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Clinical Findings and Immunological Abnormalities in Yu-Cheng Patients

by Yau-Chin Lü*† and Ying-Chin Wu*

An outbreak of poisoning caused by ingestion of rice bran oil which was accidentally contaminated with polychlorinated biphenyls (PCBs) broke out in Taiwan in February 1979. Diagnosis, management, and follow-up of the patients were performed at special clinics, and subjective symptoms and cutaneous changes such as peculiar acneform eruptions and pigmentation were recorded. The patients were divided into six age groups of both essex, and the body surface of the patients was divided into 12 sections according to the nature of skin. The prevalence of each type of cutaneous change was proved statistically by the chi-square test. The examination of the immune system function in the patients at 1 year revealed: decreased concentration of IgM and IgA but not of IgG; decreased percentage of total T-cells, active T-cells, and helper T-cells, normal percentage of B-cells and suppressor T-cells; suppression of delayed type response to recalling antigens; enhancement of lymphocyte spontaneous proliferation; and enhancement of lymphocyte proliferation with PHA, PWM, and PPD stimulation but not ConA. Follow-up studies 3 years later showed decreased blood PCB levels; some improvement of subjective symptoms and cutaneous changes; recovery of skin testing response to PPD; normal percentage of total T-cells and increased percentage of suppressor T-cells; and enhancement of lymphocyte proliferation spontaneously or under the stimulation of various mitogens.

Introduction

An outbreak of poisoning with peculiar acneform eruptions and pigmentation broke out in Taiwan in February 1979. Probably over 2000 persons were affected. The source of poisoning was found to be a specific brand of rice bran oil which was contaminated accidentally with polychlorinated biphenyls (PCBs). PCB was detected from suspected oil samples at concentrations of 4.8 to 204.9 ppm (52.0 ± 38.7 ppm mean value) (*1*). The blood levels of the poisoned patients were 3 to 1156 ppb with a mean of 89.14 ± 6.90 ppb (*2*). The district of poisoning involved mainly two prefectures, Tai-Chung and Chang-Hua.

In general, the age distribution of patients, the symptomatology, the skin pathology and the way the poisoning occurred (*3–5*), were similar to those of the Yusho outbreak in Japan in 1968 (*6–9*). The disease has been termed Yu-Cheng, which means oil disease (*10*). This paper deals with the subjective symptoms, cutaneous changes and immunological abnormalities.

Subjects and Methods

A special clinic was established for the diagnosis, management and follow-up of the patients at the Provincial Tai-Chung Hospital and the National Taiwan University Hospital. By March 1981, a total of 829 cases of suspected PCB poisoning were discovered in the special clinics among the residents of Tai-Chung Prefecture. There were 414 males and 415 females, aged between 7 days and 78 years. Half of the patients were 11 to 30 years of age. The description of subjective symptoms and cutaneous changes is based on the records for these patients. A total of 358 cases with complete description of skin findings were divided into six age groups of both sexes, and the prevalence of each cutaneous change was listed. The body surface of the patients was divided into 12 sections according to the physiological and anatomical nature of the skin: cheek, forehead, nose, chin, submandibular region, ear, trunk, seborrheic area, axilla, external genitalia, upper extremities, and lower extremities, and the distribution of each skin finding by section was also recorded. The prevalence of cutaneous changes was analyzed statistically by the chi-square test.

PCB patients suffered from various kinds of infection. Most frequent infections were those of the respiratory tract and skin, including pyoderma, tinea versicolor, dermatophytosis and warts. The low resistance of the patients to infection suggested that there was some degree of immunosuppression. Thus several immunological studies were done from the beginning of clinical observation and during follow-up investigation.

The following immunological techniques were utilized for the examination of the function of immune system in the patients. Albumin and globulin in 143 patients' sera

*Department of Dermatology, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.
†Author from whom reprints should be requested.

were studied by cellulose acetate electrophoresis at the first year. Serum immunoglobulins (IgG, IgA, and IgM) of 30 patients were measured by the single radial immunodiffusion method of Mancini et al. (*11*) with commercially available immunoplates (Behring, West Germany).

Skin testing with recalling antigens was performed by intracutaneous injection of 0.1 mL antigen of streptokinase/streptodornase in 143 patients in the first year. Tuberculin skin tests were done in 83 cases in the first year and in 30 cases 3 years later.

Thirty patients were tested for T-cell/B-cell number and subpopulation in the first year. Mononuclear cells were isolated by the method of Böyum (*12*). Active E rosette, E rosette, and erythrocyte antibody complement (EAC) rosette tests were performed according to the method of Kerman et al. (*13*) with slight modification. Ox RBC-IgG (EA_G) and ox RBC-IgM (EA_M) were prepared according to the method of Moretta et al. (14) for enumeration of T_r and T_μ cells. A monoclonal antibody technique was utilized for detection of lymphocyte subset 3 years later (*15,16*).

A lymphocyte proliferation test (*17*) with various mitogen stimulants such as PHA, ConA, PWM, and PPD was done in 83 cases in the first year and in 30 cases 3 years later.

Results and Discussion

Subjective Symptoms

Symptoms in the Early Stage. The complaints at the beginning of the disease as obtained from histories are listed in Table 1. Ocular symptoms, namely, increased discharge from eyes (29%), swelling of the eyelids (18.4%), weakness of eyesight, and soreness or easy fatigue of the eyes (14%) were the major complaints. The other complaints included cutaneous changes, constitutional symptoms and skeletomuscular disturbances.

Table 1. Symptoms in the early stage (358 cases).

Symptom	No. of patients	%
Increased discharge from the eyes	104	29
Swelling of the eyelids	66	18.4
Disturbance in vision	34	9.5
Soreness or irritation of the eye	16	4.5
Headache, dizziness	12	3.4
General malaise	17	4.7
Reduced appetite	3	1
Soreness or weakness of the limbs	26	7.3
Swelling or pain of the joints, foot	9	2.5
Numbness of the limbs	12	3.4
Acne-comedones	35	9.7
Pigmentation of the nails	9	2.5
Pruritus	20	5.6

Subjective Complaints on the First Visit to Clinics. As shown in Table 2, ocular symptoms such as disturbance of vision and easy fatiguability (51.4%) or soreness and irritation of the eye (18.8%) were still the primary complaint. Many patients developed constitutional symptoms such as general malaise (37.3%), headache or dizziness (21.9%), cough (14.2%), poor appetite (4.9%), skeletomuscular symptoms, i.e., soreness, weakness or swelling of the limbs (6.8%), neck pain or lumbago (15.6%), and numbness of the limbs (37.3%), etc. Abnormal menstruation was noted in 10.7% of female patients, pruritus in 35.6%, and hyperidrosis of the palms and soles developed in some patients.

Subjective Complaints in Follow-up Cases. Among 248 cases with complete follow-up records during the first year, the incidence of subjective complaints changed somewhat, as shown in Table 3. Ocular complaints, pruritus and cough decreased, but constitutional complaints such as headache, dizziness, malaise and reduced appetite increased. About 10% of female patients had abnormal menstruation, but none of male patients complained of impotence.

Table 2. Subjective complaints on the first visit (358 cases).

Complaints	No. of patients	%
Disturbance of vision, easy fatigability	183	51.4
Soreness, irritation of the eye	67	18.8
Headache, dizziness	78	21.9
Malaise	133	37.3
Cough	50	14.2
Reduced appetite	17	4.9
Soreness, pain or swelling of the limbs, joints	24	6.8
Neck pain, lumbago	56	15.6
Numbness of the limbs	133	37.3
Pruritus	127	35.6
Abnormal menstruation	20	10.7[a]

[a]10.7% of female patients.

