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Paraquat Intoxication on Rat Lung

—Fractional Bronchoalveolar Lavage Analyses compared with Histological and Ultrastructural Studies—

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INTRODUCTION

Paraquat(1,1′-dimethyl-4,4′-biopyridylium dichloride) is a herbicide commercially available as Gramoxone in 25% aqueous preparation. Since its first marketing in 1962, many cases of accidental or suicidal ingestions have been reported[1~3]. The lungs are particularly vulnerable and death occurs within a week in most patients due to acute respiratory failure[4]. Similar lung lesions resulting in fibrosis can be produced in mice, rats[6,7], and hamsters[8] by the administration of this compound.

In the present study pulmonary lesions of the rats were experimentally produced by the intraperitoneal injection of paraquat and were examined by light and electron microscopy.

To understand pathogenesis of the lung lesions at a cellular level, a bronchoalveolar lavage(BAL) technique was applied. Of uniqueness in this study is a new method of cell analyses, we tentatively call fractional bronchoalveolar lavage (FBAL) analyses, which can be proved more analytical and indicative of the distribution of each immune-effector cell in the respiratory system.

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MATERIALS AND METHODS

A total of 52 Wistar male rats, 200 g body weight, was classified into 8 groups (A to H). A single intraperitoneal injection was made with a dose ranging 1, 5, 10, 20, 30, 40 and 50 mg paraquat dichloride solution per kg body weight as shown in the Table 1. In the 6 rats of group A, 2 to 4 ml saline was administered in the same way and the animals were fed for up to 22 days. Under the nembuthal anesthesia were sacrificed 6 rats on 5 hours after the paraquat administration, 4 rats on days 1, 3, 6 and 10, 2 rats on days 15, 18 and 22, and 4 rats on day 40 respectively. Twenty two rats with a higher dose (group E,F,G and H) spontaneously died on days 1, 2, 4, 5, 7, 9 and 11.

The thorax and abdomen were cut open. The trachea was cannulated with a polyethylene tube and ligated firmly. Through the tube bronchoalveolar lavage (BAL) was sequentially made three times by approximately 8 ml saline. To avoid contamination of sputa and mucin, the lavage fluid was passed through 2 sheets of gauze and kept in a plastic tube. A total volume of each fluid recovered was estimated. A few drops from the fractional bronchoalveolar lavage (FBAL) fluid

Table 1. Experimental groups of paraquat intoxication

Group (mg/kg B.W.) \ Days after administration	5h	1	2	3	4	5	6	7	9	10	11	15	18	22	40	Total (52)
A (saline)	—	—	—	—	—	—	—	—	—	—	—	2	2	2	—	6
B (1)	—	—	—	2	—	—	2	—	—	2	—	—	—	—	—	6
C (5)	—	—	—	2	—	—	2	—	—	2	—	—	—	—	—	6
D (10)	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	4
E (20)	2	2	2*	—	1*	1*	—	1*	—	1*	—	—	—	—	—	10
F (30)	2	1*	3*	—	1*	—	—	—	1*	—	—	—	—	—	—	8
G (40)	—	2*	2*	—	—	—	—	—	—	—	—	—	—	—	2	6
H (50)	—	2*	2*	—	—	—	—	—	—	—	—	—	—	—	2	6

* : Rats spontaneously died

were centrifuged with a Cytospin (Shandon Co.) at 1,000 r.p.m. for 5 minutes. Following Giemsa stain by Diff Quik, cell count was made under the light microscope (×400).

The lungs were taken out together with the trachea and heart, then were slowly inflated with either 20% formalin or 2.5% glutaraldehyde in 0.1 M, pH 7.4 phosphate buffer. The whole chest organs were immersed in the same fixative. The lung tissues were cut into several sections longitudinally in the right side and tangentially in the left. The paraffin embedded sections were stained with hematoxylin eosin, Masson trichrome and periodic acid methenamine silver (PAM). For electron microscopy, the lung specimens were cut into small cubes and transferred to fresh glutaraldehyde solution for the further fixation of about 2 hours. They were then postfixed with phosphate buffered 1% osmic acid, dehydrated in graded series of alcohol and propylene oxide, and embedded in Epon 812. Semithin sections were made with glass knives and stained with aqueous toluidine blue. Ultrathin sections were made with a diamond knife and stained with uranyl acetate and lead citrate. These sections were observed with a Hitachi 800 electron microscope at an accelerating voltage 75 kV.

RESULTS

After the single injection of paraquat, the animals of the group A,B and C (saline up to 4 ml, and 1 and 5 mg/kg B.W. paraquat administrated respectively) appeared unchanged at all and lasted clinically healthy until they were sacrificed. Rats of groups D through H which received high doses up to 50 mg/kg B.W. showed no clinical signs on the same day of injection. However, 20 out of 34 animals spontaneously died either on the next day or on days 2, 4, 5, 7, 9 and 10. Their lungs were commonly enlarged and did not collapse on thoracotomy. Pleural surface was diffusely dark and reddish, and the lung was elastic and soft The cut surface showed marked edema and hemorrhage with occasional patchy consolidations.

Animals of the groups D,E,F, of a dose 10, 20, 30 mg/kg B.W. respectively were sacrificed on 5 hours and one day after the injection. These lungs did not show remarkable macroscopic changes except for scattered congestions and patchy discoloration.

In the chronic stages (group G and H), lung consistency moderately increased and grayish consolidation spread widely over more than one lobe in 2 rats. However the rest of rats examined in this stage appeared irregularly congested over the relatively stiff parenchyma.

I: Histological Observation

Groups A,B and C revealed essentially normal alveolar architectures. There was no edema, no congestion, no fibrosis and no excessive cellular infiltrates(Fig. 1).

All rats which spontaneously died within 10 days after paraquat injection had an extreme edema of proteinous fluid in the alveolar and bronchial lumens. Vascular systems including pulmonaryi veins, venules and capillaries remarkably engorged containing many red blood cells which often underwent hemolytic changes(Fig. 2). Neutrophils, mononuclear cells and lymocytes were scattered through the lung parenchyma. Hyaline membrane was evident together with fibrin strands along the alveolar ducts and wall in some animals(Fig. 3).

