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Respiratory Involvement and Immune Status in Yusho Patients

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Clinical and experimental studies on respiratory involvement and alterations in immune status were carried out. Respiratory distress occurring in these patients has improved gradually for 14 years but still remains.

Copious expectoration at an early stage of the disease may be related to the fact that a number of discrete polychlorinated biphenyls (PCBs) are distributed throughout the lung parenchyma. For accumulation in the bronchial mucosa, structural requirements and specific dose dependence of PCBs have been clearly shown; however, pathological and physiological studies have indicated that respiratory involvement in Yusho is mainly small airway disease that may be caused by involvement of cellular component (Clara cells) in bronchioles and/or associated infection.

Respiratory distress is often exacerbated by viral or bacterial infection. Changes in the immune status in PCB and polychlorinated dibenzofuran (PCDF) poisoning are as follows: IgA and IgM in the serum are decreased at an early stage of the disease and then return to normal; suppression of cellular immunity was reported in Taiwanese patients and some may remain in the later stages of the disease, as shown in our patients. PCDFs now appear to be the main causal agents in Yusho. Rats given PCDFs showed necrosis of the Clara cells in bronchioles and marked thymus atrophy, while few such changes were noted in rats given PCBs. Therefore, further examination is needed for the difference of the toxic effects between two compounds.

Introduction

Since 1968, patients with polychlorinated biphenyl (PCB) and other chemical poisoning began to appear throughout the western part of Japan. The cause was traced and confined to the contamination of edible rice-bran oil by PCBs used as a coolant in the manufacturing process (*1*). Since then, the disease has been called "Yusho," which means "the oil disease." Unusual skin lesions and the other symptoms in Yusho or PCB poisoning vary a great deal (*2,3*). Until a year after onset of the disease, respiratory symptoms had been neglected because of a lack of awareness of their relationship to PCB poisoning. Thereafter, we were asked to participate in a study on clinical, laboratory and pathological findings on respiratory involvement in the patients.

As a result, it has been observed that the incidence of respiratory symptoms correlated well with the concentration of PCBs in the blood and the definite PCB peaks were always detected in the sputa (*4*). Respiratory distress was often exacerbated by viral or bacterial infection, persisting for more than a half year in about half of the patients examined (*4*). Therefore, immunosuppressive activity of these toxic compounds was observed clinically and experimentally.

Respiratory distress occurring in these patients has improved gradually over 14 years but still remains; and furthermore, three interesting facts were found to add to this field of the study: first, structural requirements for accumulation in the bronchial mucosa reported by Brandt et al. (*5,6*); second, polychlorinated dibenzofurans (PCDFs) included in the rice oil can be hundreds to thousands of times more potent than PCBs or polychlorinated quaterphenyls (PCQs) as inducers of various clinical manifestations (*7*); third, the furan moiety was covalently bound preferentially to the nonciliated bronchiolar (Clara) cells (*8*). Therefore, we review the disease and report new facts in consideration of the above-mentioned problems.

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Materials and Methods

Clinical Study

A total of 401 patients with PCB poisoning form the basis of our study. They were subjected to the following examinations: an assessment of subject symptoms, chest x-rays, pulmonary function, microbial study of the sputum and determination of immunoglobulin levels and PCB concentration and type (4) in serum and sputum. The results of these examinations done prior to 1975 (7 years after the onset of the disease) have already been reported, and a pathological study done on the lungs from seven autopsy cases and from rats given PCBs was also described (4).

In this report, early subjective symptoms and physical findings characteristic to the disease were reviewed in detail, and the clinical course and the latest status in pulmonary function and immunological suppression in the patients observed in February and March 1983 were also described.

Table 1. Clinical course of respiratory symptoms (cough and sputa) in 79 cases, which were followed up for 11 years.

Symptoms	1972–1973	1974–1975	1977–1978	1983
Positive				
Exacerbated[a]	10 (21%)	13 (16%)	14 (26%)	4 (9%)
Unchanged[a]	18 (38%)	39 (49%)	18 (33%)	30 (70%)
Improved[a]	7 (15%)	15 (19%)	12 (22%)	0
Negative	12 (26%)	12 (15%)	10 (19%)	9 (21%)

[a]The change of symptoms over the course was compared with those in the late check-up year.