Table 3. Subjective complaints (248 cases).

Complaint	No. of patients	%
Increased eye discharge	182	73.4
Edema of eyelids	152	61.3
Vision disturbance	89	35.9
Irritation of eyes	7	2.8
Headache, dizziness	62	25.0
General malaise	107	43.1
Reduced appetite	30	12.1
Soreness, pain of joints, muscles	51	20.6
Numbness of limbs	78	31.5
Nausea, abdominal pain, diarrhea	18	7.3
Pruritus	35	14.1
Hyperidrosis of palms, soles	11	4.4
Cough	25	10.1
Abnormal menstruation	14	11.2[a]
Others[b]	21	8.5

[a]11.2% of female patients.

[b]Included decreased bodyweight (3), lumbago (3), dryness of mouth (2), tinnitus (2), leg edema (1), neck pain (1), exophthalmus (1), constipation (1), gynecomastia (1) and diabetes mellitus (1).

Cutaneous Changes

The principal dermatological findings can be divided into two groups as follows: (1) abnormal keratotic changes, including follicular keratotic changes such as follicular accentuation, horny plugs, comedo formation, acneform eruptions, cysts, Meibomian gland enlargement, and sudaminalike eruptions and xeroderma, keratotic plaques, and deformity of nails, and (2) pigmentation of mucosa, skin and nails.

Follicular keratotic change caused follicular accentuation with horny plugs and sudaminalike eruptions in the early stage. These were followed by comedo formation and acneform eruptions, then in some cases by cyst formation, including Meibomian gland enlargement. Due to immunological deficiency of patients, these follicular changes were combined with secondary bacterial infection and consequent pustules or abscess formations and residual ugly scars.

A biopsy specimen showed an opened follicular orifice filled with a layered keratinous substance, cell infiltration with giant cells around keratinous cysts or ruptured keratinous cyst wall with inflammatory cell infiltration (Fig. 1). The epidermis had no acanthosis and revealed hyperkeratosis and an increased amount of melanin in the basal layer (5).

The total of 358 cases was divided into six age groups, and the distribution of the main follicular keratotic changes is listed in Table 4. Severe acne and cyst formation appeared to be more frequent in adult group; on the contrary, accentuation of hair follicles and plug formation were more prominent in the young group. According to the physiological and anatomical natures of skin, the body surface was divided into 12 sections, and the distribution of follicular keratotic changes by

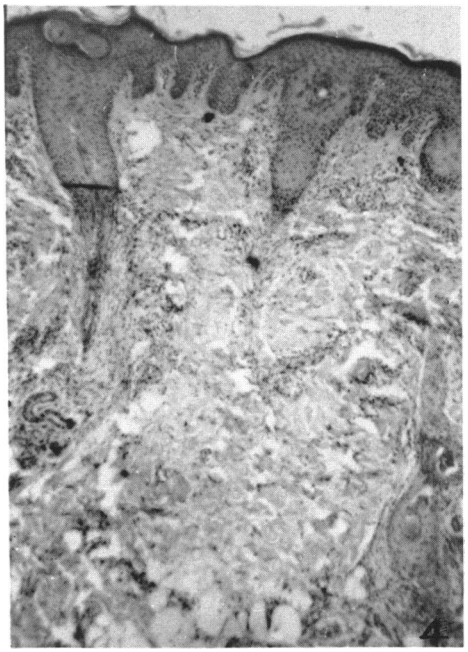

FIGURE 1. Hyperkeratosis, opened follicular orifice, and keratotic plug; increasing melanin in the basal layer.

Table 4. Acneform and hyperkeratotic follicular eruptions.

Age, yr	Age 0–10	Age 11–20	Age 21–30	Age 31–40	Age 41–50	Age > 51	Total	%
No. of patients	50	94	81	42	37	54	358	
No. of patients showing symptoms								
Comedo	30	66	51	21	21	19	208	58
Acne, localized	15	47	50	13	14	12	151	42
Acne, generalized	0	10	10	5	3	4	32	9
Cysts	0	13	7	6	2	8	36	10
Plugs, localized	10	27	14	9	7	8	75	21
Plugs, generalized	4	10	6	2	3	1	26	7

Table 5. Distribution of follicular keratotic change.

Localization	No. (%) of patients showing changes			
	Plugs	Comedo	Acne	Cyst
Cheek	47 (16.3)	210 (72.9)	83 (28.8)	24 (8.3)
Forehead	39 (13.5)	169 (58.7)	62 (21.5)	14 (4.9)
Nose	14 (4.9)	75 (26.0)	14 (4.9)	8 (2.8)
Chin	16 (5.6)	102 (35.4)	38 (13.2)	21 (7.3)
Submandibular	13 (4.5)	71 (24.7)	58 (20.1)	13 (4.5)
Ear	19 (6.6)	72 (25.0)	14 (4.9)	28 (9.7)
Trunk	20 (6.9)	72 (25.0)	69 (24.0)	25 (8.7)
Seborrheic area	24 (8.3)	40 (13.9)	36 (12.5)	15 (5.2)
Axilla	35 (12.2)	12 (4.2)	6 (2.1)	6 (2.1)
External genitalia	4 (1.4)	8 (2.8)	8 (2.8)	35 (12.2)
Extremities				
Upper	27 (9.4)	13 (4.5)	12 (4.2)	4 (1.4)
Lower	26 (9.0)	21 (7.3)	16 (5.6)	4 (1.4)
Total	288 (100.0)	288 (100.0)	288 (100.0)	288 (100.0)

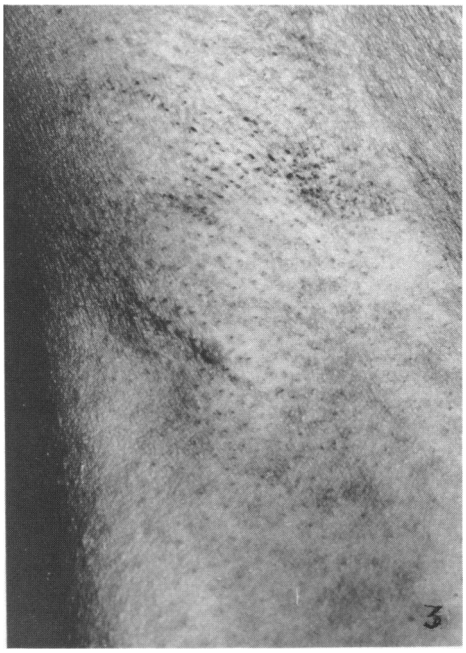

FIGURE 2. Follicular accentutation and plug in L.T.Y., a 9-year-old boy.

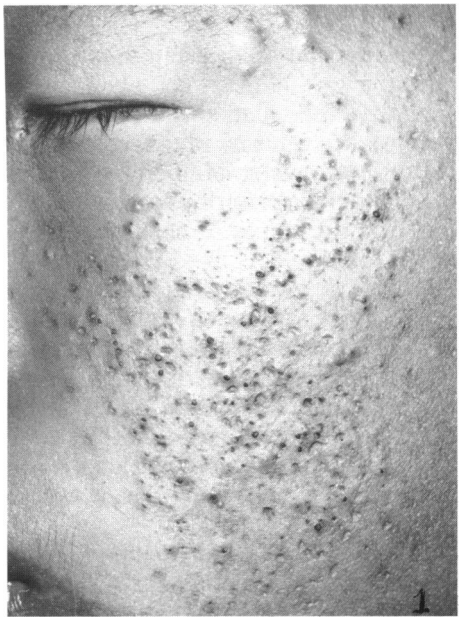

FIGURE 3. Comedo, acne and scarring in L. F. M., a 16-year-old boy.

section is shown in Table 5. The distribution of changes was tested statistically by chi-square test as follows: horny plugs in axillary cavities and on extremities; comedo formation on cheek, forehead, nose, chin, submandibular region, ear, trunk and seborrheic area; acneform eruptions on cheek, forehead, chin, submandibular region, trunk and seborrheic area; and cyst formation on external genitalia.

