It has been reported that keratinocytes exhibit phagocytic activity while indigestible and nonusable materials are eliminated from the epidermis during the keratinization process (Wolff and Hönigsmann, 1971). PCBs are known to have a cytotoxic effect on the endothelial

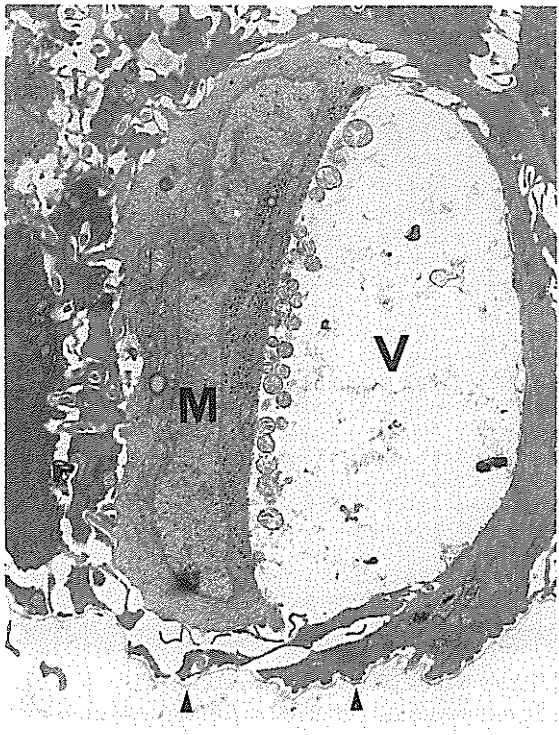

Fig. 7.7.13. Vacuoles (V) containing degenerated mitochondrias and amorphous materials are observed near the distorted merkel cell (M). arrowhead: basal lamina (× 9,400).

cells (Iatropoulos et al., 1977), which leads it to easily extravasate various materials through the blood. It is also thus suggested that the PCBs and PCDFs released in the dermis from the blood may be actively incorporated into the epidermal compartment, which then results in a metabolic disorder of glucose or lipid and the acceleration of the turnover in keratinocytes.

A light microscopic examination of the gingiva from beagle dogs given 3,4,5,3′,4′-PenCB, revealed various sized vacuoles within the basal and suprabasal cell layer in the rete pegs (Hashiguchi et al., 1989). The shapes of the keratinocytes and the clear cells around the vacuoles became distorted and resembled the shape of a crescent moon or kidney. An ultrastructural examination revealed that degenerated swollen mitochondrias and amorphous materials with high or low electron densities were observed in the vacuoles surrounded with delimiting membrane-like structures (Fig. 7.7.13). Some vacuoles ruptured thus spilling degenerated mitochondrias and amorphous materials into the intercellular space. The clear cells seen around the vacuoles were recognized to be merkel cells, because they con-

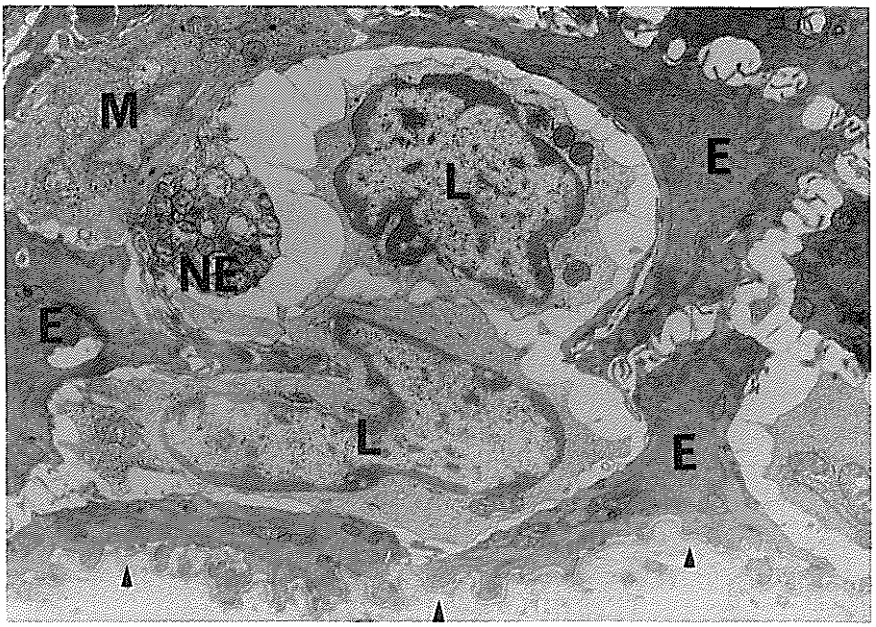

Fig. 7.7.14. The degenerated nerve terminal-like structure (NE) is observed near the distorted merkel cell (M) and the keratinocytes (E). L: lymphocyte, arrowhead: basal lamina (× 10,700).