Table 2. Serial serum immunoglobulin levels in adult Yusho patients.

Date of exam.	Cases	Level, mg/dL[a] IgG	IgM	IgA
Mar.–June 1970	28	1655 ± 414*	127 ± 57†	151 ± 77†
July–Oct. 1970	27	1843 ± 628*	186 ± 92	286 ± 100†
Jan.–Mar. 1971	9	1516 ± 471*	225 ± 103*	253 ± 149
June–Aug. 1971	13	1571 ± 341*	185 ± 65	203 ± 64
Oct.–Dec. 1971	24	1586 ± 604*	172 ± 88	232 ± 70
Jan.–Mar. 1972	29	1340 ± 447	166 ± 91	206 ± 97
Nov.–Dec. 1980	15	1307 ± 239	153 ± 61	199 ± 72
Control	57	1243 ± 329	170 ± 54	207 ± 96

[a]Mean ± SD.
*Significant increase ($p<0.01$) compared to control.
†Significant decrease ($p<0.01$) compared to control.

Animal Experiments

Male rats of the Sprague-Dawley strain, weighing about 220 g, were given, by gastric intubation, 2.5 mg of Kanechlor 400 (KC 400) in 2 mL of edible oil three times per week (Group I PCBs) and 0.25 mg of PCDF in 2 mL of oil (Group I PCDFs) and only 2 mL of oil (Group I control), and the same dose six times per 2 weeks (Group II PCBs, PCDFs and control). Each group of six animals was killed at 7 days (Group I) and 14 days (Group II) after the first ingestion. Tissues were prepared for light and electron microscopy.

Results

Respiratory Symptoms and Signs

Approximately a year after onset of the disease we were asked to see the patients. About half of the patients complained of expectoration; some showed continual mouthful expectoration and used a lot of tissue paper which filled the waste basket within a few hours. Such patients proved to have high concentration of PCBs in the blood (analysis of blood PCBs in Yusho patients was started in 1973, 5 years after the onset of the disease).

Respiratory physical findings in the patients were quite different from those in usual chronic bronchitics: that is, many patients showed no crackles except for smoking patients, even when they expectorated a mouthful of sputum, and some patients showed wheezes without radiological, physiological or immunological evidence of bronchial asthma or pulmonary emphysema.

Table 3. Pulmonary function test in 12 nonsmoking patients with reticulolinear shadows about 1 year after onset of respiratory distress and at follow-up.[a]

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	
$\dot{V}_{max50}$, L/sec	≥4.0		6	2
	>2.0		6	4
$\dot{V}_{max25}$, L/sec	≥1.5		5	2
	≥1.0		5	3
	≥0.7		2	1
$P_a O_2$, mm Hg	≥ 85	3	4	5
	> 70	2	7	1
	> 60	1	1	0

[a]Ages of patients ranged between 30 and 49 years.

Table 4. Lymphocyte subsets and function by OKT series and PHA in patients with Yusho.

Subjects	OKT-3	OKT-4	OKT-8	OKT-4/8	PHA
Control	68.3 ± 8.6	41.2 ± 5.7	26.0 ± 5.0	1.63 ± 0.45	303 ± 70
Yusho (n = 38)	63.8 ± 8.0	42.7 ± 8.1	22.8 ± 6.2	1.98 ± 0.82	268 ± 96
<10A (n = 28)[a]	63.1 ± 9.1	42.0 ± 8.8	23.5 ± 5.5	1.84 ± 0.74	286 ± 58
>10A (n = 10)[a]	65.6 ± 6.1	44.7 ± 6.3	21.0 ± 7.3	2.44 ± 0.93	248 ± 97

[a]<10A, >10A indicate PCB concentration and type (4) in the blood.