Each cutaneous change is described and discussed.

Follicular Accentuation and Horny Plug. The hair follicles became accentuated and elevated, and the orifices enlarged and plugged with blackish keratinous material (Fig. 2). The lesions were prominent in the axillary cavities and on the extremities (Table 6), especially on the extensor aspects. The prevalence of this change was similar in both sexes, but was seen with more frequency in the younger group (below 20 years of age), and the sites most often affected being cheeks, axillary cavities and extensor of extremities (Table 6).

Comedo Formation. Comedo formation was distributed at sites of predilection of ordinary comedones, namely, cheeks, forehead, nose and chin, but development of this eruption at ears, axillary cavities, external genitalia and extremities (Fig. 3) was one of the characteristics of this poisoning (Table 7). The seborrheic area is the favorite site of ordinary comedo, but this tendency was not seen in our series. As shown in Table 7, the comparison between males and females indicates that the prevalence in the submandibular region and trunk was higher in males, and a similar comparison between children and adults showed significant differences in the prevalence at these sites and at

Table 6. Prevalence of horny plugs.

Localization	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Cheek	29 (16.2)	18 (16.5)	29 (20.4)	7 (6.7)*
Forehead	23 (12.8)	16 (14.7)	20 (14.1)	7 (6.7)
Nose	11 (6.1)	3 (2.8)	8 (5.6)	2 (1.9)
Chin	9 (5.0)	7 (6.4)	8 (5.6)	5 (4.8)
Submandibular	10 (5.6)	3 (2.8)	7 (4.9)	4 (3.8)
Ear	14 (7.8)	5 (4.6)	12 (8.5)	4 (3.8)
Trunk	15 (8.4)	6 (5.5)	10 (7.0)	7 (6.7)
Seborrheic area	18 (10.1)	6 (5.5)	13 (9.2)	8 (7.6)
Axilla	21 (11.7)	14 (12.8)	26 (18.3)	7 (6.7)†
External genitalia	3 (1.7)	1 (0.9)	2 (1.4)	2 (1.9)
Extremities				
Upper	16 (8.9)	11 (10.1)	19 (13.4)	3 (2.9)*
Lower	14 (7.8)	12 (11.0)	21 (14.8)	2 (1.9)*
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)

*Significant by chi-square test, $p < 0.005$.
†Significant by chi-square test, $p < 0.01$.

the chin. The percentages of comedo formation in children and females were almost the same. There were two types of comedo (Figs. 3 and 4), but the black comedo was the primary one, and the size varied from pinhead size to rice grain size. After the removal of the black comedo, depressed scars usually remained (Fig. 3).

Acneform Eruptions. The development of acneform eruption was less frequent than the occurrence of comedo formation (Tables 4 and 5), while both lesions occurred with similar frequency in the submandibular region, trunk including seborrheic area, external genitalia and the extremities. The comparisons between males and females show the same trend as comedo formation, but the development of acneform eruption was more prominent in adults, especially on the cheeks, chin, submandibular region and trunk (Table 8). It could be that the increased secretion of sebum due to maturity was one of the main factors.

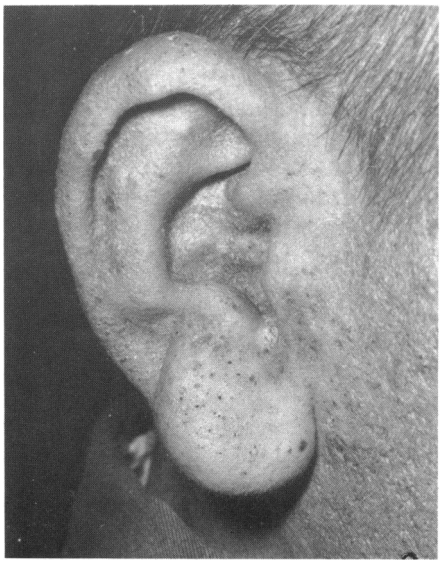

FIGURE 4. Follicular plug and comedo in C. C. K., a 16-year-old boy.

Table 7. Prevalence of comedo.

Localization	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Cheek	129 (72.1)	81 (74.3)	104 (73.2)	78 (74.3)
Forehead	100 (55.9)	69 (63.3)	89 (62.7)	57 (54.3)
Nose	43 (24.0)	32 (29.4)	33 (23.2)	33 (31.4)
Chin	66 (36.9)	36 (33.0)	39 (27.5)	51 (48.6)*
Submandibular	55 (29.6)	18 (16.5)*	19 (13.4)	41 (39.0)*
Ear	48 (26.8)	24 (22.0)	41 (28.9)	25 (23.8)
Trunk	53 (29.6)	19 (17.4)‡	25 (17.6)	34 (32.4)†
Seborrheic area	23 (12.8)	17 (15.6)	21 (14.8)	11 (10.5)
Axilla	7 (3.9)	5 (4.6)	8 (5.6)	2 (1.9)
External genitalia	7 (3.9)	1 (0.9)	5 (3.5)	2 (1.9)
Extremities				
Upper	10 (5.6)	3 (2.8)	7 (4.9)	3 (2.9)
Lower	15 (8.4)	6 (5.5)	10 (7.0)	8 (7.6)
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)

*Significant by chi-square test, $p < 0.005$.
†Significant by chi-square test, $p < 0.01$.
‡Significant by chi-square test, $p < 0.05$.

Table 8. Prevalence of acne.

Localization	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Cheek	55 (30.7)	28 (25.7)	33 (23.2)	37 (35.2)‡
Forehead	38 (21.2)	24 (22.0)	29 (20.4)	24 (22.9)
Nose	7 (3.9)	7 (6.4)	6 (4.2)	6 (5.7)
Chin	25 (14.0)	13 (11.9)	11 (7.7)	18 (17.1)‡
Submandibular	48 (26.8)	10 (9.2)*	8 (5.6)	33 (31.4)*
Ear	10 (5.6)	4 (3.7)	8 (5.6)	6 (5.7)
Trunk	50 (27.9)	19 (17.4)‡	21 (14.8)	37 (35.2)*
Seborrheic area	21 (11.7)	15 (13.8)	18 (12.7)	12 (11.4)
Axilla	4 (2.2)	2 (1.8)	2 (1.4)	4 (3.8)
External genitalia	6 (3.4)	2 (1.8)	2 (1.4)	5 (4.8)
Extremities				
Upper	10 (5.6)	2 (1.8)	6 (4.2)	4 (3.8)
Lower	11 (6.1)	5 (4.6)	11 (7.7)	3 (2.9)
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)

*Significant by chi-square test, $p < 0.005$.
‡Significant by chi-square test, $p < 0.05$.

Cyst Formation. Some lesions enlarged to rice grain- to pea-sized cysts (Figs. 5 and 6), but the prevalence was much smaller than that of comedo or acne formation (Table 4 and 5). The development on external genitalia, including pubic area, especially in male adults, was characteristic. The difference in distribution of cyst formation between males and females, and between children and adults is illustrated in Table 9, the prevalence being higher for external genitalia and chin in males; for axillary cavities in females; and for chin, trunk and external genitalia in adults.