The lungs of groups D and F in 5 hours of administration revealed accumulation of cells such as macrophage, mononuclear cells and few neutrophils in the alveolar lumens and their edematous walls(Fig. 4). Neither bronchial cuboidal cells nor Clara cells reacted at all. One day later the lungs of these groups were characterized by irregular appearance of granuloma-like lesions made up with fibroblastic cells, neutrophils, monouclear cells and macrophages over the alveolar structures(Fig. 5). Patchy congestion and hemorrhage were also found. In the group G and H (40 and 50 mg/kg B.W. administration, on the 40 th day), the lungs revealed edematous accumulation of inflammatory cells forming granuloma-like consolidations which were widely spread over alveolar architectures. There were increased cell infiltrates of lymphoid cells, mononuclear cells and fibroblastic cells(Fig. 7). And foci of the proliferation of type II alveolar cells were noticed. The alveolar wall was irregularly fibrous thickened and edema also remained particularly around the small vasculatures.

II: Ultrastructural Observation

The alveoli with essentially normal structures on a light microscopic level occasionally showed edema accumulation in the interstitium. Fibroblastic cells became swollen with electron lucent cytoplasm. Type I alveolar epithelial cells spread their flat cytoplasm over the alveolar walls joined with the cell border of round type II alveolar epithelial cell. Basal surface of the type II cells apparently protruded their cytoplasmic processes toward the interstitial fibroblastic cells through the lack of basement membrane. Endothelial cytoplasm of alveolar capillaries appeared thin membranous on the luminal side comprising air-blood barrier(Fig. 8). Interstitial side of the endothelium developed ample thickness containing a variable number of pinocytotic vesicles(Fig. 9).

In acute stage lungs edema fluid filled over the air spaces and diffusely in the interstitium. Peribronchial lymph vessels were remarkably dilated and contained mononcytes and macrophages(Fig. 10).

Became swollen by edema interstitial fibroblastic cells, endothelial cells and type I alveolar epithelial cells. Relatively intact cytoplasm of capillary endothelial cells remarkably developed pinocytotic vesicles which frequently fused each other and communicated between both surfaces(Fig. 11). Type II alveolar epithelial cells showed a quick proliferation over the limited areas making the direct contacts with underlying fibroblastic cells. These interstitial cells developed cisterns of rough-surfaced endoplasmic reticulum, free ribosomes and mitochondria in hypertrophied cytoplasm(Fig. 12).

Alveolar macrophages often accumulating in the alveolar lumens became swollen and continuing densely packed heterophagosomes with variable densities and morphology(Fig. 13). Some of them apparently adhered to irregular protrusions of the cytoplasm of type I alveolar epithelial cells(Fig. 14). This feature suggests that at least destructive alterations of these cytoplasm underwent in this period. Accumulation of collagen fibrils and elastic fibers became noticeable only some corners of the cell membrane of proliferating interstitial cells (Fig. 15). These interstitial cells were also present over the remaining alveolar interstitium(Fig. 16). The fibroblastic cells in the alveolar wall frequently developed actin-like microfilaments in their cytoplasm characterizing the ultrastructure of myofibroblast(Fig. 17). These cells often made close apposition to the microfoldings of the basal surfaces of type II alveolar epithelial cells(Fig. 18). This interaction became emphasized in chronic stages. But continuous proliferation of type II cells was

Table 2. Cell analyses of bronchoalveolar lavage fluids in combination with histological findings

Groups	Average ratio(%)			Histology
	Macrophage	Lymphocyte	Polys	
A, B & C	88.1 (57.2~99.5)	10.7 (0.5~42.8)	1.1 (0~13.4)	Essentially normal
E(6/10), F(6/8), G(4/6) & H(4/6)		Massive hemorrhage		Spontaneous death due to acute hemorrhage and edema
D, E(4/10) & F(2/8)	78.0 (44.9~99.0)	9.0 (1.0~31.4)	13.1 (0~41.7)	Acute stage
G(2/6) & H(2/6)	89.9 (78.8~98.5)	9.6 (1.5~20.9)	0.4 (0~ 1.9)	Chronic stage

Table 3. Fractional analysis of bronchoalveolar lavage(Essentially Normal Histology)

	Saline	Average ratio(%)		
	Recovered/given	Macrophage	Lymphocyte	Polys
I	7.4/10	80.7(57.2~92.1)	17.0(7.9~42.8)	2.3(0~13.4)
II	7.4/ 8.4	89.9(80.4~96.4)	9.5(3.6~16.3)	0.5(0~ 3.3)
III	7.6/ 8.1	93.6(85.7~99.5)	5.6(0.5~ 9.5)	0.6(0~ 4.8)
	22.4/26.5	88.1(57.2~99.5)	10.7(0.5~42.8)	1.1(0~13.4)

limited and not so markedly as to the extent of a whole alveolus(Fig. 19). Bronchiolar cuboidal cells were not activated to make the alveolar structure into bronchiolization(Fig. 20).

III: Fractional Analyses of Bronchoalveolar Lavage

In the fatal rats of this experiment, lavage fluid contained too many red blood cells by direct reflection of parenchymal hemorrhages.

In the conventional bronchoalveolar lavage techniques, average cell differentials of the group A, B and C were as shown in the Table 2, macrophage 88.1%, lymphocyte 10.7% and polys 1.1% respectively. Acute stage BAL was characterized by a remarkable increase of polys more than 13 times as much as the group A, B and C, while their lymphocyte maintained control level and macrophage relatively decreased.

In the chronic stage of this experiment, ratios of polys and lymphocyte returned to the control levels(Table 2).

It was obvious in the cell analyses on a fractional fluid of three sequenti al BAL performance, that lymphocyte and polys decreased in the later fractions as shown in the Table 3. The average fluid recovery of this histologically normal animals was as high as 84%. In the Table 4 lymphocyte and polys were constantly high in all the fractions of BAL. Particularly polys in the first fraction increased up to 41.7% in some animal, and the high ratio was maintained to the last fraction up to 27.2%. This increase was not due to infections such as bronchopneumonia which could be excluded by histological observation. In this stage polys and lymphocyte scattered over the bronchial regions as many as in the periphery of the lung tissue.