Clinical Course and PCBs in Blood and Sputum

Respiratory distress occurring in these patients improved gradually over 10 years after onset of the disease (Table 1), but within 5 years respiratory symptoms persisted in most cases (*9*), especially in those with high blood concentrations of PCBs and with signs of chronically infected airways. PCB concentration in blood was little changed from 1973 (5 years after the onset of the disease) to 1983 (*10*). PCB concentration in sputa was about one-third to one-tenth of the blood concentration.

In a 66-year-old male patient with bilateral cicatrical thickening of the pleura due to tuberculosis which healed in 1942, respiratory insufficiency and CO_2 narcosis had developed three times because of airflow obstruction exacerbated by bacterial infection. PCB concentration in the blood and sputum was high (27 and 8 ppb, respectively) in 1975. Chronic bronchobronchiolitis, probably related to PCB discharge from airways with secondary infection, was clinically considered by continuous proof of PCB and *Pseudomonas aeruginosa* in sputum. Continuous inhalation of antibiotics was prescribed until December 1976. After cessation of the antibiotics due to renal complication, his respiratory symptoms worsened and he died of pulmonary insufficiency in March 1977. At autopsy diffuse fibrous adhesion of bilateral pleurae was present. In the lung were mild chronic tracheo-bronchobronchiolitis (and severe edema) (*11*). The amount of PCB in the liver, fatty tissue and brain was not so increased, but the chromatogram pattern was specific to Yusho patients (*11*).

Pulmonary Function Test

The results in 12 nonsmoking patients are presented in Table 2. Vital capacity (VC) and the FEV_1/FVC ratio were almost normal, but arterial oxygen tension (P_{O2}) decreased in eight patients and maximal expiratory flow at 50 and 25% of vital capacity ($\dot{V}_{max\ 50}$ and $\dot{V}_{max\ 25}$) showed mild decreases in about half of the patients; in the latter, both inspiratory and expiratory rhonchi were audible at all times in 1970 to 1974 (*4*). The same tests were again run in 1983; and slight improvement was noted, and rhonchi were not audible at this time.

Immunological Study

Immunoglobulin levels of the patients were first determined in 1970, when the IgA and IgM levels in the serum decreased and the IgG level increased. All were restored to normal in 1972, except in three cases (one adult and two children) where the IgA level remained low (*4*). Though no statistically significant relationship between IgA levels and clinical symptoms was detected, IgA levels of less than 100 mg/100 mL were found in 5 of 29 cases with respiratory symptoms but none in 24 cases without. The IgM levels were significantly lower