The follicular keratotic changes were often complicated by bacterial infection, with formation of painful and inflammed atheromalike abscesses or pustules (Fig. 5). After the disappearance of follicular keratotic changes, these lesions remained as numerous depressed atrophic scars (Figs. 3 and 6). In our series, secondary infection occurred most frequently in the 11 to 20 year age group.

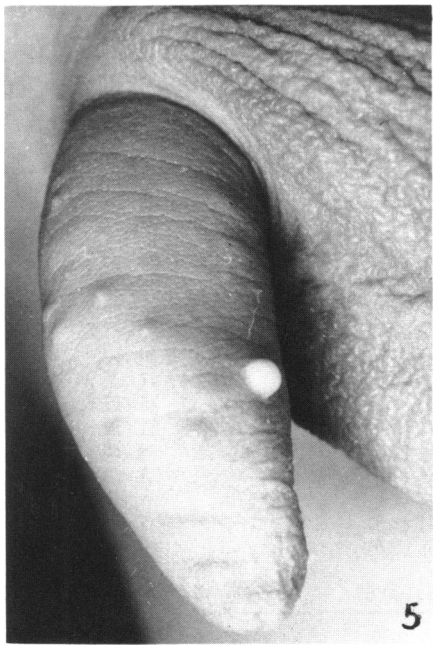

FIGURE 5. Cyst and pustule in J. H. H., a 6-year-old boy.

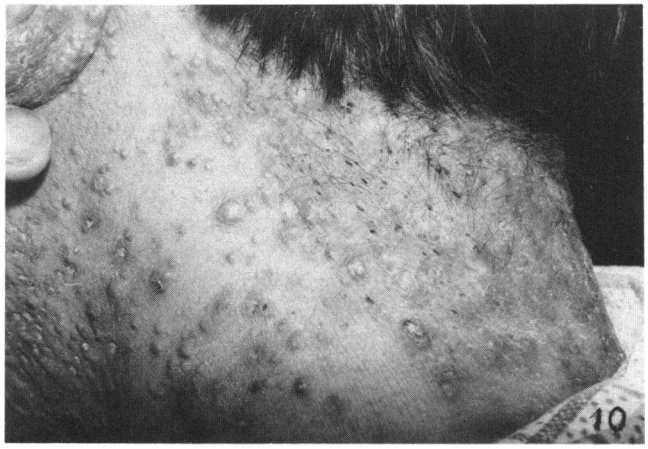

FIGURE 6. Comedo, acne, cyst and scarring at nape of neck and submandibular region in W. C. S., a 36-year-old male.

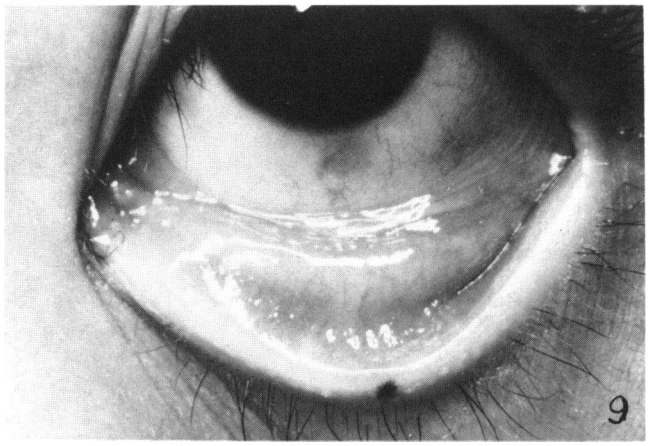

FIGURE 7. Enlargement of Meibomian glands and pigmentation of palpebral conjunctiva in K. T. M., a 10-year-old girl.

Table 9. Prevalence of cysts.

Localization	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Cheek	12 (6.7)	12 (11.0)	8 (5.6)	12 (11.4)
Forehead	9 (5.0)	5 (4.6)	4 (2.8)	8 (7.6)
Nose	4 (2.2)	4 (3.7)	4 (2.8)	2 (1.9)
Chin	18 (10.1)	3 (2.8)‡	1 (0.7)	6 (5.7)*
Submandibular	10 (5.6)	3 (2.8)	4 (2.8)	8 (7.6)
Ear	17 (9.5)	11 (10.1)	12 (8.5)	12 (11.4)
Trunk	20 (11.2)	5 (4.6)	8 (5.6)	15 (14.3)‡
Seborrheic area	10 (5.6)	5 (4.6)	5 (3.5)	5 (4.8)
Axilla	1 (0.6)	5 (4.6)‡	3 (2.1)	1 (1.0)
External genitalia	33 (18.4)	2 (1.8)*	7 (4.9)	19 (18.1)*
Extremities				
Upper	3 (1.7)	1 (0.9)	2 (1.4)	1 (1.0)
Lower	3 (1.7)	1 (0.9)	3 (2.1)	1 (1.0)
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)

*Significant by chi-square test, $p < 0.005$.
‡Significant by chi-square test, $p < 0.05$.

Table 10. Prevalence of other keratotic signs.

Symptom	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Xeroderma	26 (14.5)	26 (23.9)‡	31 (21.8)	10 (9.5)
Sudaminalike	1 (0.6)	4 (3.7)	3 (2.1)	1 (1.0)
Keratotic plaque	10 (5.6)	10 (9.2)	9 (6.3)	6 (5.7)
Meibomian swelling	34 (19.0)	18 (16.5)	25 (17.6)	16 (15.2)
Eyelid edema	104 (58.1)	82 (75.2)*	84 (59.2)	73 (69.5)
Nail deformity	62 (34.8)	46 (42.2)	56 (39.4)	33 (31.4)
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)

*Significant by chi-square test, $p < 0.0005$.
‡Significant by chi-square test, $p < 0.05$.

Enlargement of Meibomian Glands and Edema of the Eyelids. Increased discharge from the eyes was the most frequent subjective complaint (Table 3). The obstruction of the Meibomian glands was white or yellow, elevated dots at the brim of the eyelids in the beginning (Fig. 7) and enlarged cysts later. The prevalence of this gland enlargement was 15 to 20% as shown in Table 10. It caused irregularity of the lid margin.

The swelling of eyelids was another frequent subjective complaint (Table 3), and most patients, especially females, showed this symptom (Table 10).

Xeroderma. Dryness of skin was more frequent in children and females (Table 10). The skin was coarse to the touch and had very fine, diffuse, branlike scales. This phenomenon was usually combined with accentuation of hair follicles and horny plugs.

Sudaminalike Eruption. The obstruction of hair follicles and hyperkeratosis at the vestibulum of hair follicle orifices caused sudaminalike eruptions in some cases (Table 10), and many fine vesicular red eruptions densely grouped as patches. They were frequently observed at flanks, waists, lateral aspects of thighs, the anterior aspect of knees or the lateral aspect of upper extremities.

Keratotic Plaques. Tylotic, slightly yellowish thickening of the skin was observed at the eminences of palms and soles (Fig. 8). It was usually present in severe cases, with a prevalence of 5 to 10% (Table 10).

Deformity of Nails. Due to the abnormal keratotic condition, nail deformities occurred in many cases; the prevalence was 30 to 40% (Table 10). Both lateral edges of the nail were concave and entered the paronychial grooves deeply as an ingrowing nail, and the natural concavity of the nail body was obliterated. This change was most frequently observed in the big toe; severe cases showed koilonychial change (Fig. 9).

Pigmentation. This is a specific change of chronic PCB poisoning. It occurred in mucosa, nails and skin (Table 11). The hue varied from brown to brownish gray to gray, and the tint also varied from light to deep. The mucosal pigmentation had a violet or blue hue in some cases.