Polys ratio in the chronic stage (Table 5) dramatically decreased and the ranges at an each fraction were quite limited indicating scarce appearance through the lung tissue. However lymphocyte ratio in the first fraction was quite high averaging 20.1% between 19.3 and 20.9% in each animal. In contrast second and third fractions

Table 4. Fractional analysis of bronchoalveolar lavage (Acute Stage)

	Saline	Average ratio(%)		
	Recovered/given	Macrophage	Lymphocyte	Polys
I	6.4/10	71.6(44.9~93.9)	10.5(4.7~20.3)	18.0(0.4~41.7)
II	6.4/ 7	80.3(56.7~95.9)	8.4(3.9~12.2)	11.4(0 ~33.9)
III	6.2/ 7	82.2(66.7~99.0)	8.1(1.0~31.4)	9.8(0.3~27.2)
	19.0/24	78.0(44.9~99.0)	9.0(1.0~31.4)	13.1(0 ~41.7)

Table 5. Fractional analysis of bronchoalveolar lavage(Chronic Stage)

	Saline	Average ratio(%)		
	Recovered/given	Macrophage	Lymphocyte	Polys
I	6.3/10	78.9(78.8~79.1)	20.1(19.3~20.9)	1.0(0~1.9)
II	6.9/ 8	96.6(94.0~98.5)	3.4(1.5~ 6.0)	0(0)
III	6.9/ 8	94.3(91.0~96.6)	5.4(3.4~ 7.6)	0.3(0~1.3)
	20.1/26	89.9(78.8~98.5)	9.6(1.5~20.9)	0.4(0~1.9)

showed remarkable decrease of lymphocytes suggesting that they were the recruits from bronchial system, in particular of the enlarged bronchus associated lymphoid tissues (BALT) rather than from peripheral alveolar lumens. The recovery ratio of the fluid was 77% in these fibrotic animals.

DISCUSSION

Substantial lung lesions did not occur in those animals which received 1 or 5 mg/kgB.W. Two animals with a dose 10 mg/kgB.W. began to show acute changes on day 1. In our study 10 mg/kg B.W. of paraquat appears to be minimum requirement to produce lung lesions. A dose more than 20 mg/kg B.W. developed markedly hemorrhagic and edematous changes through the lung parenchyma. Passing the fatal period between day 1 to 10, the rats developed fibrotic changes in the lung.

Massive destruction of alveolar structures was evident in the fatal cases and whole capillaries and small vessels showed remarkable distension containing many red blood cells, some of which showed hemolytic changes.

These vascular changes are not specific in this experiment, but are noticed in human lungs of the patients who had acute fatal attack of myocardial infarction, and also reported in the experimental lung lesions induced by intravenous infusion of vasopressin[13]. These changes of the vasculatures are assumed due to paresis of vascular movement, called angioparesis. In those cases massive infiltration of neutrophilic leukocytes and protein-rich edema, occasional formation of hyaline membrane are common. Real cause of angioparesis is not known but can presumably result from acute circulatory disturbance in the cardio-pulmonary beds.

Polys or neutrophilc infiltration was commonly found by many authors[5,6,7,14], but the chemoattractants for the neutrophil are not determined.

Not fatal but acute changes were produced by 10 to 30 mg/kg B.W. on 5 hours and on 1 day after the administration. Destruction of the alveolar walls was not always obvious. However, type II alveolar epithelial cells proliferated at the

corners of alveolar sacs indicating insidious necrosis of type I alveolar epithelial cells. Serous edema fluid accumulated whole over the alveolar structures. Cytoplasm of the capillary endothelial cells carried a markedly increased number of pinocytotic vesicles. These findings support an increase of vascular permeability[14] as discussed by Dearden et al[3]. Fibroblastic cells were occasionally hypertrophic protruding their cytoplasmic processes to make close contact with type II alveolar epithelial cells. This epithelial-mesenchymal interaction[15,16] is quite commonly seen in mild fibrotic lungs such as so-called desquamative interstitial pneumonia and other interstitial lung diseases of man. Relationship between bronchial epithelium and interstitial connective tissue is well characterized by hemidesmosomes and anchoring fibrils in animals[17] and man[18]. Overlying cuboidal epithelial cells of the interface can be assumed their origin of bronchiolar basal cells. These characteristic findings are supposed to rise following the damages of the bronchial system[19]. In our study neither cuboidal epithelial cells nor Clara cells did appear. In this context bronchial epithelial lining is not involved in the damages due to paraquat intoxication.

In chronic stages, half animals showed irregular and relatively massive fibrosis. Besides, edematous changes also appeared in the perivascular connective tissues, where variable number of lymphocytes infiltrated in contrast to neutrophilic invasion in the acute stages. The rest of the animals showed a relatively normal architecture in the lung parenchyma with no substantial fibrotic alteration. These animals appeared less sensitive to paraquat. They might have produced edematous changes to some degrees in early stages of injection and absorptive mechanism has sufficiently occurred to a level not to leave any scarring of the interstitium. Thus it is possible that some animals only developed reversible alterations by paraquat injection even with a fatal dose for other individuals.

In the traditional techniques of bronchoalveolar lavage[10], their results simply characterized the average estimates of cell differentials including macrophage, lymphocyte and polymorphonuclear leukocyte. For instance in normal rats, they approximated 91.4%, 7.0% and 1.6% respectively. In the rats of groups A,B and C which histologically were normal, lymphocyte showed highest ratio 17.0% in the first fraction of three sequential BALs, and it gradually decreased in the second and third fractions. Polys also showed highest ratio in the first fraction ranging from 0 to 13.4%. The data are comparable to those studied by Asada who clearly showed a higher ratio of lymphocyte and neutrophil in the first fraction of three sequential BALs in the normal rats. It was proved that proximal one third of the lung tissues mainly comprised of large airways supplied most neutrophils which were situated in mucous blanket over the epithelium. Lymphocytes and polys remained up to 9.5% and 4.8% respectively in the third fraction of BAL, which makes a sharp contrast with the data by Asada[11]. We have to consider that animals of paraquat administration up to 5mg/kg B.W. were included in the same group of the table 1. Although their histology appeared normal, subdetectable infiltration of the inflammatory cells possibly took place in the tissues. The BALT certainly became more extensive in the animals surviving long after the injection. This lymphoid tissue contributed for the increase of lymphocytes exceeding the normal level.