tained Birbeck granules in the cytoplasm. In most cases, the vacuoles were seen near the merkel cells (Hashiguchi et al., 1989). Nerve terminal like structures containing swollen degenerated mitochondrias and amorphous materials, similar to those seen in the vacuoles, were observed near the keratinocytes and merkel cells, which also appeared to demonstrate irregular shapes (Fig. 7.7.14). These findings indicate that the vacuoles may have resulted from a degeneration of the nerve terminals following PenCB poisoning. It has been reported that no definitive abnormalities are found in the nervous systems of monkeys and mice after administration of PCB (Yamamoto et al., 1979; Yoshihara et al., 1979). Murai et al. (1971), however, reported that sensory neuropathy symptoms such as numbness, pain and hypoesthesia are found and sensory nerve conduction velocity is reduced, in about half of the Yusho patients examined soon after poisoning (Murai et al., 1971). Harada et al. (1981) have also reported that a decrease in the vibratory sensation is significant in Yusho patients below the age of 40 and in those older than 50 years. Our results may thus offer additional evidence for the toxicity of PCBs on the nervous systems. In this study, small vacuoles and swollen mitochondrias were also observed in the keratinocytes, but no serious changes such as dyskeratosis or keratocyst were detected (Hashiguchi et al., 1989).

7.7.2.2. Histological Findings in Dental Hard Tissue

In WKA rats with KC-400 and PCDFs poisoning, no anomalies of the dental hard tissue nor any retarded eruption occurred. There were also few morphological changes in the odontoblasts and cementoblasts, but ameloblasts and cells of stratum intermedium in the secretory and transitional stage showed severe morphological changes (Hashiguchi et al., 1985). Although cementoblasts and odontoblasts as well as ameloblasts are matrix-producing cells in the dental hard tissue, it is interesting to note that severe morphological changes were observed only in the ameloblasts and stratum intermedium cells but not in the cementoblasts and odontoblasts after PCBs and PCDFs poisoning. The reason for this discrepancy is not yet understood. The difference in their derivation may contribute to this discrepancy, because the ameloblasts and stratum intermedium cells are derived from the oral ectoderm, while the odontoblasts and cementoblasts originate from the neural crest.

In the lower incisor of 30 day old rats injected with KC-400 and PCDF intraperitoneally on day 20 after birth, secretory ameloblasts showed atrophy and became irregular in shape (Hashiguchi et al., 1985). Along with the destruction of desmosomes between ameloblasts, the intercellular spaces became dilated and distal terminal webs became obscure. In view of these above mentioned histological changes, secretory ameloblasts appeared to be vacuolar degeneration under a light microscope. Aside from the irregular shape of the nuclei, however, the cytoplasmic organelles showed few changes in their arrangement or form under an electron microscope.

In the examination of the lower molar tooth germs from newborn (1, 2, 9, 11 and 16 days after birth) WKA rats which were born to pregnant rats injected with KC-400 and PCDFs intraperitoneally from the 17th day to the 19th day of gestation (Hashiguchi et al., 1985), cystlike formations of various sizes and configurations were observed between the secretory ameloblasts (Fig. 7.7.15). Secretory ameloblasts lining cystlike formations were distorted and narrow in width. Secretory ameloblasts around cystlike formations were mostly detached from the enamel matrix. An ultrastructural examination revealed that severe degeneration and atrophy occurred in the secretory ameloblasts adjacent to cystlike formations (Fig. 7.7.16). In the degenerated ameloblasts, a rough endoplasmic reticulum (r-ER) showed changes in arrangement in accordance with an irregular outline of ameloblasts. In addition, secretory granules and coated vesicles were not detected in the degenerated Tomes' process. Amorphous materials were observed to fill cystlike formations and spaces formed between the detached ameloblasts and the enamel surface. In the transitional ameloblasts, one of the most striking alterations was a swelling of the mitochondrias in which the crista were obscure and the dilation of