The intensity of color of the mucosa varied within the individual. Some patients showed the same shade of color at every site; others had a deeper color on the

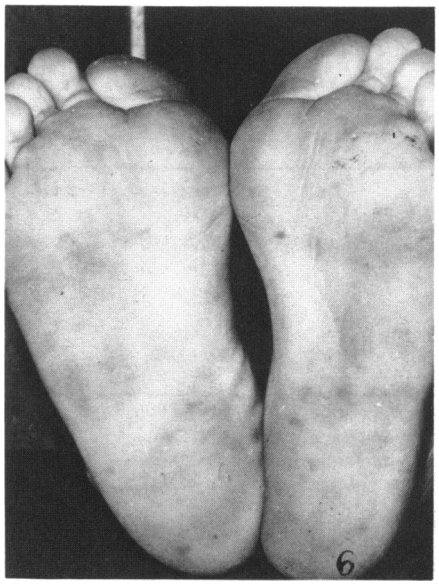

FIGURE 8. Keratotic plaque in C. C. J., a 15-year-old boy.

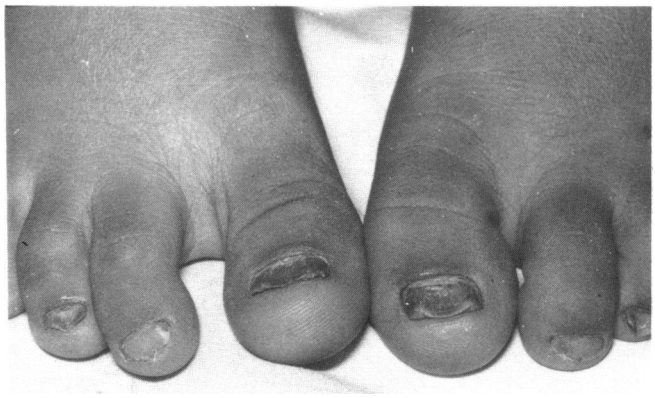

FIGURE 9. Pigmentation and koilonychial changes in toenail in H. T. I., a 7-year-old boy.

Table 11. Pigmentation by age and tissue.

Tissue	Age 0–10	Age 11–20	Age 21–30	Age 31–40	Age 44–50	Age › 51	Total	%
Mucosa	46	86	73	34	37	45	321	90
Nail	47	83	64	32	35	46	307	86
Skin	37	76	55	27	28	34	257	72
Total no. of patients	50	94	81	42	37	54	358	100

Table 12. Prevalence of pigmentation.

Localization	No. (%) of adults		No. (%) of children	
	Male	Female	Male	Female
Conjunctiva	36 (52.2)	19 (52.8)	61 (76.3)	47 (75.8)
Lip	16 (23.2)	6 (16.7)	35 (43.8)	31 (50.0)
Gingiva	49 (71.0)	30 (83.3)	67 (83.8)	56 (90.3)
Nose	27 (39.1)	26 (72.2)*	59 (73.8)	44 (71.0)
Whole body	11 (15.9)	1 (2.8)‡	15 (18.8)	7 (11.3)
Fingernail	51 (73.9)	33 (91.7)‡	70 (87.5)	59 (95.2)
Toenail	46 (66.7)	33 (91.7)†	68 (85.0)	57 (91.9)
Total	69 (100.0)	36 (100.0)	80 (100.0)	62 (100.0)

*Significant by chi-square test, $p < 0.005$.
†Significant by chi-square test, $p < 0.01$.
‡Significant by chi-square test, $p < 0.05$.

Table 13. Prevalence of pigmentation.

Localization	No. (%) of patients		No. (%) of patients	
	Male	Female	Children	Adults
Conjunctiva	115 (64.2)	74 (67.9)	108 (76.1)	55 (52.4)*
Lip	63 (35.2)	46 (42.2)	66 (46.5)	22 (21.0)*
Gingiva	141 (78.8)	96 (88.1)‡	123 (86.6)	79 (75.2)‡
Nose	98 (54.8)	77 (70.6)†	103 (72.5)	53 (50.8)*
Whole body	31 (17.3)	11 (10.1)	22 (15.5)	12 (11.4)
Fingernail	150 (83.8)	104 (95.4)*	129 (90.8)	84 (80.0)‡
Toenail	138 (77.1)	101 (92.7)*	125 (88.0)	79 (75.2)**
Total	179 (100.0)	109 (100.0)	142 (100.0)	105 (100.0)*

*Significant by chi-square test, $p < 0.005$.
†Significant by chi-square test, $p < 0.01$.
‡Significant by chi-square test, $p < 0.05$.
**Significant by chi-square test, $p < 0.001$.

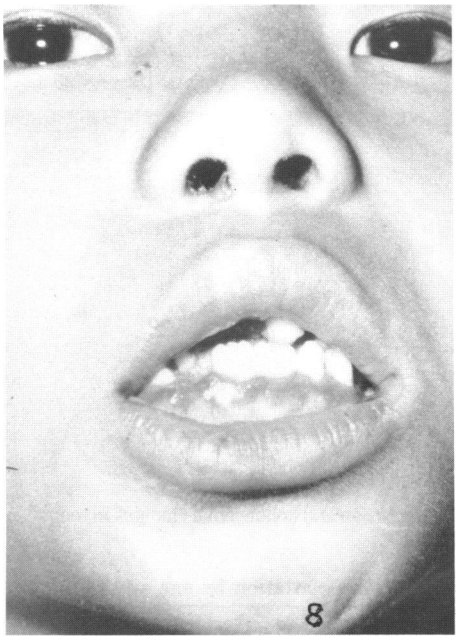

FIGURE 10. Pigmentation of the lower lip and gingiva and infection of nostril in W. S. C., an 11-year-old girl.

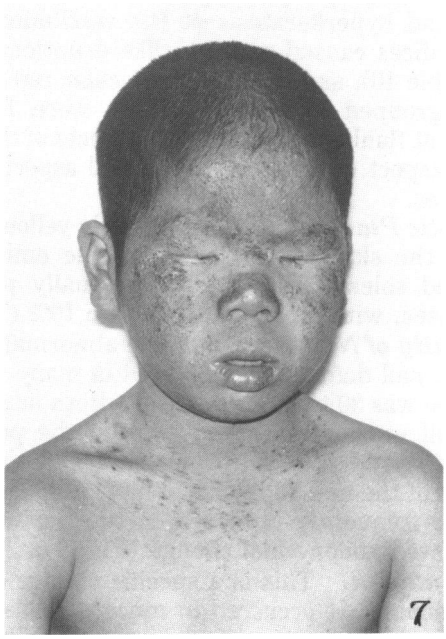

FIGURE 11. Pigmentation of the nasal tip and lower lip in L. W. T., a 10-year-old boy.

gingivae and a light color on the conjunctiva or vice versa.

Gingival pigmentation was most frequent, and the prevalence was as high as 93% in girls younger than 10 years of age although the rate of this pigmentation among healthy persons is known to be near 10%. The average prevalence was 82.3%, being higher among females, especially among female children (Table 12).

The pigmentation was a wide band formed on the portion of gingivae in diffuse contact with teeth (Fig. 10).

Conjunctival pigmentation occurred in two-thirds of the cases. Its prevalence followed the same trend as gingival pigmentation, in that it was very high in the younger group, especially among girls younger than 10 years of age. It occurred on every region of the conjunctival mucosa and more frequently on the lower palpebral conjunctiva.

Lip pigmentation occurred in one-third of the cases and more frequently among children (Table 12). It was prominent in the vermillion of the lower lip (Figs. 10 and 11) and showed diffuse, spotty, linear or mottled patterns. Of skin pigmentation, pigmentation on the nasal tip was characteristic and had high prevalence (63% in all follow-up cases). It was less frequent in adult males (Tables 12 and 13, Fig. 11).