In this study we missed the counting of a total cell number at an every fraction of BAL thus failing to complete the table. But the previous data available from our experimental studies apparently showed that the total number recovered became gradually increased to the last fraction of BAL. Based upon an absolute number of the collected cells we could have clarified whether a real increase of inflammatory cells occurred either in the regions of airways or in the peripheral parenchyma.

A decrease of lymphocyte and polys is also consistent with acute stages. Polys were always

higher than normal. This finding was comparable to the histological changes in which neutrophils increased and scattered in the lumens of bronchial system and in the alveolar structures. In the chronic stages particularly the ratio of lymphocyte was consistently as high as 20.1% in average in the first fraction of BAL. These lungs contained many lymphocytes in perivascular regions as well as along the bronchial system. Of interest is the presence of a remarkable hypertrophy of BALT in the airways.

The fractional analyses of bronchoalveolar lavage(FBAL)[11][12] characterize an approximate distribution of immune-effector cells clarifying their presence whether in bronchial system or peripheral lung tissues including alveolar structures. In this concept the results of fractional bronchoalveolar lavage will indicate whether inflammatory activities take place either in bronchial system or relatively peripheral lung tissue including alveoli. An idea of alveolitis, such as neutrophil alveolitis and lymphocyte alveolitis, commonly referred on a basis of increased cell types, should be much evaluated by the application of FBAL method.

Because the term "alveolitis"[10] was originally used from the histological viewpoint with a limited implication to inflammed alveoli, which was no relation to the BAL.

We think alveolitis should deserve its original meaning strictly. Otherwise other disease entity involving the airways particularly the terminal portions of bronchioles cannot be clarified as independent. It is our future tasks.

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=국문초록=

백서폐의 Paraquat 중독

—분할적 기관지폐포세정법과 형태학적 연구의 비교—

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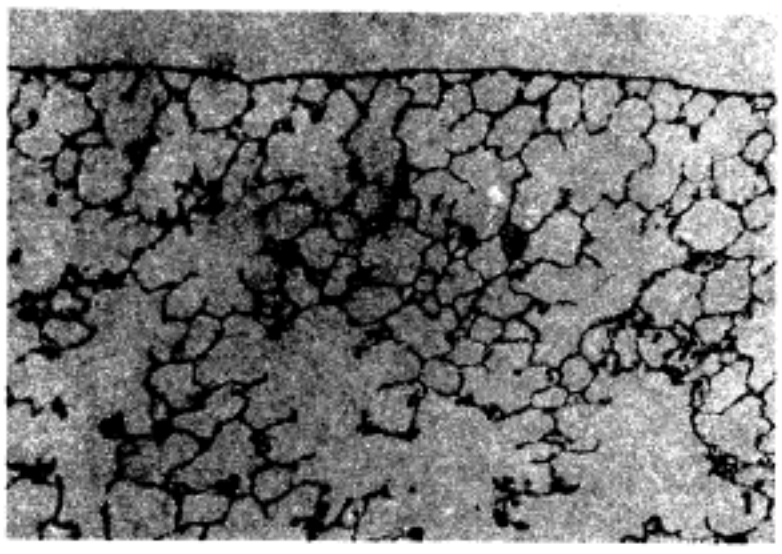
Wister 백서폐의 실험적 paraquat 중독이 잠정적으로 분할적 기관지폐포세정법(Fractional Bronchoalveolar Lavage, FBAL)이라고 불리는 새로운 방법에 의하여 연구되었다. 광학 및 전자현미경적 관찰도 동시에 행하여 졌다.

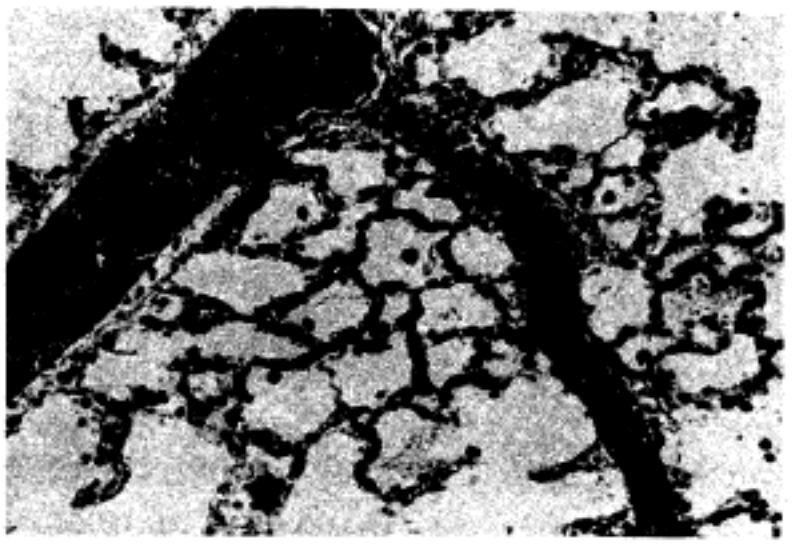
기관지폐포 세정액(BAL) 세포성분의 분할적 분석의 결과, 정상백서에서는 임파구와 다핵백혈구의 비가 첫 분설에서 가장 높고 둘째, 셋째 분설로 갈수록 점차 감소되는 것이다. 이러한 분포는 염증세포의 대체적인 분포를 나타내어 즉, 첫 분설에서 얻어진 세포들은 기관지의 기도에서 나온 것이고 나머지 분설들은 주로 최말단폐조직에서 유래된 것을 말해준다.

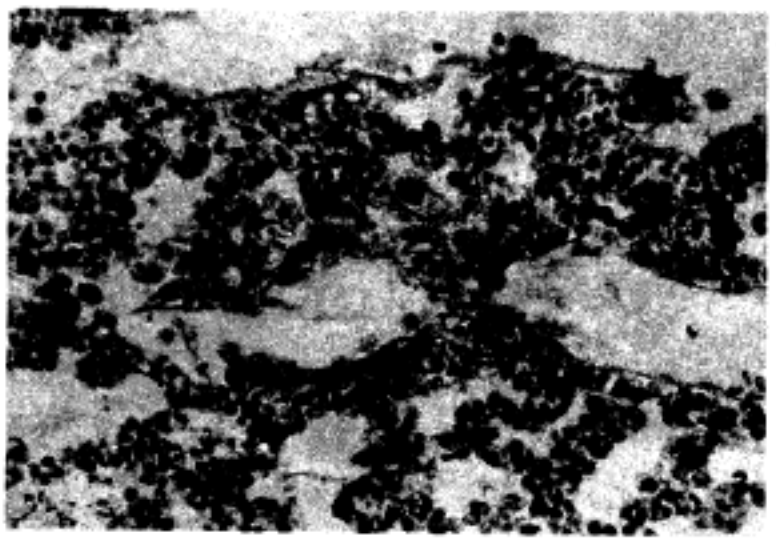
저자들의 연구에서 본 바 paraquat 중독의 급성기에서는 BAL의 모든 분설에서 호중구와 임파구의 높은 비를 보여 이는 간폐조직을 통해 호중구가 존재하는 조직소견과 일치되었다.