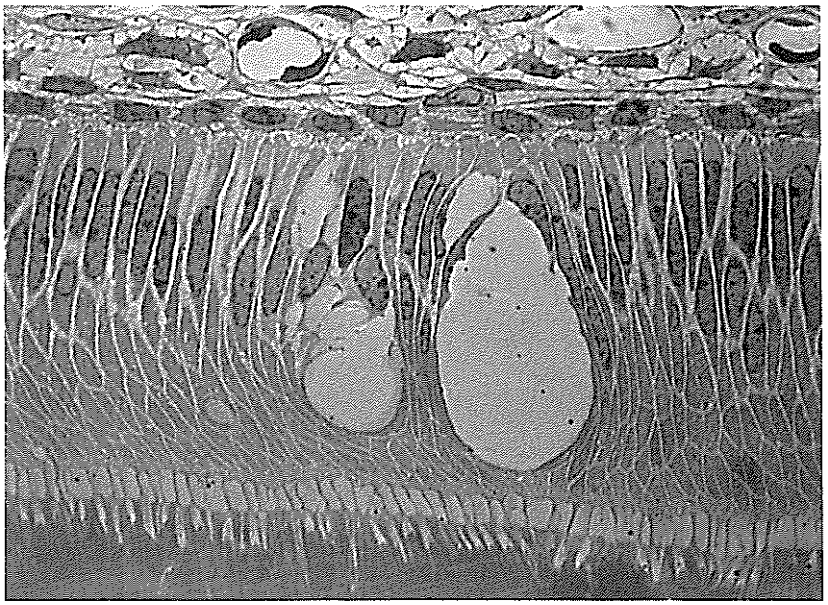

Fig. 7.7.15. Cystlike formations are observed between the secretory ameloblasts, which are detached from the enamel matrix (× 700).

the intercristal space was observed (Fig. 7.7.17). Some swollen mitochondrias exhibited a collapsing delimiting membrane. As another morphological change, various sized vacuoles either with or without a delimiting membrane were observed in the distal cytoplasma (Hashiguchi et al., 1985). The vacuoles were either empty or contained a small amount of amorphous materials of high electron density. R-ER showed morphological changes in arrangement due to swollen mitochondrias and large vacuoles. The stratum intermedium cells also showed morphological changes such as an appearance of vacuoles and a swelling of the mitochondrias, similar to those observed in the ameloblasts (Hashiguchi et al., 1985).

Findings similar to those observed in this experiment were also noted in the experiments where either fluoride, strontium, vincristin or vinblastine were administered (Kruger, 1968; Furui, 1977; Sawada, 1982). In these experiments, it was suggested that the morphological changes resulted from the disturbance of matrix-forming functions because of globular dilation of r-ER and appearance of a matrix-like substance in the intercellular spaces. In the PCB poisoned rats, however, no globular dilation of r-ER or deposition of matrix-like materials in the intercellular spaces were observed. As ameloblasts synthesize lipids (Goldberg et al., 1983), it is suggested that PCBs and PCDFs may be incorporated into lipids in ameloblasts to cause a metabolic disorder of the lipids, resulting in an alteration of ameloblasts.

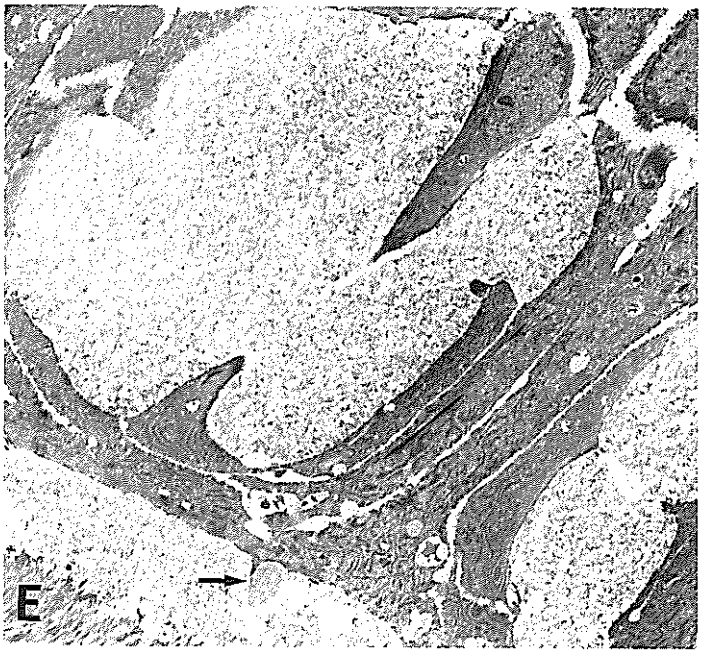

Fig. 7.7.16. A Higher Magnification of Cystlike Formations
Marked degeneration is observed to occur in the secretory ameloblasts near cystlike formations. Amorphous materials are observed in the cystlike formations and spaces between the detached ameloblasts and the enamel matrix. E: enamel matrix, arrow: degenerated Tomes' process (× 4,200).