Diffuse brownish-gray pigmentation over the whole body occurred in a small proportion of cases and was frequent in male patients (Tables 12 and 13). It was more prominent at extensor aspects such as knee and elbow, or at the sites of skin eruption.

Nail pigmentation was a very characteristic feature of chronic PCB poisoning and had the highest prevalence of all the clinical signs (Table 12). It appeared diffusely over the whole nail body and skin surrounding the nail (Figs. 9 and 12).

The severity of cutaneous lesions in hyperkeratosis and pigmentation were not parallel. Some patients with a very high blood PCB concentration and severe pigmentation had no other cutaneous eruption.

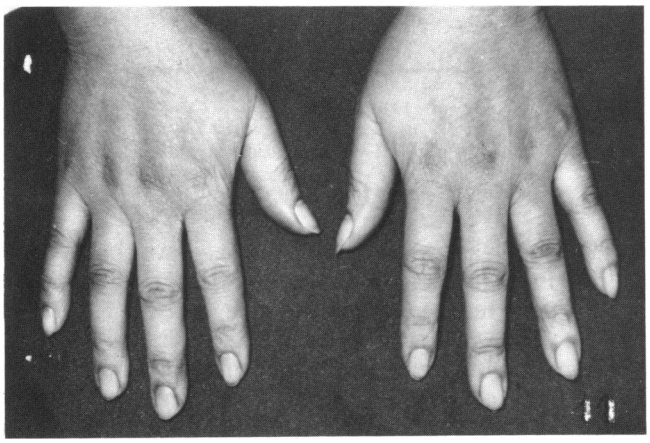

FIGURE 12. Pigmentation on the periungual region in L. Y. K., a 40-year-old female.

Grading of Disease Severity According to Clinical Signs

In the special clinics, the patients were diagnosed clinically on the basis of a positive history of exposure to the specific rice-bran oil as well as on the nature and the extent of mucocutaneous lesions.

Those patients who had a positive history and ocular symptoms, such as hypersecretion, swelling of the eyelids and enlargement of Meibomian glands, were very suggestive of having PCB poisoning and were classified as Grade I° (Table 14). In Grade I, patients presented only pigmentary changes on the mucosa and skin without developing follicular lesions. The patients in Grade II manifested localized comedones and accentuation of the hair follicles. In Grade III, the patients had localized acneform inflammatory lesions with or without external genital cysts. The patients in Grade IV showed the most prominent cutaneous lesions, with generalized acneform or keratotic follicular eruptions and were frequently associated with secondary bacterial infection (Table 14).

When classified in this way, as shown in Table 15, Grade I° included 24 cases (6.7%); Grade I, 132 cases (36.7%); Grade II, 65 cases (18.2%); Grade III, 97 cases (27.2%); and Grade IV, 40 cases (11.2%). The females outnumbered the males in the lighter grades (I°, I and II), the ratio being 1 to 0.71. However, in Grades III and IV, respectively, the number of male patients was 1.02 and 3 times higher than females. When age was considered, patients 11 to 30 years of age had a higher proportion in Grades III and IV, being 49 and 47%, respectively (Table 16). The patients under 10 years of age and above 50 were mainly in the lighter grades (I°, I and II), being 72 and 78% respectively. This tendency

Table 14. Grading according to major clinical signs.

Grade	Clinical signs
I°	Only ocular signs
I	I° + pigmentary changes only
II	I + comedones and follicular accentuation
III	II + localized acneform lesions and cysts
IV	III + widespread acneform lesions and generalized follicular accentuation, often associated with secondary infections

Table 15. Distribution of patients by clinical grade and age or sex.

Grade	Age 0–10	Age 11–20	Age 21–30	Age 31–40	Age 41–50	Age >50	Male	Female	Total	%	Male: Female
I°	2	4	7	6	1	4	17	7	24	6.7	
I	18	26	26	14	17	31	49	83	132	36.7	0.71:1
II	16	18	10	7	7	7	26	39	65	18.2	
III	11	32	28	9	9	8	49	48	358	27.2	1.02:1
IV	3	14	10	6	3	4	30	10	40	11.2	3:1
Total	50	94	81	42	37	54	171	187	358	100.0	

suggests that the severity of poisoning among patients 11 to 30 years of age, especially among males, may be caused by larger amounts of daily food intake, namely, they ingested larger amounts of the contaminated rice-bran oil.

A total of 89 cases in the special clinics were followed for 8 to 17 months (average 11.5 months) clinically, and the change of disease severity is shown in Tables 17 and 18. Most of the cases (53.9%) remained at the same grade, and 38.2 and 7.9% of the patients showed decreased severity and increased severity of disease, respectively. Many possible methods were tried for the treatment of these patients in the special clinics, but there was bitter disappointment at the lack of success, so the number of follow-up cases rapidly decreased thereafter. Although there are no reliable records, the general condition, including cutaneous changes, seemed somewhat improved in cases observed 3 years later.

The relationship between the severity of disease and blood PCB concentration is shown in Table 19. There was no notable association. Figure 13 shows the change of blood PCB in these patients. The interval between the first and the second quantitative analysis for PCB, was 72 and 453 days, the average being 293.58 days. The variation of individual PCB concentrations was very large, so no conclusions could be drawn from these results.

The quantitative analysis of PCBs in the patients' blood was continued during these years. At the Chang-Hua Prefecture, PCB concentration of the blood in 83 cases 1 year after onset and that in 17 cases 3 years later were 4 to 558 (average 149.5) and 2 to 161 (average 53.6) ppb, respectively.

Table 16. Distribution of patients by age and grade group in percent.

Grade	Age 0–10	Age 11–20	Age 21–30	Age 31–40	Age 41–50	Age >50
I° + I + II	72	51	53	65	68	78
III + IV	28	49	47	35	32	22

Table 17. Change of grade of severity of symptoms.

		Last				
Visit	Grade	I°	I	II	III	IV
First	I°	2				
	I		9	3		
	II		5	17	1	
	III		5	17	14	3
	IV			3	4	6

Table 18. Outcome of disease severity.

Outcome	No.	%
Improved	34	38.2
Stationary	48	53.9
Exacerbated	7	7.9
Total	89	100.0

Table 19. Disease severity and PCB concentration.

Grade	No. of patients	PCB concentration, ppb Highest	Lowest	Mean ± SD	*p*-Value
I°	3	157	88	116.7 ± 36.0	
I	22	316	6	72.4 ± 72.7	NS
II	39	347	8	73.3 ± 72.3	NS
III	50	152	1	71.4 ± 30.2	NS
IV	20	128	18	77.8 ± 29.7	NS

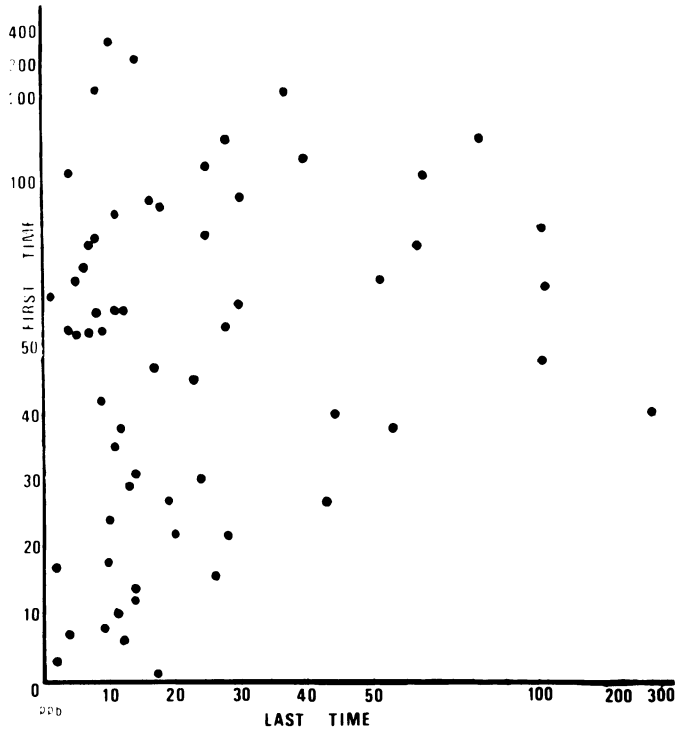

FIGURE 13. Change of PCB concentration in patients' blood with time.