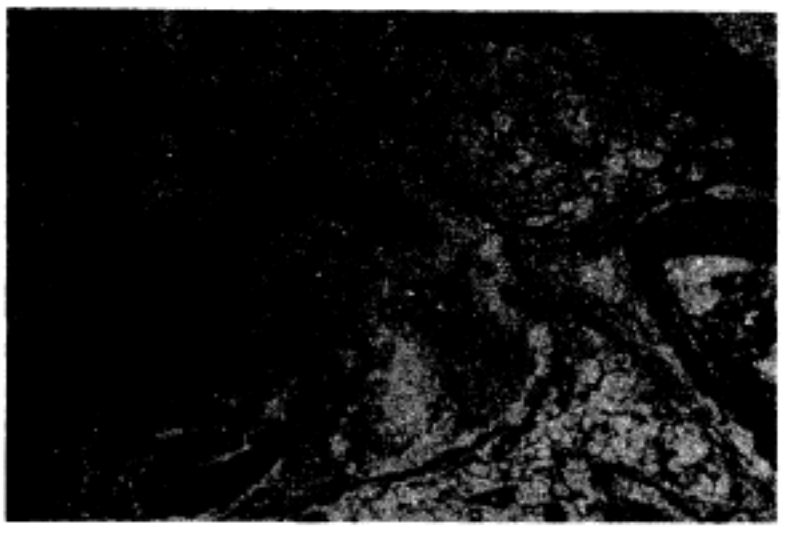
만성기에서는 임파구의 비가 BAL의 모든 분설에서 심하게 증가되었는데 이는 비대한 BALT(bronchus-associated lymphoid tissue)와 말초폐조직의 임파구 침윤과 관계되었다.

이러한 자료는 BAL액 세포성분의 분할적 분석이 세포층에서 폐질환의 병원론을 유추하는데 중요한 역할을 하는 호흡계의 각각의 immune-effector cells의 분포를 추정하는데 도움을 준다고 믿어지는 바이다.









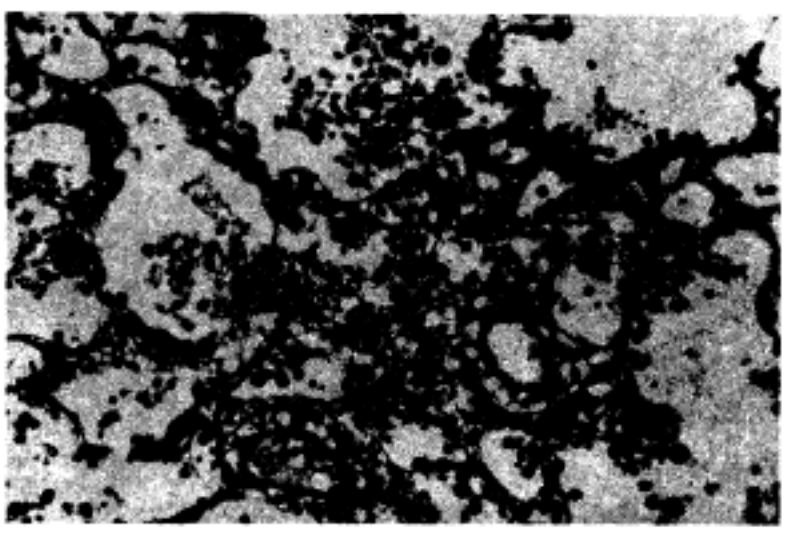


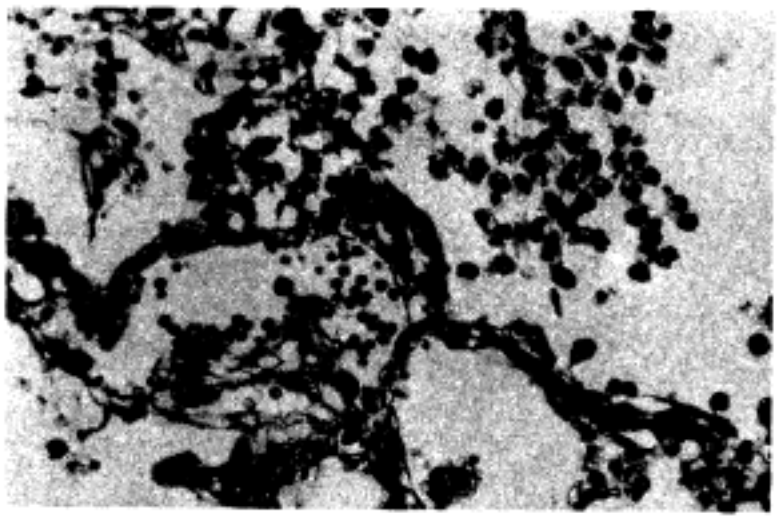
Fig. 1. Lung of control rat, 18 days after saline injection. The alveolar structure is neat and intact. Essentially normal architecture. Hematoxylin and eosin. (H-E, ×100)