Since the systemic effects of PCBs and PCDFs are severe, it is of course possible that some secondary changes may also occur as a result of the systemic disturbance caused by PCBs and PCDFs. A disturbance of endocrine glands has been demonstrated to be one of the factors which cause hypoplasia of the dental hard tissue, retarded eruption and anomalies of the dental root shape (Anderson et al., 1970). PCBs have also been reported to disturb the thyroid function (Murai et al., 1985). Following a thyroidectomy, ameloblasts have also been reported to show severe changes similar to those in the PCB poisoned rats (Taniguchi and Kitamura, 1984). These findings may thus indicate that PCBs and PCDFs cause severe changes in ameloblasts indirectly through the suppression of the thyroid function. The alteration of ameloblasts, however, seemed to be more severe in the molar teeth than in the incisors. The differential response to PCBs and PCDFs in the incisors and molar teeth may be due to differences in the ability of continuous cell turnover, because the supply of the immature ameloblasts and the migration of ameloblasts occur in the continuously erupting incisors but not in molar teeth.

The origin and nature of the cystlike formations observed between ameloblasts

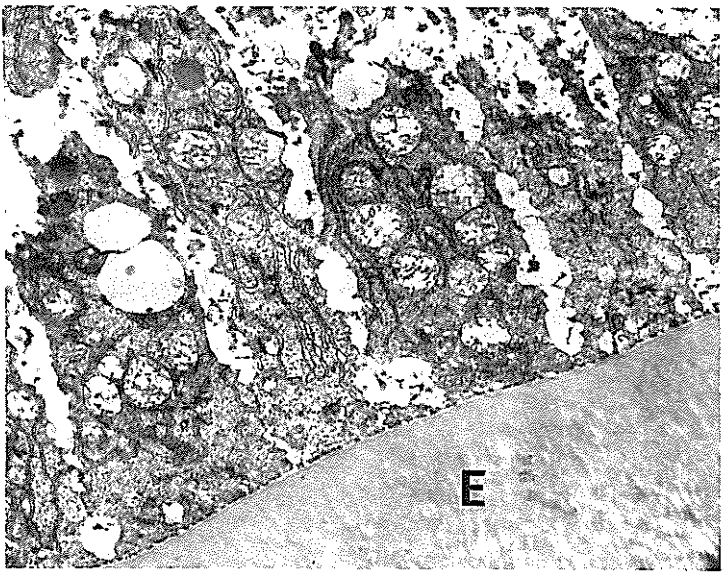

Fig. 7.7.17. Some transitional ameloblasts show marked degeneration. Numerous degenerated swollen mitochondrias are observed in the ameloblasts. E: enamel matrix (× 8,800).

remain obscure. It is generally accepted that the capillaries are observed to be sparse in number and are located somewhat remotely from the ameloblast layer in the presecretory stage. On the other hand, capillaries are also observed to invade the outer enamel epithelium, closely approaching the stratum intermedium, which is a uniform mono-layer adjacent to the basal end of ameloblasts after the secretory stage (Eisenmann, 1989). In addition, the active transport of several materials into the enamel organ through capillaries also increases in the secretory stage. It is known that when PCBs are administered they are partly incorporated into the lipoprotein in the serum and have a cytotoxic effect on endothelial cells (Iatropoulos et al., 1977), which may lead to an extravasation of PCBs from the capillaries. Crenshaw and Takano (1982) have demonstrated that the intravasculary perfused lanthanum penetrates into the intercellular space of the ameloblasts from the capillaries but its penetration toward the enamel matrix is stopped by the distal terminal webs. In our experiment, amorphous materials similar to those in cystlike formations were also observed in the capillaries adjacent to the stratum intermedium cell layer (Hashiguchi et al., 1985). On the basis of these findings, we hypothesize that PCBs released from the capillaries located near the ameloblast layer may enter into and may be stored in the intercellular spaces between the secretory ameloblasts, and thus result in the appearance of cystlike formations. On the other hand, cystlike formations may result from the lysis of some ameloblasts as is observed in some

keratinocytes in the keratocyst.