Immunological Studies

Albumin, Globulin, and Immunoglobulin. This study was carried out by Chang et al. in the first year (*18*). As shown in Table 20, α_2-globulin in serum of patients was mildly increased (0.72 ± 0.20 g%), while the γ-globulin level was mildly decreased (1.17 ± 0.39 g%). In view of this result, some suppression in humoral immunity may be suspected.

Table 20. serum albumin and serum globulin.

	Albumin, g-%	Globulin, g-% α_1	α_2	β	γ
PCB patients	4.39 ± 0.48	0.25 ± 0.07	0.72 ± 0.20	0.75 ± 0.16	1.17 ± 0.39
Controls	4.06 ± 0.84	0.26 ± 0.07	0.67 ± 0.09	0.82 ± 0.09	1.32 ± 0.19

The concentration of blood immunoglobulins was also studied by Chang et al. (*19*). Table 21 shows the serum levels of immunoglobulin in the patients and normal control. Significant decreases in serum IgA and IgM were noted in the poisoning group ($p < 0.01$ and $p < 0.001$, respectively), while the concentration of IgG was in normal range. These data also suggest the suppression in humoral immunity by PCB poisoning.

Skin Testing. Delayed hypersensitivity was studied by skin testing with recalling antigens. An intracutaneous test with a solution containing streptokinase and streptodornase was performed in 143 patients by Chang et al. in the first year (*17*). As illustrated in Table 22, it showed a significant low positive rate (35.7%) in the patients.

Tuberculin tests have been done twice, in 83 patients the first year and in 26 patients 3 years later. As shown in Table 23, the positive rate during the first year in PCB-poisoned individuals was 48.2%, while that in healthy controls was 73.7%. These data suggest that suppression of cellular immunity occurred in the patients. Three years later, positive response of PPD skin test increased to 61.5% in PCB patients, suggesting some recovery of cellular immunity in PCB-poisoned patients.

Table 21. Serum immunoglobulin levels.

	IgG, mg-%	IgA, mg-%	IgM, mg-%
Controls (n = 23)	1377 ± 214	245 ± 70	173 ± 48
PCB patients (n = 30)	1469 ± 566	185 ± 88	105 ± 58
p-value[a]	NS	< 0.01	< 0.001

[a]Significance by Mann-Whitney test.

Table 22. Skin test with SK/SD antigen.[a]

	No. tested	Positive No.	Positive %	p-Value (chi-square)
PCB patients	143	51	35.7	
Controls	300	238	79.0	< 0.05

[a]Contains streptokinase (5 μg) and streptodornase (0.25 μg) in 0.1 mL.

Table 23. Tuberculin (PPD) skin test.

Subject	No. tested	Positive No.	Positive %	p-Value (chi-square)
PCB patients 1st year	83	40	48.2	< 0.01
PCB patients 4th year	26	16	61.5	< 0.05
Controls	38	28	73.7	

Lymphocytes in Peripheral Blood. By using different rosette techniques to enumerate the percentage of lymphocyte subpopulation, the percentage of total T-cells, active T-cells and T_μ-cells (helper T-cells) were shown to be decreased as relative to controls (Table 24). The number of total lymphocytes and the percentage of B-cells and T_γ-cells (suppressor T-cells) were not affected. This study was performed in the first year (*18*).

A newly developed method, a monoclonal antibody technique, was used to identify the lymphocyte subset 3 years later. As shown in Table 24, the percentage of total T-cells by the E rosette method and OKT-3 were recovered. The percentage of OKT-4 (helper T-cells) was still low but that of OKT-8 (suppressor T-cells) increased. Thus the immunoregulating index (percentage of OKT-4/OKT-8) in PCB patients was lower than that of healthy control (1.2 ± 0.4 vs. 1.9 ± 0.4). These data suggest that the cellular immunity in PCB victims recovered partially.

Lymphocyte Proliferation Test. This test was performed in the first year with the culture media of supplemented RPMI-1640 containing 10% fetal calf serum. The spontaneous proliferation of lymphocytes of PCB patients was slightly enhanced but not statistically significantly. Among the tests stimulated by various mitogens, the response to PHA (phytohemagglutinin) and PWM (pokeweed mitogen) showed some increase, but the response to ConA (Concanavalin A) was not significant (Table 25). The test stimulated by PPD (tuberculin) was also done in the first year, and the response was also significantly enhanced (Table 26).

The lymphocyte proliferation test was studied again 3 years later, with AB serum instead of fetal calf serum being used in the culture media. As shown in Tables 27 and 28, the enhancement of spontaneous proliferation of lymphocytes in PCB patients was very significant, and

Table 24. Total lymphocyte count and percentage of subpopulation.

	1st year Control (n = 23)	1st year PCB patients (n = 30)		4th year Control (n = 27)	4th year PCB patients (n = 30)
Total lymphocytes/mm^3	3005 ± 971	3103 ± 908			
Active T-cells	22.1 ± 4.4	11.3 ± 6.7**	OKT-3	70.7 ± 7.4	70.6 ± 11.0
T-cells	63.3 ± 9.5	41.7 ± 16.3**	T-cell	71.0 ± 3.6	71.5 ± 5.9
B-cells	26.6 ± 6.1	28.9 ± 7.1	B-cell	17.0 ± 2.6	13.9 ± 5.4
T_μ-(helper)	36.9 ± 12.1	21.6 ± 6.9**	OKT-4	45.0 ± 6.4	36.1 ± 12.2*
T_γ-(suppressor)	24.9 ± 8.7	22.2 ± 8.7	OKT-8	24.8 ± 3.2	30.9 ± 8.3*
			OKIa	13.7 ± 5.0	11.0 ± 3.9‡
			T4/T8	1.9 ± 0.4	1.2 ± 0.4*

**Significant by Mann-Whitney U test, $p < 0.001$.
*Significant by Mann-Whitney U test, $p < 0.005$.
‡Significant by Mann-Whitney U test, $p < 0.05$.

Table 25. Lymphocyte proliferation at third culture day, stimulated by various mitogens, first year.

	Lymphocyte proliferation, cpm ± SEM	
	Control (n = 35)	PCB patients (n = 83)
Blank	893 ± 110	1,466 ± 238
PHA[a]	224,211 ± 30,244	294,143 ± 15,550‡
	(SI = 351 ± 60)	(SI = 388 ± 46)[d]
ConA[b]	210,677 ± 24,910	257,334 ± 14,934
	(SI = 338 ± 55)	(SI = 310 ± 34)
PWM[c]	61,960 ± 9,910	82,489 ± 6,482‡
	(SI = 98 ± 17)	(SI = 98 ± 10)

[a]PHA = 1:25 dilution, Difco, M form.
[b]ConA = 20 μg/mL.
[c]PWM = 1:100 dilution, Gibco.
[d]Stimulation index (SI) = (absolute Δ cpm - blank Δ cpm)/blank Δ cpm.
‡Significant, $p < 0.05$.