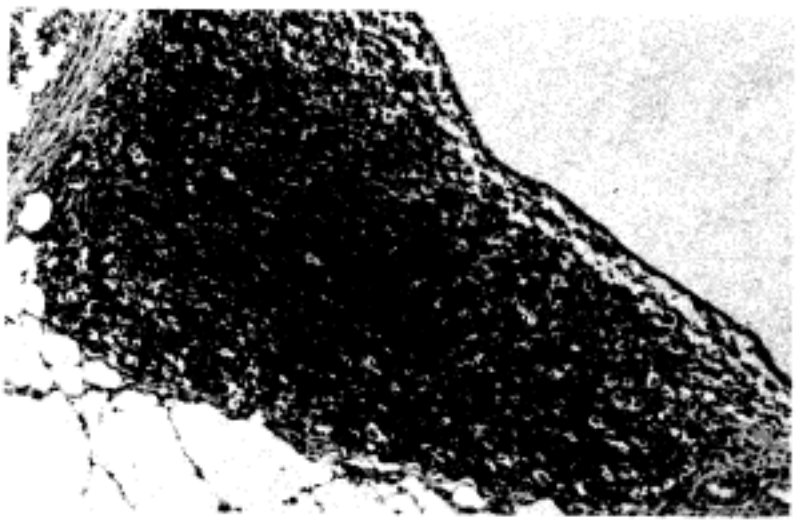
Fig. 2. Rat spontaneously died, 1 day after 50 mg/kg B.W. paraquat administration. Remarkable engorgement of the pulmonary veins and alveolar capillaries. Red blood cells often show hemolytic change. (H-E, ×200)

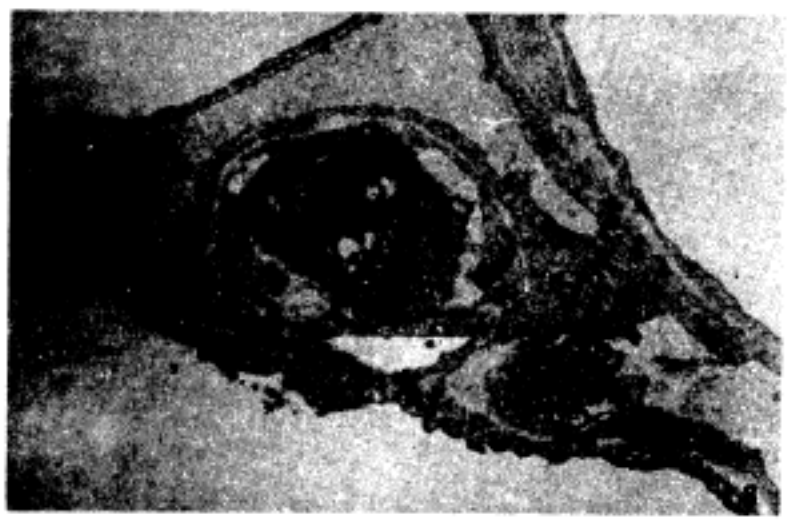
Fig. 3. Rat spontaneously died, 2 days after 30 mg/kg B.W. paraquat administration. Massive hemorrhages occur over the alveolar wall, spreading-out into the lumen. Neutrophils and macrophages accumulate over the destroyed alveolar wall. Hyaline membrane structure is formed along the alveolar lining. (H-E, ×400)

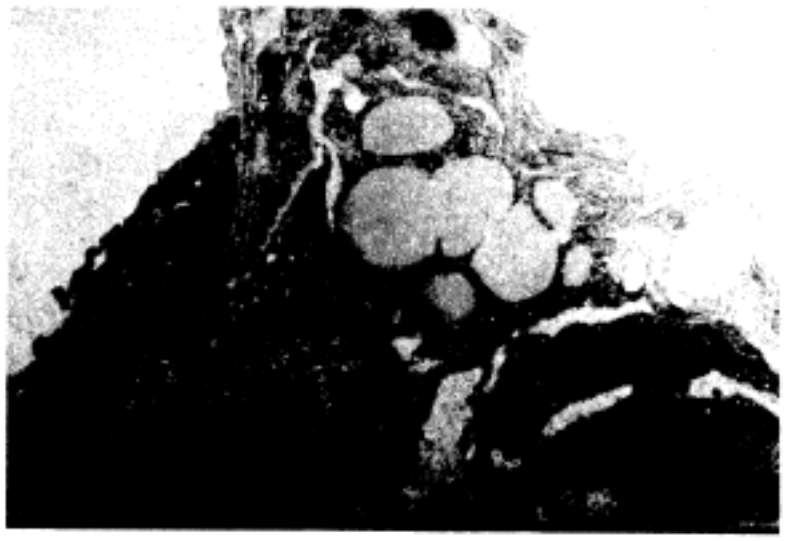
Fig. 4. Five hours after 20 mg/kg B.W. paraquat administration. Massive interstitial edema with marked dilatation of lymph vessels. (H-E, ×100)

Fig. 5. The same animal as in Fig. 4. Alveolar walls are edematously swollen with occasional destruction, leading to hemorrhage and inflammatory cell infiltration. (H-E, ×200)