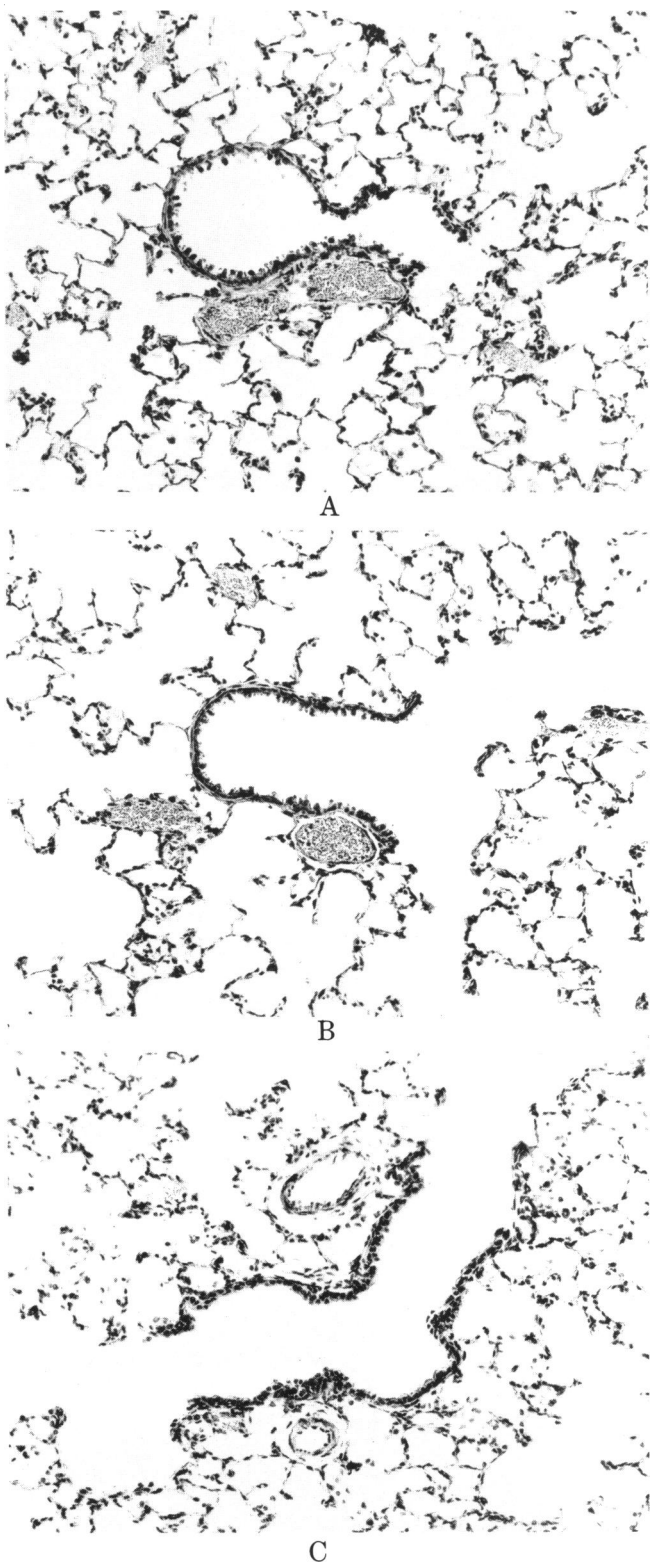

FIGURE 1. Tissue from lungs of rats given PCDFs for 2 weeks. Clara cells are hyperplastic but more scarcely seen in terminal bronchiole, probably due to (A) necrotic changes, compared with (B) hyperplastic changes in rat lungs given PCB for 2 weeks and (C) controls. H-E stain × 80.