In spite of severe alteration of ameloblasts, no marked hypoplasia of enamel matrix was observed in our experiment (Hashiguchi et al., 1985). The explanation for this discrepancy is still unclear. In this study, however, not all the ameloblasts were subject to degeneration. In addition, Reith (1970) and Moe (1979) have found that physiological cell death occured frequently in the ameloblast layer during their maturation process, which indicated that not all the ameloblasts may be necessary to form the enamel matrix. Based on these findings, we therefore consider that the surviving ameloblasts may probably be adequate to form the enamel matrix.

References

Akamine, A., Hashiguchi, I., Kishi, T., et al. (1983) Alteration in oral pigmentation of patients with Yusho. Fukuoka Acta Med. 74, 284–288 (in Japanese).

Akamine, A., Hashiguchi, I., Maeda, K., et al. (1985) Prevalence of periodontal disease in patients with Yusho. Fukuoka Acta Med. 76, 248–252 (in Japanese).

Anderson, H., Holst, G. (1970) Endocrine disorders. In: Gorlin, R. J., and Goldman, H. M., eds., Thoma's Oral Pathology. Vol 2, 6th ed. pp. 618–635. C.V. Mosby, St. Louis.

Aoki, A. (1975) Ocular findings of chlorobiphenyls intoxication and histological changes of the palpebral conjunctiva in rats fed with kaneclor 500. Fukuoka Acta Med. 66, 642–645 (in Japanese).

Aono, M., Okada, H. (1969) Oral findings in Yusho. Fukuoka Acta Med. 60, 468–470 (in Japanese).

Bleehen, S. S., Ebling, F. J. (1979) Disorders of skin colour. In: Rook, A., Wilkinson, D. S., Ebling, F. J., eds., Textbook of Dermatology. 3rd ed. Vol 2, pp. 1377–1431, Blackwell Scientific Publications, Oxford.

Crenshaw, M. A. Takano, Y. (1982) Mechanisms by which the enamel organ controls calcium entry into developing enamel. J. Dent. Res. 61 (Sp Iss), 1574–1579.

Eisenmann, D. R. (1989) Amelogenesis. In: Ten Cate, A. R., ed., Oral Histology. pp. 197–212. C. V. Mosby, St. Louis.

Fukuyama, H., Hidaka, Y., Sano, S., et al. (1977) Relation between blood PCB level and oral pigmentation in Yusho patients. Fukuoka Acta Med. 68, 128–132 (in Japanese).

Fukuyama, H., Yumiko, A., Akamine, A., et al. (1979) Alteration in stomatological findings of patients with Yusho (PCB poisoning) in the general examination. Fukuoka Acta Med. 70, 187–198 (in Japanese).

Furui, A. (1977) Electron microscopy on formative disturbance of rat incisor enamel following administration of strontium. Shikwa Gakuho 77, 847–879 (in Japanese).

Glickman, I. (1972) Clinical Periodontology, 4th ed. pp. 290–414, W. B. Saunders, Philadelphia.

Goldberg, M., Lelous, M., Escaig, F., et al. (1983) Lipids in the developing enamel of the rat incisor. Parallel histochemical and biochemical investigations. Histochemistry 78, 145–156.

Hall, W., Douglass, G. (1977) Plaque control. In: Schluger, S., Yuodelis, R. A., Page, R. C., eds., Periodontal Disease. pp. 344–369, Lea & Febiger, Philadelphia.

Hara, Y., Yoshimura, S., Maeda, K., et al. (1985) Immunohistological study on movement of immune cells in periodontitis. 1. Difference of immunoglobulin-bearing cell populations in patients'