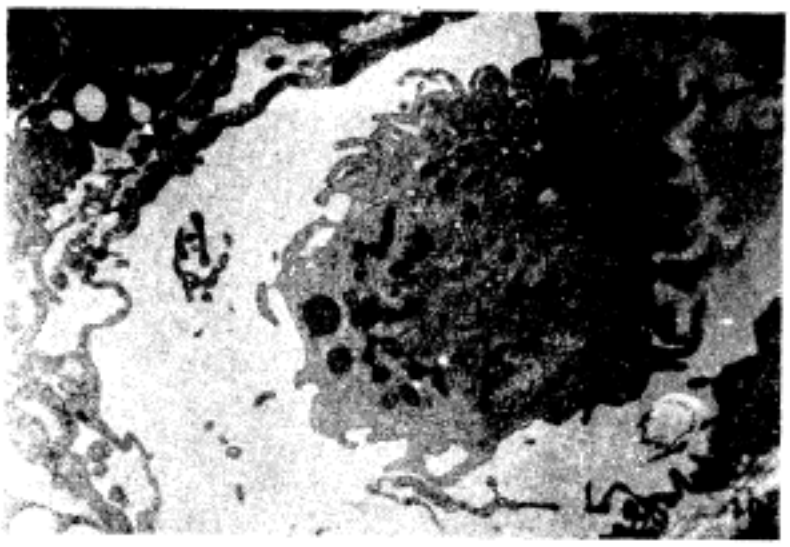


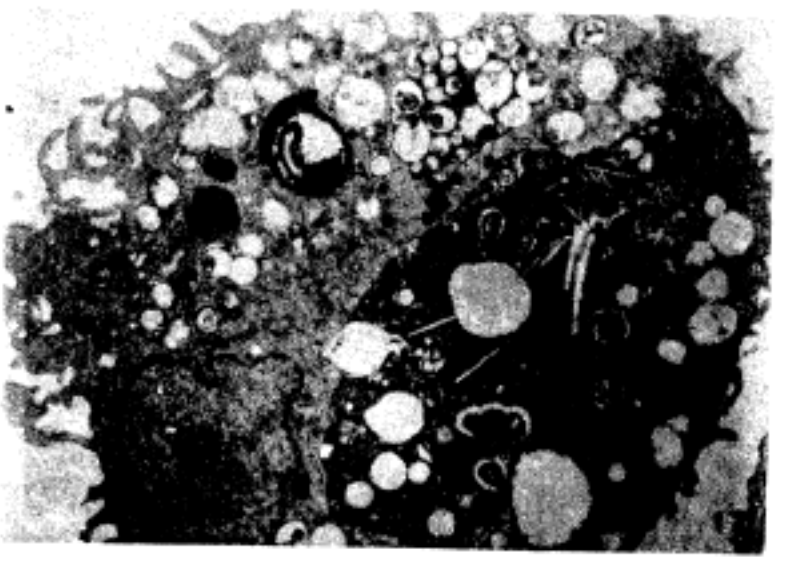
Fig. 6. One day after 20 mg/kg B.W. paraquat administration. A high-power-view of the damaged alveolar structure. Alveolar lining epithelial cells desquamated and dense fibrin strands dispersed in alveolar lumen. Many neutrophils, monocytes and macrophages are evident over the alveolar wall and in the lumen. (H-E, ×400)

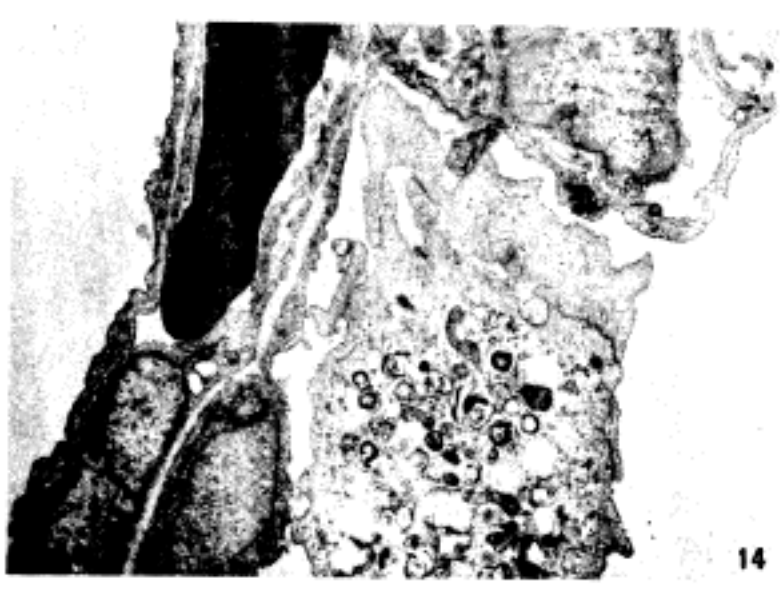
Fig. 7. The same animal as in Fig. 6. Bronchus-associated lymphoid tissue is gradually hypertrophic, protruding into the bronchial lumen. (H-E, ×200)

Fig. 8. Three days after 1 mg/kg B.W. paraquat administration. Air-blood barrier is fine and delicate. Type II cell, flat cytoplasm of type I cells are intact. A fibroblastic cytoplasm intervenes between capillary and type II alveolar epithelial cells. Electron micrograph (EM, ×5,000)

Fig. 9. Six days after 5 mg/kg B.W. paraquat administration. Fibroblastic interstitial cells are situated in the center of alveolar interstitium. Their cytoplasms contain a number of lipid droplets and develop rough endoplasmic reticulum. Capillary endothelial cells are swollen, containing many organellae in the cytoplasm. (EM, ×5000)

Fig. 10. Five hours after 20 mg/kg B.W. paraquat administration. Lymphatic vessels generally show marked dilatation, containing a few macrophages which surfaces developirregularly elongated pseudpod. (EM, ×3,500)





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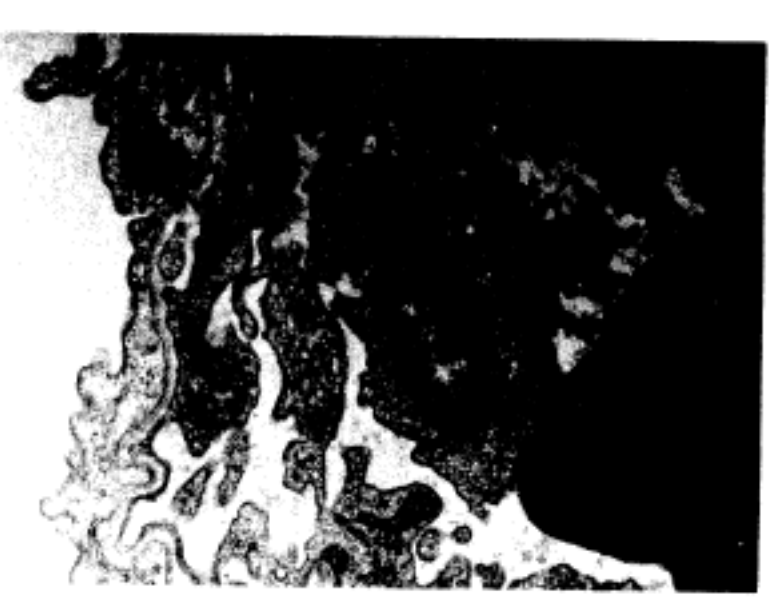