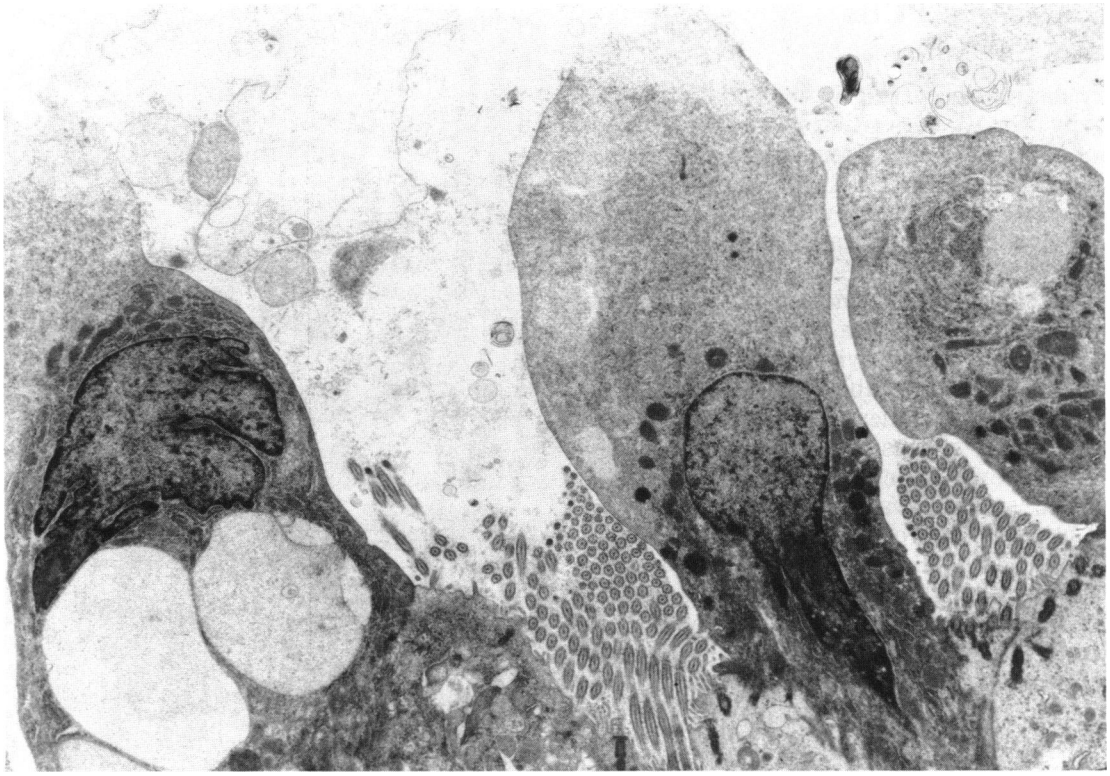

FIGURE 2. Ultrastructural view of rat lungs given PCDFs for 2 weeks. Necrosis of the Clara cells is clearly seen. ×2600.

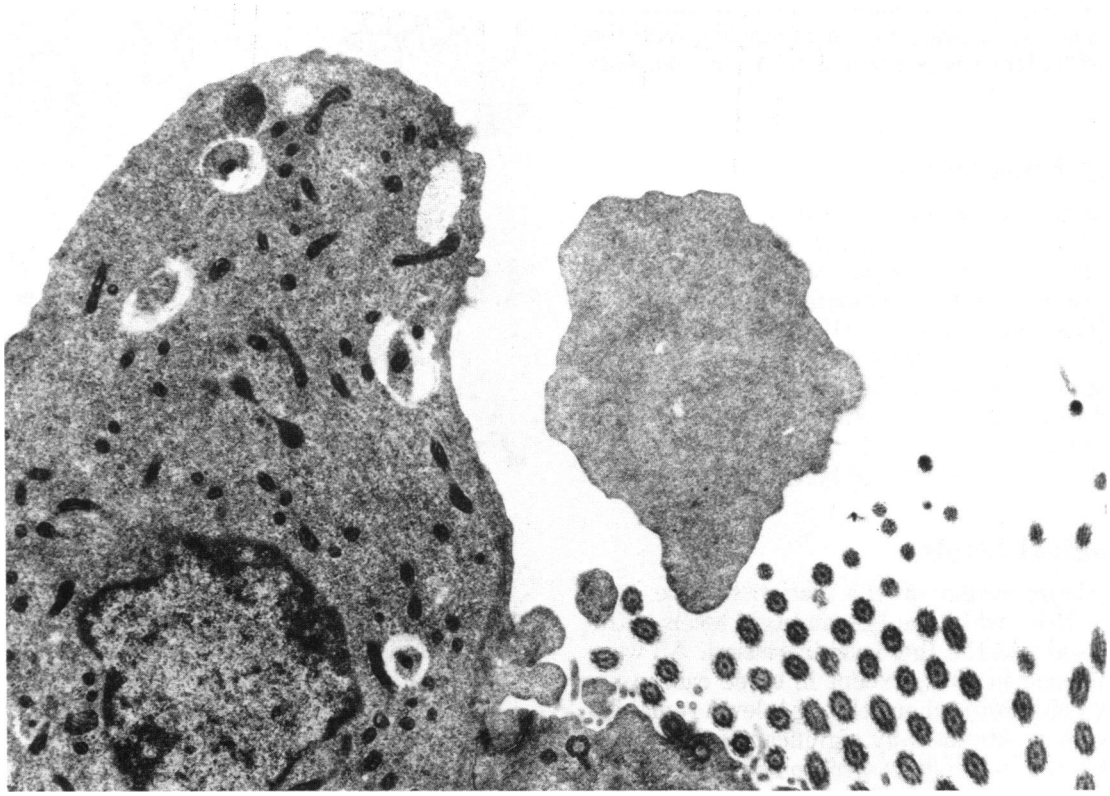

FIGURE 3. Ultrastructural view of rat lungs given PCB. A proliferation of agranular endoplasmic reticulum in the cytoplasm is shown, but no necrotic changes are present. × 5000.

in patients with severe dermatological symptoms, while the IgA levels in sputum were not low (*4*). Immunoglobulin levels were again examined in 1980, when all were in normal range (Table 3).