generations. J. Japan Ass. Periodont. 27, 795-806 (in Japanese).
Harada, T., Tominaga, H., Tanaka, K., et al. (1981) Study on vibratory sensation of patients with Yusho (PCB poisoning). Fukuoka Acta Med. 72, 214–215 (in Japanese).
Hashiguchi, I., Akamine, A., Nakano, T., et al. (1983) Ultrastructural changes in the gingival epithelium on the experimental PCB poisoning in the crab eating monkeys. Fukuoka Acta Med. 74, 246-254 (in Japanese).
Hashiguchi, I., Akamine, A., Hara, Y., et al. (1985) Effects on the hard tissue of teeth in PCB poisoned rat. Fukuoka Acta Med. 76, 221–228 (in Japanese).
Hashiguchi, I., Akamine, A., Miyatake, S., et al. (1987) Histological study on the gingiva in the patient with Yusho and the PCB poisoned monkeys. Fukuoka Acta Med. 78, 259–265 (in Japanese).
Hashiguchi, I., Akamine, A., Hara, Y., et al. (1989) Histological study on the gingiva in PenCB poisoned beagle dogs. Fukuoka Acta Med. 80, 263–268 (in Japanese).
Hashiguchi, I., Akamine, A., Miyatake, S., et al. (1991) Immunohistochemical and histopathological study of the effect of PCB on the periodontal tissue. Fukuoka Acta Med. 82, 256–261 (in Japanese).
Hashimoto, K. (1979) Electron microscopy of the skin. Kanehara & Co. Ltd., Tokyo (in Japanese).
Hirayama, C. (1979) Hepatocellular dysfunction in patients with PCB poisoning. Fukuoka Acta Med. 70, 238–245 (in Japanese).
Honbo, S., Hori, Y., Toshitani, S., et al. (1991) Dermatological findings in the annual examination of the patients with Yusho in 1989–1990. Fukuoka Acta Med. 82, 345–350 (in Japanese).
Iatropoulos, M. J., Felt, G. R., Adams, H. P., et al. (1977) Chronic toxicity of 2,5,4′-trichlorobiphenyl in young rhesus monkeys. II. Histopathology. Toxicol. Appl. Pharmacol. 41, 629–638.
Ikui, H., Sugi, K., Uga, S. (1969) Ocular signs of chronic chlorobiphenyl poisoning ("Yusho"). Fukuoka Acta Med. 60, 432–439 (in Japanese).
Inao, S. (1970) Adrenocortical insufficiency induced in rats by prolonged feeding of PCB. Kumamoto Med. J. 23: 27 (in Japanese).
Kitamura, T., Miyatake, S., Hara, Y., et al. (1987) Immunohistological study on movement of immune cells in periodontitis. 3. Analysis of T cell subsets. J. Japan Ass. Periodont. 29, 1084–1093 (in Japanese).
Kruger, B. J. (1968) Ultrastructural changes in ameloblasts from fluoride treated rats. Archs. Oral Biol. 13, 969–977.
Löe, H., Karring, T., Hara, K. (1972) The site of mitotic activity in rat and human oral epithelium. Scand. J. Dent. Res. 80, 111–119.
Moe, H. (1979) Physiological cell death of secretory ameloblasts in the rat incisor. Cell Tissue Res. 197, 443–451.
Murai, Y., Kuroiwa, Y. (1971) Peripheral neuropathy in chlorobiphenyl poisoning. Neurology 21, 1173–1176.
Murai, K., Tsuji, H., Kajiwara, E., et al. (1985) Thyroid function in patients with PCB poisoning. Fukuoka Acta Med. 76, 233–238 (in Japanese).
Nakanishi, Y., Kurita, Y., Kanegae, H., et al. (1985) Respiratory involvement and immune status in polychlorinated biphenyls and polychlorinated dibenzofurans poisoning. Fukuoka Acta Med. 76, 196–203 (in Japanese).
Newman, M. G., Sang, M., Nisengard, R. (1990) Host-bacterial interactions in periodontal diseases. In: Carranza, F. A., ed., Glickman's Clinical Periodontology, 7th ed. pp. 372–386, W. B. Saunders, Philadelphia.

Ohgami, T., Nonaka, S., Irifune, H., et al. (1991) A comparative study on the concentration of polychlorinated biphenyls (PCBs) and polychlorinated quaterphenyls (PCQs) in the blood and hair of "Yusho" patients and inhabitants of Nagasaki prefecture. Fukuoka Act Med. 82, 295–299.

Okumura, H., Masuda, N., Akamine, A., et al. (1987) Concentration levels of the PCB and PCQ, pattern of the PCB and ratio of CB% in buccal mucosa of patients with the PCB poisoning (Kanemi-Yusho). Fukuoka Acta Med. 78, 358–364 (in Japanese).

Reith, E. J. (1970) The stage of amelogenesis as observed in molar teeth of young rats. J. Ultrastruct. Res. 30, 111–151.