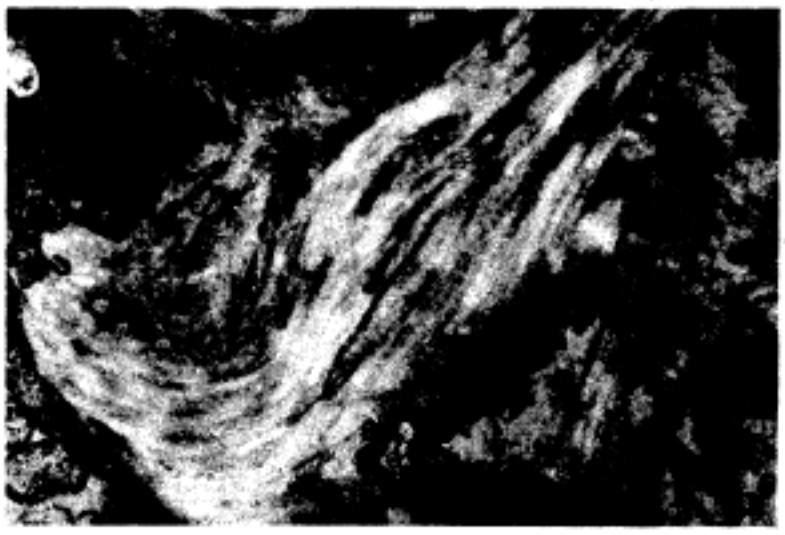
Fig. 11. Five hours after 30 mg/kg B.W. paraquat administration. Fibroblastic cell in the alveolar interstitium becomes swollen due to edema. (EM, ×12,000)

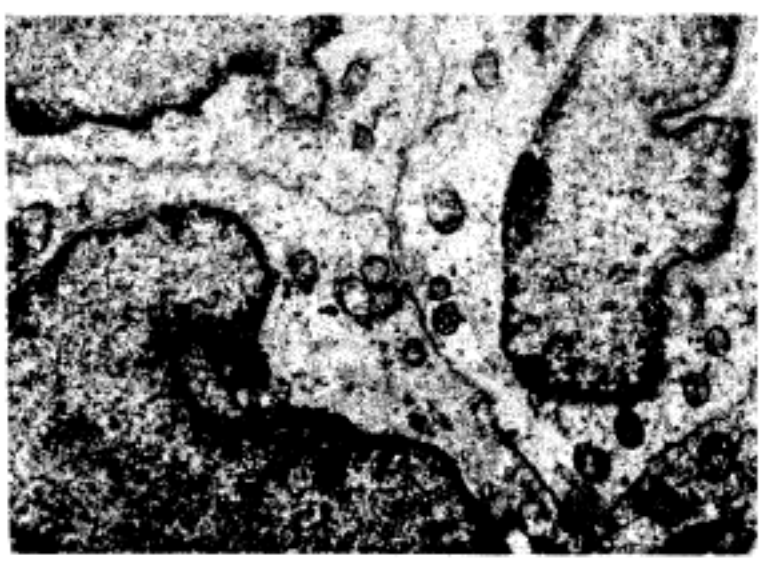
Fig. 12. The same animal as in Fig. 11. Alveolar macrophages contain a variably shaped heterophagosomes in the swollen cytoplasm. Cell surface shows very irregular filopodia. (EM, ×3,500)

Fig. 13. Five hours after 10 mg/kg B.W. paraquat administration. Alveolar macrophage contains a remarkably increased number of lamellar bodies which undergo necrotic alteration. (EM, ×10,000)

Fig. 14. Ten days after 5 mg/kg B.W. paraquat administration. Cytoplasmic processes of two alveolar macrophages show very close apposition each other. One of those cytoplasms engulfs a cytoplasmic process of the degenerating type I alveolar epithelium. (EM, ×3,500)

Fig. 15. The same animal as in Fig. 14. A high-power-view of the endothelial cytoplasm which shows a remarkably increased phagocytotic vesicles and free ribosomes. (EM, ×10,000)





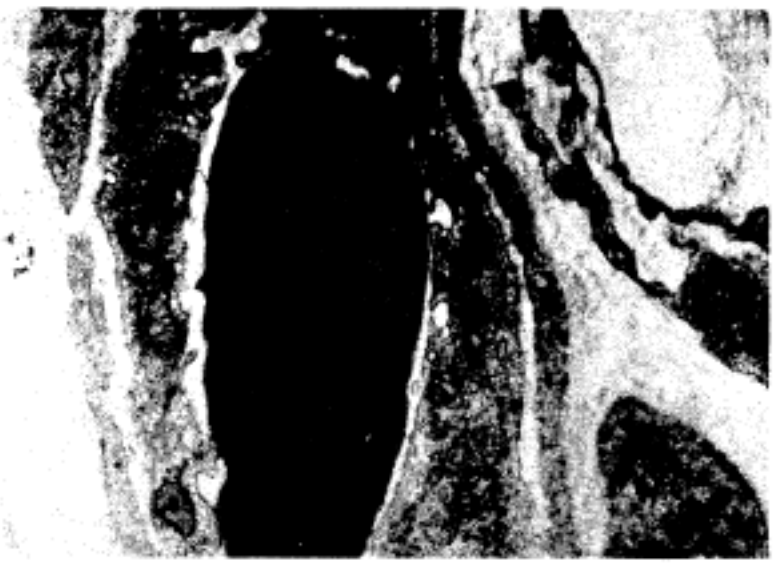


Fig. 16. Forty days after 40 mg/kg B.W. paraquat administration. Alveolar structure appears in some of the chronic cases. However fibroblastic cells in the interstitium stretched out the cytoplasmic process to get a direct contact with basal surface of type II alveolar epithelial cell. Interstitium occasionally shows edematous changes. (EM, ×3,500)

Fig. 17. Forty days after 50 mg/kg B.W. paraquat administration. Two of the alveolar type II cells are situated, side by side, suggesting the proliferation of those cells. (EM, ×4,000)

Fig. 18. The same animal as in Fig. 17. A cytoplasm of the arterioles, situated in the fibrotic interstitium, shows hypertrophic cytoplasm, containing a large number of pinocytotic vesicles, dilated vesicles, free ribosomes, rough endoplasmic reticulums and mitochondria. (EM, ×8,000)

Fig. 19. The same animal as in Fig. 17. Large lymphocytes aggregate together with close interdigitation of the adjacent cytoplasms in the bronchial-associated lymphoid tissue. (EM, ×7,000)

Fig. 20. The same animal as in Fig. 17. Alveolar interstitium occasionally accumulates bundles of collagen fibrils, where elastic fibers and connective tissue microfibrils exist. (EM, ×10,000)