Since T-cells appeared to be sensitive to PCDF poisoning in the animal experiment described below, the effect of PCDF on two T-cell subsets, i.e., suppressor T-cells and helper T-cells were studied. Table 4 shows that the percentage of helper T-cells (OKT-4) was slightly increased but that of suppressor T-cells (OKT-8) was slightly decreased in the patients with higher blood PCB concentration (> 10A). The response to nonspecific mitogen-PHA was also lowered in the same patients.

Pathologic Changes in the Lungs and Thymus

Pathological study of lung specimens from seven autopsy patients disclosed lymphocyte infiltration in bronchial and bronchiolar walls and macrophage infiltration in alveoli, particularly around bronchioles associated with alveolar collapse in four patients dying at 13 to 48 years of age (*4*). Focal hemorrhage and/or pulmonary edema and pleural and pericardial effusion or adhesion were also detected in three patients who died within 4 to 12 months after the onset of the poisoning (*12,13*). Marked hyperemia, atelectasis, and alveolar hemorrhage were noted in a stillborn case (*14*).

In rat lungs given PCDFs, necrosis of the Clara cells was seen mildly at a week and clearly at 2 weeks after the start of the experiment (Figs. 1A and 2). Other histologic changes were mild pulmonary edema and vascular congestion at 2 weeks (Fig. 1A). These findings were mild in rats given PCBs (Figs. 1B and 3).

Rat thymus given PCDFs decreased in size, and microscopic features showed severe atrophy (Fig. 4A), but rats given PCBs showed the same type of changes but of less severity (Fig. 4B).

Discussion

Cause, Clinical Symptoms and Pathophysiological Changes in Yusho

The causative agents proved to be PCBs, PCQs and PCDFs by chemical and activation analysis. Recent studies in mice showed that a number of discrete PCBs were not taken up by the bronchi but were distributed very evenly throughout the lung parenchyma (*5*). However, specific dose dependence (*15*) and structural requirements (*6*) of PCBs were shown to exist for accumulation in the mouse bronchial mucosa. A large amount of expectoration at an early stage of the disease may relate to this. Nevertheless, pathophysiological findings in the patients reveal that respiratory involvement in Yusho is mainly small airways disease which may be caused by involvement of the cellular component in bronchioles and/or associated infections. Recent studies that appear to be related to these problems are