Saito, R., Shigematsu, N., Ishijima, S. (1972) Immunoglobulin levels in serum and sputum of patients with PCB poisoning. Fukuoka Acta Med. 63, 408–411 (in Japanese).

Sawada, T. (1982) Ultrastructural changes in ameloblasts of rats administered with vincristin and vinblastine. Shikwa Gakuho 82, 347–383 (in Japanese).

Seymour, G. J., Greenspan, J. S. (1979) The phenotypic characterization of lymphocyte subpopulations in established human periodontal disease. J. Periodont. Res. 14, 39–46.

Taniguchi, K., Kitamura, K. (1984) An electronmicroscopic study of the effect of thyroidectomy on amelogenesis. Jpn. J. Oral Biol. 26, 786–807 (in Japanese).

Vos, J. G., De Roij, T. H. (1972) Immunosuppressive activity of a polychlorinated biphenyl preparation on the humoral immune response in guinea pigs. Toxicol.Appl.Pharmacol. 21, 549–555.

Wolff, K., Hönigsmann, H. (1971) Permeability of the epidermis and the phagocytic activity of keratinocytes. Ultrastructural studies with thorotrast as a marker. J. Ultrastruct. Res. 36, 176–190.

Yagi, N., Kimura, M., Itokawa, Y. (1976) Sodium, potassium, magnesium and calcium levels in polychlorinated biphenyl (PCB) poisoned rats. Bull. Environ. Contam. Toxicol. 16, 516–519.

7.8. Autopsy Findings of Patients with Yusho and Stillborn Fetuses

Masanori Kuratsune

The autopsy findings of ten deceased patients with Yusho and two stillborn fetuses delivered by an affected mother have been previously reported by Kikuchi and his associates (Kikuchi et al., 1969, 1971, 1977, 1979; Kikuchi and Masuda, 1973, 1976; Kikuchi, 1972, 1984).

7.8.1. Autopsied Patients with Yusho

The ten autopsied patients consisted of 9 males and 1 female, and ranged from 13 to 76 years of age. Five of them died less than 4 years after the onset of Yusho, while the remaining five died from 7 years to 12 years and 6 months after onset. Their clinical severity ranged from grade I to IV (severest) and four of the five patients who died less than 4 years after onset were grade III or IV patients.

The major autopsy diagnoses given to these patients were as follows:

Liquor sanguinas: a boy aged 13 who died of acute heart failure 1 year after the onset, with a clinical severity of grade III

Multiple miliary myocardial necrosis and fibrosis: a 25-year-old male (grade IV) died of acute heart failure 1 year after onset

Generalized amyloidosis: a 73-year-old male (grade III) died of heart failure 1 year and 4 months after onset

Osteitis fibrosa generalizata: a 46-year-old male (grade III) died of heart failure 3 years and 10 months after onset

Chronic bronchitis pyothorax: a 59-year-old male (grade II) died 9 years after onset

Liver cell carcinoma: 2 patients consisting of a 48-year-old female (grade I) who died 2 years and 10 months after onset, and a 69-year-old male (grade I) who died 9 years and 6 months after onset

Pulmonary carcinoma: 2 patients consisting of a 72-year-old male (grade II) who died 7 years after onset, and a 69-year-old male (grade I) who died 11 years and 8 months after onset

Esophageal carcinoma: a 76-year-old male (grade I) died 12 years and 6 months after onset

The skin of these autopsied patients all showed specific changes, either mild or severe, such as atrophic epidermis, hyperkeratosis and the cystic dilatation of hair follicles, keratinization of the duct epithelium of the sebaceous gland and heavy

deposits of melanin in the basal layer.

It is noteworthy that carcinomas were found among half of these autopsied cases. Since the hepatocarcinogenicity of PCBs and PCDFs and the promoting effect of PCBs are well known (see Section 5.5), the fact that two patients were affected with liver cell carcinoma seems to be particularly important. According to Kikuchi, however, "these two patients showed no symptoms of liver lesions in the early stage of poisoning and revealed C pattern gaschromatograms, that is, the pattern of healthy persons". He thus concluded that "these liver lesions hardly seem to be associated with Yusho". He also mentioned that ". . . five patients in our series had carcinoma in the organs related to intake or excretion of PCBs. It is, however, hard to consider that these cancers had been caused by PCBs, because of the short interval period between the intake of PCBs and the occurrence of the carcinoma in some patients, a small quantity of residual PCBs in organs, and moreover no reliable epidemiological evidence available to support an increased risk of malignancies in the patients with Yusho" (Kikuchi, 1984). Without a doubt, further careful studies, particularly epidemiological ones, are needed before any definitive conclusions can be reached on this issue (see Chapter 10).