Table 26. Lymphocyte proliferation stimulated by tuberculin (100 μg/mL) at fifth and seventh culture day, first year.

	Lymphocyte proliferation, cpm ± SEM	
	Controls (n = 32)	PCB patients (n = 60)
Day 5		
Blank	2,971 ± 487	4,756 ± 929
PPD	12,228 ± 3,196	27,375 ± 5,053*
	(SI = 3.65 ± 0.67)[a]	(SI = 7.10 ± 1.31)‡
Day 7		
Blank	10,006 ± 1,891	13,481 ± 2,237
PPD	19,209 ± 4,353	43,354 ± 7,318†
	(SI = 2.31 ± 0.37)	(SI = 4.92 ± 0.81)‡
Maximum		
Blank	10,087 ± 1,887	13,616 ± 2,227
PPB	22,316 ± 4,740	49,633 ± 7,689‡
	(SI = 2.97 ± 0.47)	(SI = 667 ± 1.08)‡

[a]Stimulation index = (absolute Δ cpm - blank Δ cpm)/blank Δ cpm.
*Significant, $p < 0.005$.
†Significant, $p < 0.01$.
‡Significant, $p < 0.05$.

Table 27. Lymphocyte proliferation stimulated with various mitogens, after three years.

	Lymphocyte proliferation, cpm ± SEM	
	(n = 14)	(n = 30)
Blank	195 ± 37	761 ± 117*
PHA	64,613 ± 6,764	252,886 ± 25,723*
	(SI = 485 ± 92)[a]	(SI = 553 ± 104)
ConA	79,296 ± 11,249	185,300 ± 26,702†
	(SI = 568 ± 109)	(SI = 400 ± 88)
PWM	29,021 ± 3,476	57,789 ± 8,213‡
	(SI = 200 ± 35)	(SI = 114 ± 22)‡

[a]Stimulation index = (absolute Δ cpm - blank Δ cpm)/blank Δ cpm.
*Significant, $p < 0.005$.
†Significant, $p < 0.01$.
‡Significant, $p < 0.05$.

the tests stimulated with PHA, ConA, PWM and PPD also revealed significant enhancement of lymphocyte proliferation. This may be an abnormal rebound phenomenon caused by sublethal immunotoxic dosages of PCB and its derivatives, but this hypothesis should be studied.

In summary, at present in Yu-Cheng patients the positive rate of the tuberculin test recovered somewhat with time. The total number of T-cells returned to normal. The suppressor T-cells increased, but helper T-cells were still lower, so the immunoregulating index (OKT-4 /OKT-8) was still very low. The number of B-cells remained in the normal range. Lymphocyte proliferation stimulated by various mitogens is still enhanced.

Table 28. Lymphocyte proliferation stimulated by tuberculin at fifth and seventh culture day, after three years.

	Lymphocyte proliferation, cpm ± SEM	
	Controls (n = 8)	PCB patients (n = 24)
Day 5		
Blank	322 ± 73	1,070 ± 209‡
PPD	1,763 ± 1,024	30,682 ± 8,773‡
	(SI = 6.54 ± 4.15)[a]	(SI = 31.12 ± 8.07‡
Day 7		
Blank	134 ± 39	1,870 ± 571‡
PPD	525 ± 186	20,177 ± 4,532†
	(SI = 3.85 ± 0.85)	(SI = 14.65 ± 3.51)‡
Maximum		
Blank	338 ± 73	1,872 ± 492‡
PPD	1,832 ± 1,018	34,774 ± 8,681‡
	(SI = 7.21 ± 4.10)	(SI = 30.95 ± 8.00)†

[a]Stimulation index = (absolute Δ cpm - blank Δ cpm)/blank Δ cpm.
†Significant, $p < 0.01$.
‡Significant, $p < 0.05$.

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REFERENCES

1. Ko, Y. C., Chang, C. E., Liu, S. S., and Hu, H. T. Epidemiological study of PCB poisoning. J. Formosan Med. Assoc. 80: 406–417 (1981).
2. Ko, Y. C., Jao, L. T., Cheng, C. T., Hsu, S. T., Hsiao, H. C., and Hu, H. T. The blood level of PCB in the poisoned patients. J. Formosan Med. Assoc. 80: 774–779 (1981).
3. Lee, Y. Y., Lü, Y. C., and Chen, J. S. An endemic dermatosis due to PCB intoxication. A brief communication. J. Formosan Med. Assoc. 79: 357–359 (1980).
4. Lee, Y. Y., Wong, P. N., Lü, Y. C., Sun, C. C., Wu, Y. C., Lin, R. Y., Jee, S. H., Ng, K Y., and Yeh, H. P. An outbreak of PCB poisoning. J. Dermatol. 7: 435–441 (1980).
5. Lü, Y. C. Skin change of chronic PCB poisoning. J. Formosan Med. Assoc. 79: 856 (1980).
6. Goto, M., and Higuchi, K. The symptomatology of Yusho (PCB poisoning) in dermatology. Fukuoka Acta Med. 60: 409–431 (1969).
7. Higuchi, K. PCB Poisoning and Pollution. Kodansha, Tokyo, 1976.
8. Masuda, Y., Kagawa, R., Shimamura, K., Takada, M., and Kuratsune, M. PCB in the blood of Yusho patients and ordinary persons. Fukuoka Acta Med. 65: 25–27 (1974).
9. Urabe, H., Koda, H., and Asahi, M. Present state of Yusho patients. Ann. N. Y. Acad. Sci. 320: 273–276 (1979).
10. Chang, K. J., Lu, F. J., Tung, T. C., and Lee, T. P. Studies of patients with PCB-poisoning (2). Determination of urinary corpoporphyrin, uroporphyrin, δ-aminolevulinic acid and prophobilinogen. Res. Commun. Chem. Pathol. Pharmacol. 30: 547–553 (1980).
11. Mancini, G., Carbonara, A. O., and Heremans, J. F. Immuno-

chemical quantitation of antigens by single radical immunodiffusion. Int. J. Immunochem. 2: 235–254 (1965).
12. Böyum, A. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21(Suppl. 97): 77–89 (1966).
13. Kerman, R., Smith, R., Ezdinli, E., and Stefani, S. S. Unification and technical aspects of total T, active T and B-lymphocyte rosette assays. Immunol. Commun. 5: 685–694 (1976).
14. Moretta, L., Ferrarini, M., Durante, M. L., and Mingari, M. C. Expression of a receptor for IgM by human T cells in vitro. Eur. J. Immunol. 5: 565–569 (1975).
15. Reinherz, E. L., Kung, P. C., Goldstein, G., and Schlossman, S. F. Separation of functional subsets of human T cells by a monoclonal antibody. Proc. Natl. Acad. Sci. (U. S.) 76: 4051–4055 (1979).
16. Reinherz, E. L., Kung, P. C., Goldstein, G., and Schlossman, S. F. Further characterization of the human inducer T cell subset defined by monoclonal antibody. J. Immunol. 123: 2894–2896 (1979).
17. Penhale, W. J., Farmer, A., Maccuish, A. C., and Irvine, W. J. A rapid micro-method for the phytohemagglutinin induced hyman lymphocyte transformation test. Clin. Exp. Immunol. 18: 155–167 (1974).
18. Chang, K. J., Chen, J. S., Huang, P. C., and Tung, T. C. Study of patients with PCB poisoning. I. Blood analysis of patients. J. Formosan Med. Assoc. 79: 303–313 (1980).
19. Chang, C. J., Hsieh, K. H., Lee, T. P., Tang, S. Y., and Tung, T. C. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. Toxicol. Appl. Pharmacol. 61: 58–63 (1981).