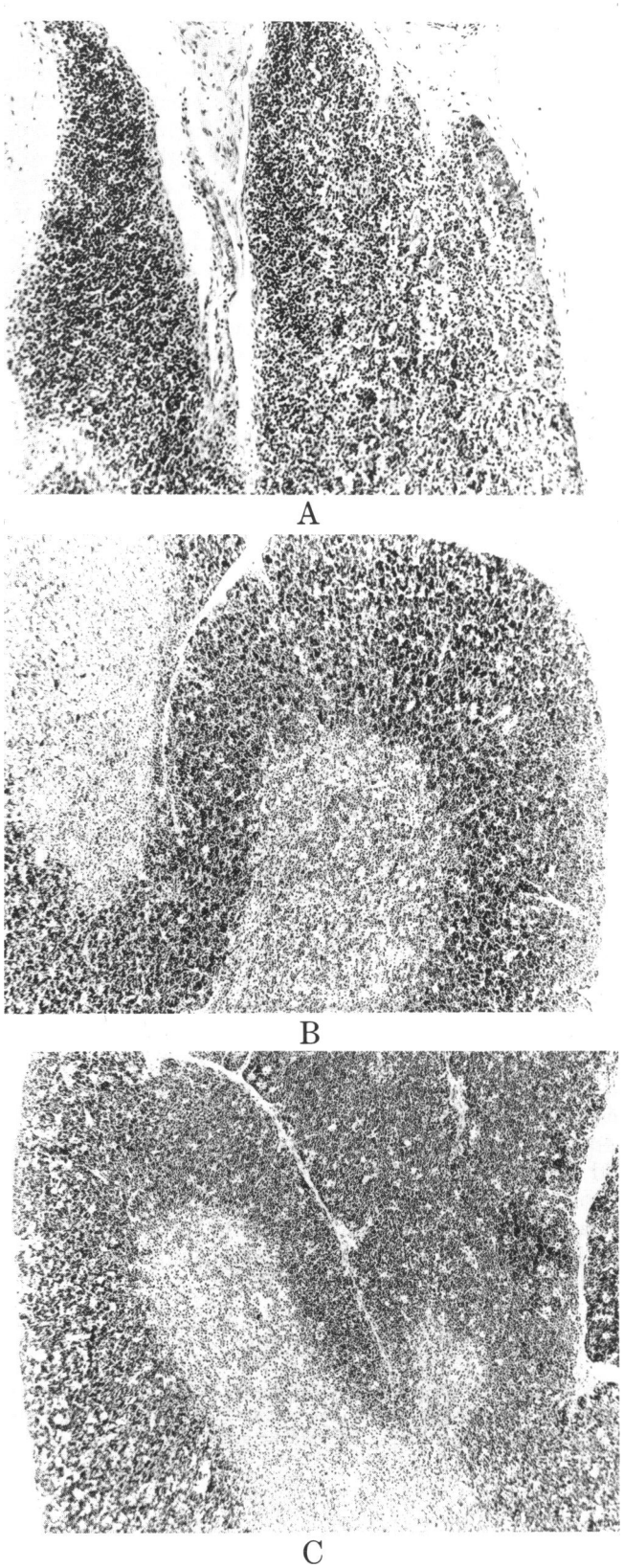

FIGURE 4. Thymic tissue (A) of rats given PCDFs shows marked atrophy; (B) thymic tissue of rats given PCB shows mild atrophy, compared with (C) control rat thymus. H-E stain, × 80.

the following. The main causative agents inducing Yusho are PCDFs (*7*), and a furan derivative (4-ipomeanol) that produces a characteristic lesion in several laboratory animal species which is necrosis of the nonciliated bronchiolar (Clara) cells. The initial damage to this cell population appears to be highly selective. Relatively late occurring manifestations of lung toxicity after administration of comparatively large doses of furan derivatives sometimes may include pulmonary edema, pleural effusions, vascular congestion and hemorrhage (*8*). Lungs of rats given PCDFs in this study also showed the same pathologic features as those in 4-ipomeanol (furan derivative) intoxication.

Alterations in Immune Status

Respiratory distress is often exacerbated by viral or bacterial infection. In the latter, Gram negative bacilli were often detected and found to persist in about half of the cases examined (*4*).

The changes in immune status in Japanese and Taiwanese patients (*16*) were almost the same in humoral immune response; that is, IgA and IgM in the serum decreased in both Japanese and Taiwanese patients about 2 years after the onset of the disease, and then returned to normal after 3 years.

Yau-Chin Lü (*16*) reported low OKT 4/8 ratio (namely, low helper/suppressor T-cell ratio) and enhanced responsiveness to nonspecific mitogen-PHA in Chinese PCB-poisoned patients 3 years after onset. Conversely, our data showed, in Japanese Yusho patients 14 years after onset, high OKT 4/8 ratio and lowered responsiveness to PHA. The differences may be attributed to phenomena in the course of the disease.

Data on the effect of PCBs on the immune mechanism are sparse, although a general lowering of gamma-globulin levels in nonpregnant rodents has been noted by some authors (*17–19*), and the suppression of cellular immunity such as the delayed-type skin repsonse to streptokinase and streptodornase was reported in PCB-poisoned patients (*20*).

In our experimental animals, feeding of PCBs caused mild thymus atrophy, and feeding of PCDFs, marked atrophy. As mentioned above, PCDFs are the main causative agents in Yusho. Therefore, further examination is needed to ascertain in detail the difference in effects between PCBs and PCDFs.

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