Histologically, a multiplication of the duct epithelium of the esophageal gland was found in six cases and peribronchial and peribronchiolar inflammatory changes with lymphocytes and histiocytes were noted in five cases. The results of the chemical analyses of the tissue and organs of these autopsied patients for PCBs and PCDFs are explained in Chapter 4. It should be noted here, however, that the heart of the above male patient who died of multiple miliary myocardial necrosis and fibrosis contained a level of PCBs as high as 5.2 ppm at whole base (Kikuchi, 1984).

7.8.2. Stillborn Fetuses

A 25-year-old woman unknowingly consumed Kanemi rice oil during her pregnancy from January to October 1968 and was diagnosed as having Yusho at the end of pregnancy because of acneiform eruptions appearing on her face and thigh. On October 24, 1968, she delivered a female stillborn weighing 2,600 g. On autopsy, the skin of the stillborn, except for that of the palm and sole, was diffusely dark-brown colored, due to the presence of abundant melanin pigment (Fig. 7.2.9; see color frontispiece). No acneiform eruptions were noticed, but histologically, hyperkeratosis, atrophy of the epidermis and cystic dilatation of hair follicles with keratotic plugs were observed, especially on the head. Hyperemia of all organs and atelectasis of the lungs were also seen. The cause of death was determined to be coiling of the umbilical cord. The concentration of PCBs in the fatty tissue of this fetus was 0.02 ppm, which was considerably lower than that in the

liver, which was 0.07 ppm. This finding was considered to be rather strange because fatty tissue usually contains much higher concentrations of PCBs than do any other tissues. The gaschromatographic pattern of PCBs for this fetus was pattern A, which is peculiar for Yusho. This was the first case of the so-called "black babies" delivered by women suffering from Yusho (Kikuchi et al., 1969; Kikuchi, 1984).

Another female stillborn fetus was delivered by the same woman as described above, at the end of her pregnacy, 6 years and 6 months after the onset of Yusho. The fetus showed neither any abnormal pigmentation of the skin nor other dermal lesions except for very mild follicular hyperkeratosis. However, the heart of the fetus weighed 19 g and showed a cardiac anomaly, namely, a ventricular septal defect measuring 4 mm in diameter, and right ventricular hypertrophy with dilatation. No other pathological changes were found. The gaschromatographic pattern of PCBs contained in the tissue of this fetus was pattern B, which is often seen in lightly affected patients with Yusho but rarely seen in normal persons (Kikuchi et al., 1977; Kikuchi, 1984).

References

Kikuchi, M. (1972) An autopsy case of PCB poisoning with liver cirrhosis and liver cell carcinoma. Fukuoka Acta Med. 63, 387–391 (in Japanese).

Kikuchi, M. (1984) Autopsy of patients with Yusho. Am. J. Indust. Med. 5, 19–30.

Kikuchi, M., Masuda, Y. (1973) PCB poisoning. Autopsy findings of so-called Yusho patients. Jap. J. Clin. Path. 21, 422–428 (in Japanese).

Kikuchi, M., Masuda, Y. (1976) The pathology of Yusho. In: Higuchi, K. ed., PCB poisoning and pollution. Tokyo, Kodansha Ltd., New York, Academic Press, 6, 69–86.

Kikuchi, M., Hashimoto, M., Hozumi, M., et al. (1969) An autopsy case of stillborn of chlorobiphenyls poisoning. Fukuoka Acta Med. 60, 489–495 (in Japanese).

Kikuchi, M., Mikagi, Y., Hashimoto, M., et al. (1971) Two autopsy cases of chronic chlorobiphenyls poisoning. Fukuoka Acta Med. 62, 89–103 (in Japanese).

Kikuchi, M., Kurihara, K., Higuchi, Y., et al. (1977) Two autopsy cases of Yusho patients in 1975. A case of pulmonary carcinoma and a stillborn from a Yusho patient. Fukuoka Acta Med. 68, 156–161 (in Japanese).

Kikuchi, M., Shigematsu, N., Umeda, G. (1979) Autposy report of two Yusho patients who died nine years after onset. Fukuoka Acta Med. 70, 215–222 (in Japanese).